# *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) Polymorphisms Affect Therapeutic Efficacy of Nateglinide in Chinese Patients with Type 2 Diabetes

Jin-Fang Song[1, 2], Qian Lu[1], Xue-Yan Zhou[1], Cheng-Lin Li[1], Xiao-Xing Yin[1], Tao Wang[1, 3*]

[1]Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, Xuzhou, China

[2]Department of Pharmacy, Affiliated Hospital of Jiangnan University, Wuxi, China

[3]Department of Pharmacy, Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

Objective:

Methods: A total of 200 T2DM patients and 200 healthy volunteers were enrolled to identify PPARD rs2016520 and NOS1AP rs12742393 genotypes using the polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). Sixty newly diagnosed T2DM patients (39 men, 21 women) were enrolled and treated with nateglinide (360 mg/day) for 8 weeks. Anthropometric measurements, clinical laboratory tests were obtained at baseline and after 8 weeks treatment.

Results: The minor C allelic frequencies of the PPARD rs2016520 (T/ C) polymorphism were 21.70% and 24.50% in T2DM patients and healthy controls, respectively. In this study, no significant differences were found between T2DM patients and control subjects in allelic frequencies of PPARD rs2016520(T/C) polymorphisms. The frequency of the C allele at the NOS1AP rs12742393 locus was higher in patients with T2DM than in healthy controls (33.25% vs. 21.50%, $P < 0.05$). Patients with the C allele of the PPARD rs2016520 polymorphism showed higher levels of body mass index (BMI), waist to hip ratio (WHR) and homeostasis model assessment for beta cell function (HOMA-B) at baseline ($P < 0.05$). After nateglinide treatment, patients with at least one C allele of PPARD rs2016520 showed a smaller decrease in post plasma glucose (PPG), HOMA-B than those with the TT genotype did ($P < 0.05$). Patients with the CC genotype of NOS1AP rs12742393 (A/C) had higher low-density lipoprotein-cholesterol (LDL-C) levels and lower HOMA-B ($P < 0.05$). In patients with the AA genotype, the drug showed better efficacy with respect to levels of fasting plasma glucose (FPG), fasting serum insulin (FINS), HOMA-B and homeostasis model assessment for insulin resistance (HOMA-IR) than in patients with the AC+CC genotype ($P < 0.05$). NOS1AP rs12742393 genotype distribution and allele frequency were associated with responsiveness of nateglinide treatment ($P < 0.05$).

Conclusion: These data suggest that the variant of PPARD and NOS1AP were associated with nateglinide monotherapy efficacy in newly diagnosed Chinese T2DM patients.

**Objective:** Nateglinide has been widely used clinically and display excellent safety and efficacy, the response to nateglinide varies among individuals. Among various reasons under discussion is genetic polymorphism, especially the genes related to drug absorption, distribution, metabolism and targeting. Recent studies have described the importance of *PPARD* and *NOS1AP* in regulating the secretion and resistance of insulin. However, little is known about the impacts of *PPARD* and *NOS1AP* genetic polymorphism on the efficacy of nateglinide. Therefore, the current study was designed to investigate a potential association of *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) polymorphisms with efficacy of nateglinide in newly diagnosed Chinese type 2 diabetes mellitus(T2DM) patients.

**Methods:** A total of 200 T2DM patients and 200 healthy volunteers were enrolled to identify *PPARD* rs2016520 and *NOS1AP* rs12742393 genotypes using the polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). Sixty newly diagnosed T2DM patients (39 men, 21 women) were enrolled and treated with nateglinide (360 mg/day) for 8 weeks. Anthropometric measurements, clinical laboratory tests were obtained at baseline and after 8 weeks treatment.

**Results:** The minor C allelic frequencies of the *PPARD* rs2016520 (T/ C) polymorphism were 21.70% and 24.50% in T2DM patients and healthy controls, respectively. In this study, no significant differences were found between T2DM patients and control subjects in allelic frequencies of *PPARD* rs2016520(T/C) polymorphisms. The frequency of the C allele at the *NOS1AP* rs12742393 locus was higher in patients with T2DM than in healthy controls (33.25% vs. 21.50%, $P < 0.05$). Patients with the C allele of the *PPARD* rs2016520 polymorphism showed higher levels of body mass index (BMI), waist to hip ratio (WHR) and homeostasis model assessment for beta cell function (HOMA-B) at baseline ($P < 0.05$). After nateglinide treatment, patients with at least one C allele of *PPARD* rs2016520 showed a smaller decrease in post plasma glucose (PPG), HOMA-B than those with the TT genotype did ($P < 0.05$). Patients with the CC genotype of *NOS1AP* rs12742393 (A/C) had higher low-density lipoprotein-cholesterol (LDL-C) levels and lower HOMA-B ($P < 0.05$). In patients with the AA genotype, the drug showed better efficacy with respect to levels of fasting plasma glucose (FPG), fasting serum insulin (FINS), HOMA-B and homeostasis model assessment for insulin resistance (HOMA-IR) than in patients with the AC+CC genotype ($P < 0.05$). *NOS1AP* rs12742393 genotype distribution and allele

frequency were associated with responsiveness of nateglinide treatment ($P < 0.05$).

**Conclusion:** These data suggest that the variant of *PPARD* and *NOS1AP* were associated with nateglinide monotherapy efficacy in newly diagnosed Chinese T2DM patients.

**Keywords:** *PPARD*, *NOS1AP*, genetic polymorphism, type 2 diabetes mellitus, nateglinide

## INTRODUCTION

Oral hypoglycemic agents such as sulfonylureas, glinides, metformin, α-glucosidase inhibitors, dipeptidyl peptidase 4 inhibitors and sodium glucose coordinated transporter 2-inhibitor are commonly used in the treatment of type 2 diabetes. Variations in genes encoding key proteins involved in insulin secretion, insulin action, and metabolism may alter susceptibility to type 2 diabetes mellitus (T2DM) and are the main factors affecting individual differences in response to hypoglycemic drug therapy [1, 2].

*PPARD* gene is located on chromosome 6p21.1-p21.2, and its coding product PPAR-δ (also named PPAR-β) is a member of the peroxisome proliferator activated receptor family, which is widely distributed in the liver, kidneys, cardiac and skeletal muscle, adipose tissue, brain, pancreatic and vasculature [3, 4]. PPAR-δ plays an important role in insulin resistance and islet β-cell function [5-8]. Activation of PPAR-δ in the liver may decrease hepatic glucose output, thereby contributing to improved glucose tolerance and insulin sensitivity [9, 10]. More recently, PPAR-δ activation came into focus as an interesting novel approach for the treatment of metabolic syndrome. Meanwhile, both preclinical and clinical studies have shown that PPAR-δ specific agonist therapy enhanced β-oxidation, decreased free fatty acid, and improved insulin sensitivity [11, 12]. Large-scale clinical studies in the Chinese population have shown that *PPARD* rs2016520 polymorphism (also named +294T > C or -87T > C) is associated with fasting blood glucose, postprandial blood glucose, insulin level and insulin resistance, and is a key factor affecting the development of metabolic syndrome and T2DM [13-14]. Studies in a Mexican population have produced similar results [15].

*NOS1AP*, located on chromosome 1q22.3, and its coding product is known as carboxy-terminal PDZ ligand of neuronal nitric oxide synthase (nNOS), which regulates nNOS activity through interaction with the PDZ binding region of nNOS [16]. Studies have shown that nNOS is also localized on insulin secreted granules and can be activated by increasing β cells intracellular calcium

which is a known response to glucose stimulation [16, 17]. Functional studies have shown that dysfunction of nNOS may in fact be directly involved in insulin secretion as well as insulin resistance [18-20]. A novel mechanism for beta cell dysfunction has been recently described, namely that elevated cholesterol inhibits insulin secretion by modifying nNOS activity [21]. In addition, genetic studies have implied that the variations of *NOS1AP* are associated with individual differences in the efficacy of sulfonylureas as well as increased safety risk of developing new-onset diabetes in patients taking calcium channel blockers [22, 23]. One clinical study showed that *NOS1AP* rs12742393 C allele gene was associated with an increased susceptibility to T2DM in the Chinese population [24]. Although many studies have been conducted on the association between *NOS1AP* variants and T2DM susceptibility and the metabolic related traits has been investigated, however, the results were inconsistent in different populations, such as the European population [23-27]. Though the studies on how the variants influenced the diseases were limited, one functional study showed that rs12742393 could affect *NOS1AP* gene expression through influencing transcription factor binding [28].

Nateglinide is an important non-sulfonylurea oral hypoglycemic agent that improves blood glucose levels. It promotes insulin secretion from pancreatic islet beta cells by inhibiting ATP-sensitive $K^+$ channels and activating $Ca^{2+}$ channels. However, considerable interindividual di□erences in the therapeutic efficacy of nateglinide have been reported in T2DM patients [29, 30]. The underlying mechanism that lead to variations is still unclear. It is hypothesized that genetic polymorphisms of genes that code drug metabolizing enzymes, drug transporters, drug targets, or susceptibility genes related to T2DM pathogenesis may affect the pharmacokinetic or pharmacodynamics process of drugs, and eventually lead to interindividual variation in therapeutic efficacy of nateglinide [30]. Cytochrome P450 (CYP) 2C9 and CYP3A4 have been identified as the main metabolic enzymes involved in the biotransformation of nateglinide. SLCO1B1 gene encoding organic anion transporting polypeptide 1B1 (OATP1B1), which is involved in cellular uptake and transport of nateglinide. Some studies attributed interindividual di□erences in the pharmacokinetic process of nateglinide to genetic polymorphism of *CYP2C9* and *SLCO1B1*, but not any of the *CYP3A4* polymorphisms [31]. It is hypothesized that due to the genetic polymorphism of the enzymes and transporters mentioned above and their influence on the pharmacokinetic process of nateglinide might contribute to individual differences in pharmacodynamics. But, this could not

elucidate the complete mechanism of action by which the same nateglinide therapy results in various therapeutic responses [32-34].

Based on the facts that *PPARD* and *NOS1AP* play crucial roles in functional regulation of β-cells, insulin resistant and metabolism, we conduct this study to evaluate the association *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) polymorphisms with susceptibility to development of type 2 diabetes and identify the impact of these polymorphisms on nateglinide efficacy in Chinese T2DM patients.

## MATERIALS AND METHODS

### Participants and study Design

A total of 200 T2DM patients and 200 healthy controls were recruited for analysis of *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) polymorphisms. All subjects were evaluated for their medical history, physical examination, and clinical laboratory examinations. T2DM patients were diagnosed referring to the 1999 World Health Organization criteria. The inclusion criteria for T2DM patients were (i) a BMI in the range of 18.5 - 30 kg/m$^2$ and (ii) that the subject should not have received any insulin secretagogue or any agonist or inhibitor of CYP2C9, CYP3A4, and OATP1B1 in the previous 3 months. Patients who were receiving insulin treatment, pregnant or lactating women, and patients with serious diseases such as acute myocardial infarction, cerebral vascular accident, trauma, and kidney or liver disease were excluded from the study. A total of 60 newly diagnosed T2DM patients (39 male, 21 female) with various *PPARD* rs2016520(T/C) or *NOS1AP* rs12742393(A/C) genotypes and the same with the same *CYP2C9**1 and *SLCO1B1* 521TT genotype were randomly selected to take 360mg nateglinide per day (120 mg once before meal) orally for 8 consecutive weeks. The study was registered in the Chinese Clinical Trial Register (No. ChiCTRCCC13003536), in which the protocol used was approved by the ethics committee of the Affiliated Hospital of Xuzhou Medical University. Written informed consent was obtained from each participant before the study.

### Anthropometric and Biochemical Measurements

The general anthropometric parameters considered for this study were height (in meters), weight (in kilograms), and waist and hip circumferences (in centimeters). After an overnight fast by

the study subjects, blood samples for measurements of plasma glucose and insulin were obtained both in the fasting state and 2 h later during a standard 75-g oral glucose tolerance test. Plasma glucose and insulin, hemoglobin A1c (HbA1c), and serum lipids were measured as previously described [35]. These parameters were measured at the end of weeks 0 and 8 after administration of nateglinide.

The homeostasis model assessment for insulin resistance (HOMA-IR) and beta cell function (HOMA-B) are given by: HOMA-IR = fasting insulin level (mU/L) × fasting glucose level (mmol/L)/22.5; HOMA-B = 20 × fasting serum insulin (FINS)/(FPG-3.5) [36].

## Genotyping

Genomic DNA was extracted from peripheral blood leucocytes using a SiMax Genome DNA Kit(Sbsbio, Shanghai, China). In the present study, the *PPARD* rs2016520 locus was amplified by the polymerase chain reaction (PCR) with the following primers: 5-TGGGAAGGGTGATAGGGCA-3 (forward) and 5'-CTGGTGAGTGGCAGAGCAGA-3(reverse). The 602bp PCR products were digested by FoKI (NEB, Beijing, China). For the *NOS1AP* rs12742393 locus, the following primer pairs were used 5-GGTGAATGTGTACAAAGGAGAAGG-3 (forward) and 5-CAAACTGAAATGGACCACAAAGAG-3(reverse). The PCR products were digested by BsrI(NEB, Beijing, China). All obtained DNA fragments were separated by 2% agarose gel electrophoresis followed by ethidium bromide staining and visualization with UV transillumination. To confirm the assay results, 5.0% of all samples were directly sequenced.

## Definition of the Response to Nateglinide

T2DM subjects were classified into two groups based on changes in HbA1c after treatment with nateglinide: responder and non-responder. According to previous studies, nateglinide monotherapy improved HbA1c in patients with type 2 diabetes by an average of 10% to 20% from baseline levels [37-40]. In the present study, HbA1c levels were reduced by an average of 19.95% in all subjects treated with nateglinide. Therefore, we identified a 20% improvement in HbA1c after 8 weeks of nateglinide treatment as an intermediate value, with responders were defined as patients with 20% or greater decrease in HbA1c and non-responders defined as patients who failed to achieve this level.

## Statistical Analysis

Statistical analyses were carried out using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA). All data were expressed as mean standard ± deviation (SD), or percentages as appropriate. Hardy-Weinberg equilibrium, frequencies of genotypes and alleles were assessed using $\chi^2$ tests in the study sample. A comparison of baseline characteristics in patients with T2DM and healthy controls was carried out using independent samples t tests. Characteristics among genotypes were compared using one-way analysis of variance. Paired t tests and independent samples t tests were used to estimate the effects of nateglinide on biochemical index among genotypes. Parameters with nonnormal distribution were analyzed by the Kruskal-Wallis test. Statistical power was calculated by power calculator software (http://www.ncss.com). A value of $P < 0.05$ was considered statistically significant.

# RESULTS

## Genotyping Analysis and Allelic Frequencies

The genotypes and allelic frequencies of the *PPARD* rs2016520 (T/C) and *NOS1AP* rs12742393 (A/C) polymorphisms in the T2DM patients and in control subjects are shown in Table1. The minor C allelic frequencies of the *PPARD* rs2016520 (T/ C) polymorphism were 21.70% and 24.50% in T2DM patients and healthy controls, respectively. In this study, no significant differences were found between T2DM patients and control subjects in allelic frequencies of *PPARD* rs2016520(T/C) polymorphisms. The frequency of the C allele at the *NOS1AP* rs12742393 locus was higher in patients with T2DM than in healthy controls (33.25% vs. 21.50%, $P = 0.000$). The genotype distributions of *PPARD* rs2016520 and *NOS1AP* rs12742393 SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$).

## The Baseline Clinical Characteristics of Different *PPARD* rs2016520 and *NOS1AP* rs12742393 Genotypes

The baseline clinical characteristics of 200 T2DM patients with different *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393 (A/C) genotypes were measured before therapy (Table

2). The *PPARD* rs2016520(T/C) polymorphism was noticeably associated with levels of BMI, WHR and HOMA-B; individuals with the C allele had obviously higher BMI and WHR levels, but markedly lowerHOMA-B as compared with those of other genotypes ($P < 0.05$) (Table 2, Figure S1). However, patients with the CC genotype at *NOS1AP* rs12742393 (A/C) had higher levels of LDL-C ($P < 0.030$) but lower levels of HOMA-B ($P = 0.000$) than those patients with genotypes AA and AC (Table 2, Figure S2).

## Effects of the rs2016520 and rs12742393 Polymorphisms on Therapeutic Efficacy of Nateglinide in T2DM Patients

To evaluate the e□ects of *PPARD* and *NOS1AP* variations on the efficacy of nateglinide, newly diagnosed T2DM patients with various *PPARD* rs2016520 (C/T) and *NOS1AP* rs12742393 (A/C) genotypes but with the same *SLCO1B1* T521C and *CYP2C9**1 genotype were enrolled. Nateglinide significantly decreased the levels of FPG, PPG, HbA1c, TG, and TC, and increased the levels of FINS, PINS, HOMA-B and HDL-C levels in T2DM patients after 8 weeks of nateglinide treatment (Table S). Patients with *PPARD* rs2016520 TT genotypes had a significantly decrease in PPG and notably increase HOMA-B as compared with patients with the TC+ CC genotypes, which indicated that patients with genotype TT had better efficacy of nateglinide monotherapy (Table S2-1, Fig 1). Moreover, patients with *NOS1AP* rs12742393 AC+CC genotypes had diluted response of nateglinide in the case of FPG, FINS, HOMA-IR, and HOMA-B compared with AA genotype carriers (Table S2-2, Fig 2).

## Association of *PPARD* rs2016520 and *NOS1AP* rs12742393 Genetic Polymorphisms With Response Rate to Nateglinide Treatment

In order to evaluate the association between *PPARD* and *NOS1AP* polymorphisms and the response to nateglinide treatment, genotypes and allelic frequency distributions were analyzed in the responder and non-responder groups (Table3). No signifficant e□ects of the variation in *PPARD* rs2016520(T/C) on nateglinide treatment were detected. According to predetermined criteria of 20% reduction from baseline, *NOS1AP* rs12742393 A allele carriers exhibited higher response rate to nateglinide treatment; AA allele homozygotes had the highest response rate (70.83%), while AC heterozygous and CC homozygous had 44.44% and 22.22%, respectively ($P = 0.027$).

## DISCUSSION

In the present study, we found for the first time that genetic polymorphisms of *PPARD* and *NOS1AP* may affect the therapeutic efficacy of nateglinide in Chinese patients with T2DM. We observed that, in T2DM patients with at least one C allele of *PPARD* rs2016520(T/C) or one C allele of *NOS1AP* rs12742393(A/C), may be less responsive to treatment with nateglinide, indicating that the *PPARD* and *NOS1AP* genotype may serve as nateglinide response prognosticator. Therefore, we suggest that prior genotyping and individualized administration of nateglinide may be beneficial for those T2DM patients who require treatment with nateglinide.

*PPARD* and *NOS1AP* are directly or indirectly involved in the regulation of β-cell function and insulin resistance; this suggests that genetic polymorphisms in the two genes may contribute to interindividual differences in nateglinide response [5-8, 18-21]. To date, there has been no report on the influence of *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) polymorphism on nateglinide response. Further pharmacogenetic and functional studies are necessary to investigate the potential mechanism and lay the foundation for Individualized administration for T2DM.

The data from this study also showed that *PPARD* rs2016520(T/C) and *NOS1AP* rs2016520(T/C) polymorphisms may affect some clinical indicators related to T2DM. The C allele of *NOS1AP* rs2016520(T/C) polymorphism is associated with the susceptibility of T2DM, but no association was found between *PPARD* polymorphism and the development of T2DM. Previous studies have shown that PPAR-δ is involved in glucose and lipid metabolism and plays an important role in fat storage, insulin resistance and the regulation of islet β-cell function [5-8]. Studies in Korean and Chinese populations have found that *PPARD* gene polymorphism may be associated with obesity, dyslipidemia, insulin resistance and other risk factors for T2DM [14, 41]. Currently, no direct evidence has been found that *PPARD* gene polymorphism affects T2DM susceptibility. Common variations in *NOS1AP* has been associated with T2DM in Caucasian and African American patients treated with calcium-channel blockers [23, 26]. Our data suggest that *NOS1AP* rs12742393 is associated with T2DM susceptibility in Chinese, which is consistent with the findings of a previous clinical study in a Chinese population [24]. In contrast, two other studies failed to find this association in Caucasians [26, 27]. These results of our study are not completely consistent with those from some previous studies, and the reasons for the differences may be the race and

environment.

Studies have reported that *CYP2C9* and *SLCO1B1* gene polymorphisms could affect the pharmacokinetic process of nateglinide, resulting in differences in drug concentrations in plasma and therapeutic efficacy [42-45]. Therefore, in this study we selected patients with the same *CYP2C9**1 and *SLCO1B1* 521TT genotype to avoid any possible changes in the pharmacokinetics and pharmacodynamics of nateglinide caused by *OATP1B1* or *CYP2C9* polymorphism. Our study is an exploratory study on the effect of *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) polymorphisms on the efficacy of nateglinide in patients with T2DM. Our data show that nateglinide monotherapy has good clinical effect with respect to reduce FPG, PPG, HbA1c, TG and TC levels in patients with T2DM, and improve FINS, PINS, HOMA-B and HDL-C levels. Also, patients with *PPARD* rs2016520 TC + CC genotypes had attenuated efficacy of nateglinide monotherapy with respect to PPG, and HOMA-B compared with TT genotype carriers. Finally, our results showed that the *NOS1AP* rs12742393(A/C) polymorphism was associated with an attenuated nateglinide effect in Chinese patients with T2DM, and that individuals with AC+CC genotypes showed a smaller increase in FINS and HOMA-B, but a smaller decrease in FPG and HOMA-IR levels as compared to individuals with the TT genotype.

The *PPARD* gene encoding PPAR-δ, which is related to islet function and insulin resistance, might directly or indirectly participate in the pathogenesis of T2DM [7, 46-48]. In the present study, we preliminarily found that the polymorphism of *PPARD* gene affected the impact of nateglinide on insulin secretion in Chinese, which may be realized by the role of PPAR-δ in insulin secretion, as measured by HOMA-B. The biological effect of PPAR-δ overlaps with the therapeutic mechanism of nateglinide to a certain extent, which can partly explain the mechanism of *PPARD* gene polymorphism affecting the efficacy of nateglinide. However, the exact molecular mechanism remains to be further studied.

*NOS1AP* mainly regulates nNOS activity, and nNOS can inhibit intracellular $Ca^{2+}$ level and thereby regulate insulin secretion [17, 22, 49]. In addition, lateral ventricular injection of nNOS inhibitors can affect insulin secretion and insulin sensitivity [19]. Therefore, it is speculated that *NOS1AP* may increase T2DM susceptibility by affecting insulin secretion and sensitivity, and nateglinide also acts on islet β cells to promote insulin secretion, and *NOS1AP* gene polymorphism may affect the efficacy of nateglinide. In this study, we observed that subjects with at least one C

allele of the *NOS1AP* rs12742393 showed a smaller decrease in FPG and HOMA-IR and more obvious increase in FINS and HOMA-B levels than those with the AA genotype, which suggested that the *NOS1AP* rs12742393 C allele confers the poor nateglinide response through induce insulin resistance, as measured by HOMA-IR. Animal studies have shown that knockout of mouse nNOS gene may induce insulin resistance in mice [50]. Meanwhile, it has been reported that the dysfunction of nNOS in islet β cells is related to insulin secretion [17-20]. Therefore, it is speculated that *NOS1AP* rs12742393 risk gene C affects the efficacy of nateglinide in patients with T2DM, which is at least partially associated with insulin resistance and islet β cell. However, the exact mechanism by which *NOS1AP* gene polymorphism affects the efficacy of nateglinide needs to be further investigated.

## Limitation

In interpreting the results of our study, several shortcomings must be addressed. According to research reports, *PPARD* rs2016520 and *NOS1AP* rs12742393 have the strongest correlation with the risk factors and susceptibility of T2DM [14, 23-25, 41]. Therefore, our study focused only on the effect of mutations in *PPARD* rs2016520 and *NOS1AP* rs12742393 on nateglinide efficacy. However, the possibility still exists that other susceptibility loci for T2DM may a□ect the therapeutic efficacy of nateglinide [51, 52]. Second, the sample size was relatively small, we may have missed some meaningful results. Further studies with a larger sample size are required to confirm the effects of *PPARD* and *NOS1AP* polymorphisms on the therapeutic efficacy of nateglinide. Third, our study only investigated the effects of gene polymorphism on the efficacy of nateglinide. However, the mechanisms by which the two SNPs in *PPARD* and *NOS1AP* affect the therapeutic efficacy of nateglinide are not fully understood. In the future, more functional studies are needed to explore the mechanism by which *PPARD* and *NOS1AP* gene polymorphism affect drug efficacy.

## Conclusion

The variations of *PPARD* rs2016520 and *NOS1AP* rs12742393 seem to be associated with the therapeutic efficacy of nateglinide in newly diagnosed Chinese patients with T2DM. We therefore suggest that prior genotyping for *PPARD* rs2016520(T/C) and *NOS1AP* rs12742393(A/C) single-nucleotide polymorphisms may be beneficial for T2DM patients who need be treated with nateglinide. Further pharmacogenomic and functional studies to confirm the exact effects of *PPARD*

and *NOS1AP* variants on nateglinide therapeutic efficacy are necessary to achieve individualized drug administration.

## REFERENCES


[1] Heo CU, Choi CI. Current Progress in Pharmacogenetics of Second-Line Antidiabetic Medications: Towards Precision Medicine for Type 2 Diabetes. *J Clin Med*. 2019;8(3):393. Published 2019 Mar 21. doi:10.3390/jcm8030393

[2] Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol.* 2020;16(7):377-390. doi:10.1038/s41581-020-0278-5

[3] Wagner N, Wagner KD. PPAR Beta/Delta and the Hallmarks of Cancer. *Cells*. 2020;9(5):1133. Published 2020 May 4. doi:10.3390/cells9051133

[4] Fredenrich A, Grimaldi PA. PPAR delta: an uncompletely known nuclear receptor. *Diabetes Metab.* 2005;31(1):23-27. doi:10.1016/s1262-3636(07)70162-3

[5] Cao M, Tong Y, Lv Q, et al. PPARδ Activation Rescues Pancreatic β-Cell Line INS-1E from Palmitate-Induced Endoplasmic Reticulum Stress through Enhanced Fatty Acid Oxidat io n. *PPAR Res*. 2012;2012:680684. doi:10.1155/2012/680684

[6] Iglesias J, Barg S, Vallois D, et al. PPARβ/δ affects pancreatic β cell mass and insulin secretion in mice. J Clin Invest. 2012;122(11):4105-4117. doi:10.1172/JCI42127

[7] Lee CH, Olson P, Hevener A, et al. PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A*. 2006;103(9):3444-3449. doi:10.1073/pnas.0511253103

[8] Wang YX, Lee CH, Tiep S, et al. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell*. 2003;113(2):159-170. doi:10.10 16/s0092-8674(03) 00 269-1

[9] Barak Y, Liao D, He W, et al. Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. *Proc Natl Acad Sci U S A*. 2002;99(1):303-308. doi:10.1073/pnas.012610299

[10] Kostadinova R, Montagner A, Gouranton E, et al. GW501516-activated PPARβ/δ promotes liver fibrosis via p38-JNK MAPK-induced hepatic stellate cell proliferation. *Cell Biosci.* 2012;2(1):34. Published 2012 Oct 10. doi:10.1186/2045-3701-2-34

[11] Bojic LA, Telford DE, Fullerton MD, et al. PPARδ activation attenuates hepatic steatosis in Ldlr-/- mice by enhanced fat oxidation, reduced lipogenesis, and improved insulin sensitivity. *J*

*Lipid Res*. 2014;55(7):1254-1266. doi:10.1194/jlr.M046037

[12] Greene NP, Fluckey JD, Lambert BS, et al. Regulators of blood lipids and lipoproteins? PPARδ and AMPK, induced by exercise, are correlated with lipids and lipoproteins in overw eight/o bese men and women. *Am J Physiol Endocrinol Metab*. 2012;303(10):E1212-E1221. doi:10.1 152/ajpendo.00309.2012

[13] Tang L, Lü Q, Cao H, et al. PPARD rs2016520 polymorphism is associated with metabolic traits in a large population of Chinese adults. *Gene*. 2016;585(2):191-195. doi:10.1016/j.gene.2 016.02.035

[14] Hu C, Jia W, Fang Q, et al. Peroxisome proliferator-activated receptor (PPAR) delta genetic polymorphism and its association with insulin resistance index and fasting plasma glucose concentrations in Chinese subjects. *Diabet Med.* 2006;23(12):1307-1312. doi:10.1111/j.1464-5491.2006.02001.x

[15] Carrillo-Venzor MA, Erives-Anchondo NR, Moreno-González JG, et al. Pro12Ala PPAR-γ2 and +294T/C PPAR-δ Polymorphisms and Association with Metabolic Traits in Teenagers from Northern Mexico. *Genes (Basel)*. 2020;11(7):776. Published 2020 Jul 10. doi:10.339 0/genes11070776

[16] Aspinwall CA, Qian WJ, Roper MG, et al. Roles of insulin receptor substrate-1, phosphati dy linositol 3-kinase, and release of intracellular Ca2+ stores in insulin-stimulated insulin secre tion in beta-cells. *J Biol Chem.* 2000;275(29):22331-22338. doi:10.1074/j bc.M 909647199

[17] Lajoix AD, Reggio H, Chardès T, et al. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion [published correction appears in Diabetes 2001 Sep;50(9):2177-8]. *Diabetes.* 2001;50(6):1311-1323. doi:10.2337/diabetes.50.6.1311

[18] Rizzo MA, Piston DW. Regulation of beta cell glucokinase by S-nitrosylation and association with nitric oxide synthase. *J Cell Biol. 2*003;161(2):243-248. doi:10.1083/jcb.200301063

[19] Shankar R, Zhu JS, Ladd B, et al. Central nervous system nitric oxide synthase activity regulates insulin secretion and insulin action. *J Clin Invest.* 1998;102(7):1403-1412. doi:10.1 172/JCI3030

[20] Shankar RR, Wu Y, Shen HQ, et al. Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. *Diabetes.* 2000;49(5):684-687. doi:10.2 337/d iabetes.49.5.684

[21] Hao M, Head WS, Gunawardana SC, et al. Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. *Diabetes*. 2007;56(9):2328-2338. doi:10.2337/db07-0056

[22] Becker ML, Aarnoudse AJ, Newton-Cheh C, et al. Common variation in the NOS1AP gene is associated with reduced glucose-lowering effect and with increased mortality in users of sulfonylurea. *Pharmacogenet Genomics*. 2008;18(7):591-597. doi:10.1097/FPC.0b013e328300e8c5

[23] Becker ML, Visser LE, Newton-Cheh C, et al. Genetic variation in the NOS1AP gene is associated with the incidence of diabetes mellitus in users of calcium channel blockers. *Diabetologia.* 2008;51(11):2138-2140. doi:10.1007/s00125-008-1143-4

[24] Hu C, Wang C, Zhang R, et al. Association of genetic variants of NOS1AP with type 2 diabetes in a Chinese population. *Diabetologia*. 2010;53(2):290-298. doi:10.1007/s00125-009-1594-2

[25] Chu AY, Coresh J, Arking DE, et al. NOS1AP variant associated with incidence of type 2 diabetes in calcium channel blocker users in the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia.* 2010;53(3):510-516. doi:10.1007/s00125-009-1608-0

[26] Andreasen CH, Mogensen MS, Borch-Johnsen K, et al. Lack of association between PKLR rs3020781 and NOS1AP rs7538490 and type 2 diabetes, overweight, obesity and related metabolic phenotypes in a Danish large-scale study: case-control studies and analyses of quantitative traits. *BMC Med Genet*. 2008;9:118. Published 2008 Dec 26. doi:10.1186/1471-2350-9-118

[27] Prokopenko I, Zeggini E, Hanson RL, et al. Linkage disequilibrium mapping of the replicated type 2 diabetes linkage signal on chromosome 1q. *Diabetes*. 2009;58(7):1704-1709. doi:10.2337/db09-0081

[28] Wratten NS, Memoli H, Huang Y, et al. Identification of a schizophrenia-associated functional noncoding variant in NOS1AP [published correction appears in Am J Psychiatry. 2010 Jul;167(7):870]. *Am J Psychiatry*. 2009;166(4):434-441. doi:10.1176/appi.ajp.2008.08081266

[29] Cai XL, Luo YY, Han XY, Ji LN. A meta-analysis of efficacy and safety of nateglinide in type 2 diabetes mellitus in Asia. *Chinese J of Diabet,* 2012;21:913-917. doi:10.396/j.issn.1006-6187.2013.10.014

[30] Cheng Y, Xiong QX, Liu Q, et al. A comparative study of the clinical efficacy of Naglinide and Acarbose in the treatment of type 2 diabetes mellitus. *Modern Chinese Drug Use*, 2010;4:166-167. doi: 10.3969/j.issn.1673-9523.2010.12.144

[31] Kalliokoski A, Neuvonen M, Neuvonen PJ, et al. Different effects of SLCO1B1 polymorphism on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *J Clin Pharmacol*. 2008;48(3):311-321. doi:10.1177/0091270007311569

[32] Barroso I, Luan J, Middelberg RP, et al. Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action [published correction appears in Plos Biol. 2003 Dec;1(3):445]. *PLoS Biol.* 2003;1(1):E20. doi:10.1 371/journal.pbio.0000020

[33] Schwanstecher C, Meyer U, Schwanstecher M. K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channe ls. *D iabetes*. 2002;51(3):875-879. doi:10.2337/diabetes.51.3.875

[34] Barhanin J, Lesage F, Guillemare E ,et al. K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature*. 1996;384(6604):78-80. doi:10.1038/38 4078a0

[35] Wang T, Wang XT, Lai R, et al. MTNR1B Gene Polymorphisms Are Associated With the Therapeutic Responses to Repaglinide in Chinese Patients With Type 2 Diabetes Mellitus. *Fr ont Pharmacol.* 2019;10:1318. Published 2019 Nov 7. doi:10.3389/fphar.201 9.01318

[36] Kong X, Xing X, Hong J, et al. Association of a type 2 diabetes genetic risk score with insulin secretion modulated by insulin sensitivity among Chinese Hans. *Clin Genet*. 2017;91(6):832-842. doi:10.1111/cge.12817

[37] Bolen S, Wilson L, Vassy J, et al. Comparative Effectiveness and Safety of Oral Diabetes Medications for Adults With Type 2 Diabetes. *Rockville (MD): Agency for Healthcare Resea rch and Quality (US)*; July 2007.

[38] Kawamori R, Kaku K, Hanafusa T, et al. Efficacy and safety of repaglinide vs nateglinide for treatment of Japanese patients with type 2 diabetes mellitus. *J Diabetes Investig.* 2012;3(3):30 2-308. doi:10.1111/j.2040-1124.2011.00188.x

[39] Kim MK, Suk JH, Kwon MJ, et al. Nateglinide and acarbose for postprandial glucose control after optimizing fasting glucose with insulin glargine in patients with type 2 diabetes. *Diabetes*

*Res Clin Pract.* 2011;92(3):322-328. doi:10.1016/j.diabres.2011.01.022

[40] Ning G, Chen LL, Chen MD, et al. Chinese expert consensus on clinical application of naglini de [J]. *Chinese Journal of Endocrinology and Metabolism*,2011(05):451-453. doi:10. 3760/cm a.j.issn.1000-6699.2011.05.029

[41] Shin HD, Park BL, Kim LH, et al. Genetic polymorphisms in peroxisome proliferator-activated receptor delta associated with obesity. *Diabetes.* 2004;53(3):847-851. doi:10.2337/diabetes.5 3.3.847

[42] Kirchheiner J, Meineke I, Müller G, et al. Influence of CYP2C9 and CYP2D6 polymorphisms on the pharmacokinetics of nateglinide in genotyped healthy volunteers. *Clin Pharmacokinet.* 2004;43(4):267-278. doi:10.2165/00003088-200443040-00005

[43] Cheng Y, Wang G, Zhang W, et al. Effect of CYP2C9 and SLCO1B1 polymorphisms on the pharmacokinetics and pharmacodynamics of nateglinide in healthy Chinese male volunte ers. *Eur J Clin Pharmacol*. 2013;69(3):407-413. doi:10.1007/s00228-012-1364-9

[44] Niemi M, Backman JT, Kajosaari LI, et al. Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther.* 2005;77(6): 468-478. doi:10.1016/j.clpt.2005.01.018

[45] Izumi S, Nozaki Y, Maeda K, et al. Investigation of the impact of substrate selection on in vitro organic anion transporting polypeptide 1B1 inhibition profiles for the prediction of drug-drug interactions. *Drug Metab Dispos.* 2015;43(2):235-247. doi:10.1124/dmd.114.059105

*[46]* Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med*. 2002;53:409-435. doi:10.1146/annurev.med.53.082901.104018

[47] Reilly SM, Lee CH. PPAR delta as a therapeutic target in metabolic disease. *FEBS Lett.* 2008;582(1):26-31. doi:10.1016/j.febslet.2007.11.040

[48] Winzell MS, Wulff EM, Olsen GS, et al. Improved insulin sensitivity and islet function after PPARdelta activation in diabetic db/db mice. *Eur J Pharmacol*. 2010;626(2-3):297-305. doi:10. 1016/j.ejphar.2009.09.053

[49] Gunawardana SC, Rocheleau JV, Head WS, et al. Mechanisms of time-dependent potentiation of insulin release: involvement of nitric oxide synthase. *Diabetes.* 2006;55(4):1029-1033. doi:10.2337/diabetes.55.04.06.db05-1532

[50] Turini P, Thalmann S, Jayet PY, et al. Insulin resistance in mice lacking neuronal nitric oxide

synthase is related to an alpha-adrenergic mechanism. *Swiss Med Wkly.* 2007;137(49-50):700-704.

[51] Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis [published correction appears in Nat Genet. 2011 Apr;43(4):388]. *Nat Genet.* 2010;42(7):579-589. doi:10.1038/ng.609

[52] Shi LX, Zhang L, Zhang DL, et al. Association between TNF-α G-308A (rs1800629) polymorphism and susceptibility to chronic periodontitis and type 2 diabetes mellitus: A meta-analysis. *J Periodontal Res.* 2021;56(2):226-235. doi:10.1111/jre.12820

**Table1** Comparison of genotype and frequencies of *PPARD* rs2016520 and *NOS1AP* rs12742393 polymorphism between T2DM patients and healthy subjects.

| Genotypes | Healthy subjects n =200 (frequency) | T2DM patients n =200 (frequency) | *P* values |
|---|---|---|---|
| *PPARD* rs2016520 | | | |
| TT | 110(55.00%) | 124(62.00%) | |
| TC | 82(41.00%) | 65(32.50%) | |
| CC | 8(4.00%) | 11(5.40%) | 0.194[b] |
| Alleles | | | |
| T | 302(75.50%) | 313(78.30%) | |
| C | 98(24.50%) | 87(21.70%) | 0.356[b] |
| *NOS1AP* rs12742393 | | | |
| AA | 123(61.50%) | 86(43.00%) | |
| AC | 68(34.00%) | 95(47.50%) | |
| CC | 9(4.50%) | 19(9.50%) | 0.001[b] |
| Alleles | | | |
| A | 314(78.50%) | 267(66.75%) | |
| C | 86(21.50%) | 133(33.25%) | 0.000[b] |

The allelic frequencies are indicated in absolute values (percentage). [b]*P* values are determined by the Pearson chi-square test.

**Table 2** The Baseline Clinical Characteristics of Different *PPARD* rs2016520 and *NOS1AP* rs12742393 Genotypes in patients with T2DM (n=200)

| Parameters | *PPARD* genotypes | | | *P* value | *NOS1AP* genotypes | | | *P* value |
|---|---|---|---|---|---|---|---|---|
| | TT | TC | CC | | AA | AC | CC | |
| N(male/female) | 123(69/54) | 66(36/30) | 11(6/5) | 0.967 [b] | 86(45/41) | 95(52/43) | 19(11/8) | 0.898[b] |
| Age (years) | 48.37±12.05 | 50.71±12.37 | 48.11±12.49 | 0.274 | 48.72±11.89 | 49.77±11.79 | 50.25±10.48 | 0.218 |
| BMI (kg/m$^2$) | 25.41±3.14 | 26.61±3.25 | 27.82±4.01 | 0.012* | 25.94±3.27 | 26.32±4.11 | 25.79±3.21 | 0.899 |
| WHR | 0.91±0.07 | 0.93±0.05 | 0.94±0.04 | 0.028* [c] | 0.92±0.07 | 0.94±0.07 | 0.92±0.08 | 0.468[c] |
| $HbA_{1c}$ (%) | 9.26±2.16 | 9.08±2.19 | 9.46±1.48 | 0.332 | 9.41±2.31 | 9.42±2.04 | 9.31±2.34 | 0.898 |
| FPG (mmol/L) | 9.85±2.77 | 9.71±2.75 | 9.63±2.18 | 0.926 [c] | 9.72±2.75 | 9.87±2.24 | 10.01±2.71 | 0.706 |
| PPG (mmol/L) | 16.21±4.40 | 16.14±4.52 | 15.53±2.54 | 0.885 [c] | 15.89±4.69 | 16.31±4.32 | 15.79±4.13 | 0.726 |
| FINS (mU/L) | 9.81±6.32 | 10.14±6.87 | 11.73±7.79 | 0.642 | 10.15±6.91 | 10.42±7.81 | 9.82±6.63 | 0.676[c] |
| PINS (mU/L) | 37.12±34.23 | 41.49±44.84 | 31.53±22.35 | 0.622 | 37.13±26.53 | 36.76±27.41 | 34.81±25.01 | 0.729 |
| HOMA-IR | 4.25±2.87 | 4.29±3.23 | 4.87±3.12 | 0.807 | 4.28±3.11 | 4.59±3.85 | 4.38±2.69 | 0.525 |
| HOMA-B | 38.31±7.32 | 35.26±6.94 | 34.73±5.98 | 0.011 | 37.31±8.23 | 32.26±7.94 | 31.43±4.38 | 0.041 |
| TG (mmol/L) | 2.51±2.93 | 2.25±1.84 | 2.53±1.56 | 0.793 | 2.25±1.63 | 2.45±2.08 | 2.21±1.86 | 0.113 |
| TC (mmol/L) | 5.29±1.36 | 5.36±1.21 | 4.87±0.92 | 0.509 | 5.19±1.21 | 5.31±1.31 | 5.41±1.12 | 0.320 |
| HDL-C (mmol/L) | 1.43±0.47 | 1.45±0.42 | 1.29±0.21 | 0.543 [c] | 1.39±0.49 | 1.42±0.47 | 1.39±0.49 | 0.527 |
| LDL-C (mmol/L) | 3.26±1.19 | 3.25±0.91 | 3.21±1.02 | 0.989 | 3.11±0.82 | 3.08±1.01 | 3.69±0.93 | 0.030[c*] |

BMI = body mass index; WHR = waist to hip ratio; $HbA_{1c}$ = hemoglobin $A_{1c}$; 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-B = homeostasis model assessment for beta cell function; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol.

Data are given as mean ± SD. *P* values represent statistical difference among three different genotypes assessed by the one-way ANOVA. [b]*P* values are determined by the Pearson chi-square test. [c]*P* values are determined by the Kruskal-Wallis test. *$P<0.05$.

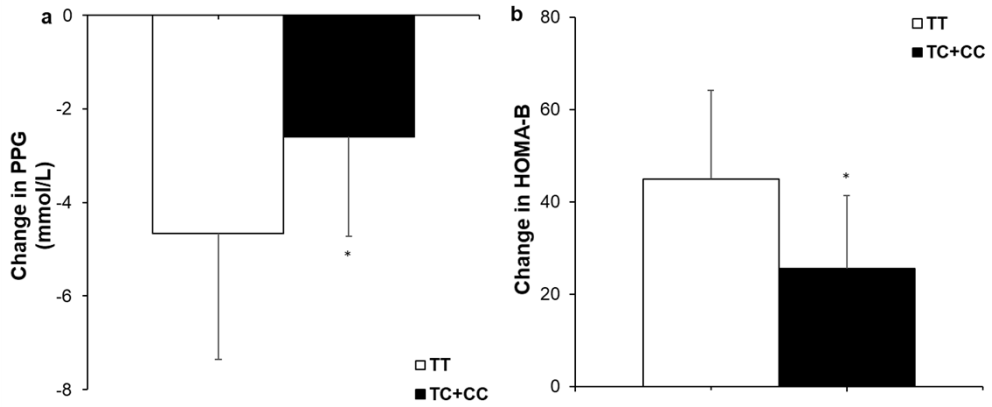


**Fig 1** Comparisons of DV(postadministration minus preadministration) of PPG(a) and HOMA-B (b) between the different *PPARD* rs2016520 genotypes in T2DM patients after treatment of nateglinide. Data are expressed with mean ± standard deviation. $^{*}P<0.05$ compared with TT genotype group.

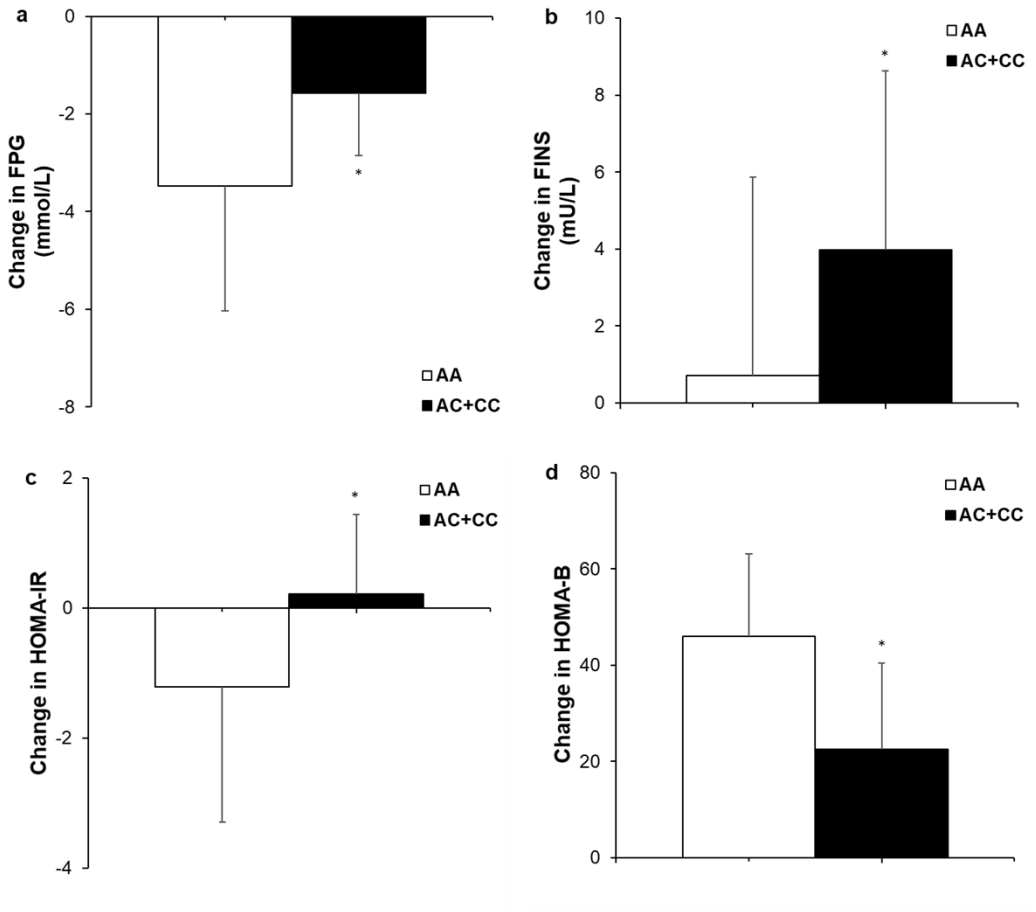


**Fig 2** Comparisons of DV(postadministration minus preadministration) of FPG (a), FINS (b), HOMA-IR (c) and HOMA-B (d) among the different *NOS1AP* rs12742393 genotypes in T2DM patients after treatment of nateglinide. Data are expressed with mean ± standard deviation. $^{*}P<0.05$ compared with AA genotype group.

**Table 3** Genotype and allele distributions between responders and non-responders of *PPARD* rs2016520 and *NOS1AP* rs12742393 variants (n = 60)

| | **Genotype** | | | ***P* value** | **Allele frequency** | | ***P* value** |
|---|---|---|---|---|---|---|---|
| *PPARD* rs2016520 | TT | TC | CC | | T | C | |
| Responder (%) | 19(54.29%) | 11(47.83%) | 1(50.00%) | | 49(52.69%) | 13(48.15%) | |
| Non-responder (%) | 16(45.71%) | 12(52.17%) | 1(50.00%) | 0.964 | 44(47.31%) | 14(51.85%) | 0.678 |
| *NOS1AP* rs12742393 | AA | AC | CC | | A | C | |
| Responder (%) | 17(70.83%) | 12(44.44%) | 2(22.22%) | | 46(61.33%) | 16(35.56%) | |
| Non-responder (%) | 7(29.17%) | 15(55.56%) | 7(77.78%) | 0.027 | 29(38.67%) | 29(64.44%) | 0.006 |

**Table S1** Clinical characteristics of T2DM patients before and after nateglinide treatment

| Parameters | Before treatment | After treatment | *P* values |
|---|---|---|---|
| FPG (mmol/L) | 8.70±2.23 | 6.67±1.17 | 0.000 |
| PPG (mmol/L) | 14.26±2.94 | 10.46±1.59 | 0.000 |
| FINS (mU/L) | 9.26±5.87 | 14.09±13.47 | 0.007 |
| PINS (mU/L) | 39.33±33.11 | 70.21±52.89 | 0.000 |
| HOMA-IR | 3.61±2.42 | 4.27±4.28 | 0.246 |
| HOMA-B | 28.26±16.01 | 60.31±35.32 | 0.000 |
| HbA1c (%) | 8.35±1.74 | 6.71±1.00 | 0.000 |
| TG (mmol/L) | 2.15±1.34 | 1.85±1.10 | 0.002 |
| TC (mmol/L) | 4.98±1.31 | 4.58±1.08 | 0.001 |
| HDL-C (mmol/L) | 1.30±0.42 | 1.49±0.68 | 0.026 |
| LDL-C (mmol/L) | 2.82±0.78 | 2.68±0.74 | 0.066 |

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-B = homeostasis model assessment for beta cell function; $HbA_{1c}$ = hemoglobin $A_{1c}$; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol.

Data are expressed as mean ± SD. *P* values are determined by the Student's *t* test. $^{**}P<0.01$.

**Table S2-1** Effects of different *PPARD* rs2016520 genotypes in T2DM patients on clinical characteristics determined before and after naglinide treatment

| Parameters | | *PPARD* rs2016520 | | *P* values |
|---|---|---|---|---|
| | | TT | TC+CC | |
| N(male/femal) | | 35(24/11) | 25(15/10) | 0.493[b] |
| FPG (mmol/L) | Before | 9.07±2.63 | 8.19±1.42 | 0.134 |
| | After | 6.74±1.36 | 6.57±0.87222 | 0.579 |
| | DV | -2.32±1.72 | -1.61±1.44 | 0.099 |
| PPG (mmol/L) | Before | 15.23±2.78 | 12.90±2.66 | 0.002 |
| | After | 10.57±1.71 | 10.31±1.42 | 0.538 |
| | DV | -4.66±2.70 | -2.59±2.13 | 0.002 |
| FINS (mU/L) | Before | 9.36±5.93 | 9.13±5.91 | 0.883 |
| | After | 16.06±15.72 | 11.32±9.05 | 0.182 |
| | DV | 6.70±15.03 | 2.19±10.33 | 0.201 |
| PINS (mU/L) | Before | 41.55±30.72 | 36.23±36.61 | 0.544 |
| | After | 75.14±54.71 | 63.29±50.51 | 0.397 |
| | DV | 33.59±43.71 | 27.07±29.25 | 0.519 |
| HOMA-IR | Before | 3.81±2.65 | 2.72±1.47 | 0.443 |
| | After | 4.94±4.94 | 3.34±2.97 | 0.157 |
| | DV | 1.12±4.85 | -0.01±3.53 | 0.338 |
| HOMA-B | Before | 28.25±16.60 | 25.22±14.80 | 0.469 |
| | After | 73.20±42.81 | 50.74±26.43 | 0.015 |
| | DV | 44.95±19.23 | 25.48±15.93 | 0.000 |
| HbA1c (%) | Before | 8.58±1.81 | 7.02±1.60 | 0.221 |
| | After | 6.58±1.12 | 6.42±0.80 | 0.543 |

| | | | | |
|---|---|---|---|---|
| | DV | -1.87±1.55 | -1.60±1.44 | 0.714 |
| TG (mmol/L) | Before | 2.19±1.58 | 2.09±0.92 | 0.782 |
| | After | 1.90±1.26 | 1.78±0.83 | 0.690 |
| | DV | -0.29±0.78 | -0.31±0.64 | 0.926 |
| TC (mmol/L) | Before | 4.67±1.20 | 4.45±0.88 | 0.906 |
| | After | 4.74±1.19 | 4.52±0.87 | 0.439 |
| | DV | -0.29±0.75 | -0.55±1.12 | 0.283 |
| HDL-C (mmol/L) | Before | 1.29±0.45 | 1.31±0.38 | 0.788 |
| | After | 1.38±0.41 | 1.64±0.92 | 0.158 |
| | DV | 0.09±0.38 | 0.32±0.87 | 0.183 |
| LDL-C (mmol/L) | Before | 2.79±0.80 | 2.87±0.78 | 0.720 |
| | After | 2.75±0.79 | 2.58±0.67 | 0.364 |
| | DV | -0.04±0.61 | 0.29±0.57 | 0.110 |

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-B = homeostasis model assessment for beta cell function; $HbA_{1c}$ = hemoglobin $A_{1c}$; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol.

Data are given as mean ± SD. *P* values represent statistical difference among the three different genotypes assessed by the one-way ANOVA. [b]*P* values are determined by the Pearson chi-square test. [c]*P* values are determined by the Kruskal-Wallis test. [**]$P<0.01$.

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

**Table S2-2** Comparisons of clinical characteristics in T2DM patients with different *NOS1AP* rs12742393 genotypes before and after nateglinide treatment.

| Parameters | | AA | AC+CC | *P* value |
|---|---|---|---|---|
| N(male/female) | | 24(11/13) | 36(19/17) | 0.598[b] |
| FPG (mmol/L) | Before | 9.97±2.54 | 10.32±2.02 | 0.580 |
| | After | 6.51±1.34 | 8.75±1.44 | 0.000 |
| | DV | -3.48±2.55 | -1.57±1.28 | 0.000[c] |
| PPG (mmol/L) | Before | 17.25±4.31 | 16.73±4.50 | 0.651 |
| | After | 10.47±3.45 | 12.95±3.68 | 0.016 |
| | DV | -6.78±4.41 | -4.81±3.57 | 0.000[c] |
| FINS (mU/L) | Before | 9.33±6.49 | 8.92±5.96 | 0.802 |
| | After | 10.04±6.26 | 12.87±6.81 | 0.110 |
| | DV | 0.72±5.15 | 3.98±4.65 | 0.014 |
| PINS (mU/L) | Before | 31.63±22.32 | 31.90±21.62 | 0.963 |
| | After | 46.91±26.82 | 47.82±26.90 | 0.028 |
| | DV | 14.31±14.23 | 17.36±15.33 | 0.441 |
| HOMA-IR | Before | 4.04±2.96 | 4.02±2.58 | 0.978 |
| | After | 2.81±1.66 | 4.32±2.21 | 0.006 |
| | DV | -1.22±2.07 | 0.21±1.23 | 0.001 |
| HOMA-B | Before | 25.45±17.21 | 27.01±16.92 | 0.730 |
| | After | 75.20±43.81 | 47.7±39.31 | 0.014 |
| | DV | 45.95±37.23 | 22.48±21.93 | 0.003 |
| HbA1c (%) | Before | 9.81±1.89 | 9.68±1.96 | 0.809 |
| | After | 7.02±0.78 | 7.01±1.74 | 0.979 |
| | DV | -2.79±1.58 | -2.71±1.28 | 0.830 |
| TG (mmol/L) | Before | 2.21±1.53 | 2.51±2.26 | 0.572 |
| | After | 1.84±1.04 | 2.06±2.02 | 0.625 |
| | DV | -0.36±1.13 | -0.37±2.04 | 0.983 |
| TC (mmol/L) | Before | 5.10±1.01 | 5.32±1.78 | 0.585 |
| | After | 5.04±0.91 | 4.74±1.23 | 0.311 |
| | DV | -0.06±0.91 | -0.54±1.47 | 0.806 |
| HDL-C (mmol/L) | Before | 1.41±0.42 | 1.39±0.49 | 0.871 |
| | After | 1.37±0.39 | 1.30±0.43 | 0.524 |
| | DV | -0.06±0.45 | -0.11±0.66 | 0.747 |
| LDL-C (mmol/L) | Before | 3.10±0.82 | 3.19±1.16 | 0.744 |
| | After | 3.31±0.91 | 3.02±1.12 | 0.295 |
| | DV | 0.21±0.92 | -0.03±1.37 | 0.455 |

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-B = homeostasis model assessment for beta cell function; $HbA_{1c}$ = hemoglobin

$A_{1c}$; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein-cholesterol; LDL-C = low-density lipoprotein-cholesterol.

Data are given as mean ± standard deviation. *P* values represent statistical difference among the three different genotypes assessed by one-way ANOVA. [b]*P* values are determined by Pearson chi-square test. [c]*P* values are determined by Kruskal-Wallis test.

DV, differential values (postadministration minus preadministration).

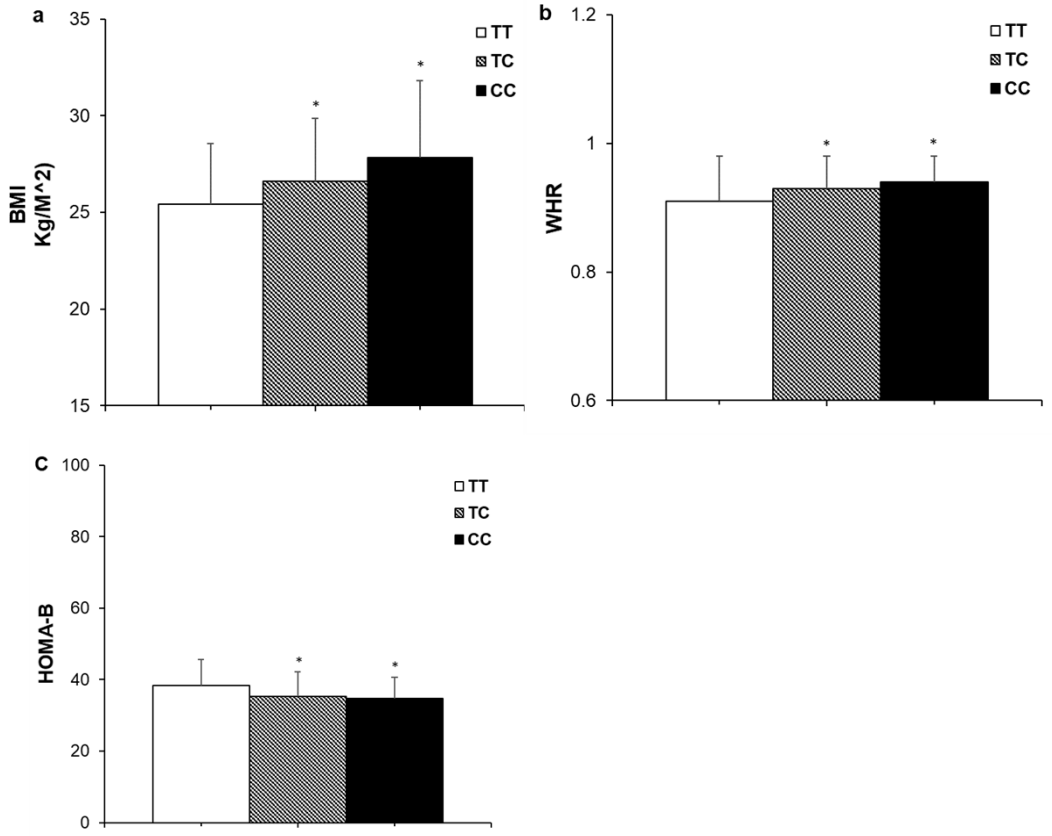


**Fig. S1** Baseline levels of BMI, WHR, and HOMA-B in T2DM patients with different *PPARD* rs2016520 genotypes. Data are expressed with mean ± standard deviation. *P<0.05 compared with TT genotype group.

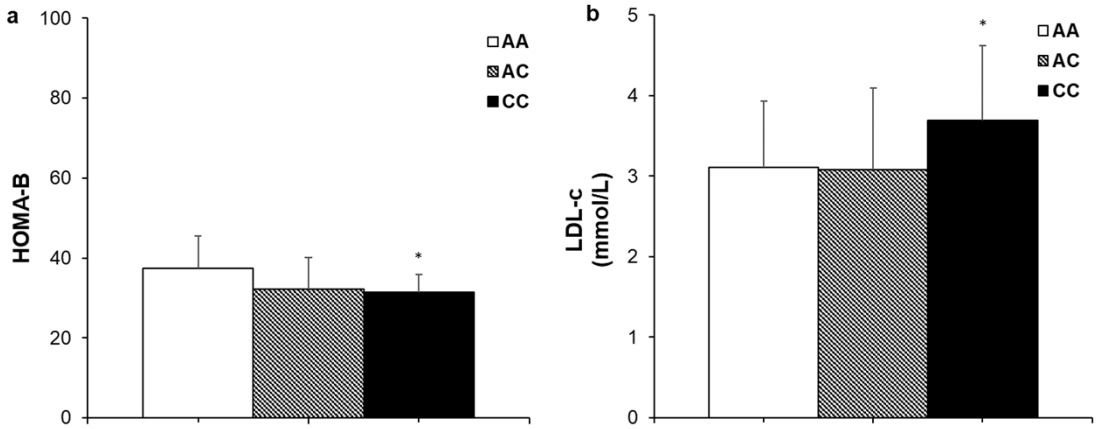


**Fig S2** Baseline levels of HOMA-B(a) and LDL-C(b) in T2DM patients with different *NOS1AP* rs12742393 genotypes. Data are expressed with mean ±standard deviation. $^{*}P<0.05$ compared with AC and AA genotype groups respectively.