The Effect of PTPRδ rs17584499 C/T Polymorphism on Therapeutic Efficacy of Metformin in Chinese Patients with Type 2 diabetes

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Abstract

Aim: To investigate whether the protein tyrosine phosphates receptor type delta gene (PTPRδ) rs17584499 C/T genetic polymorphism is associated with development of type 2 diabetes mellitus (T2DM) and metformin therapeutic efficacy in Chinese T2DM patients.

Methods: A case-control study of 402 T2DM patients and 171 healthy controls was conducted to identify the genotypes for PTPRδ rs17584499 polymorphism using the ABI 3700 automatic sequence assay. 44 first-onset T2DM patients were selected to orally 500 mg metformin daily for 12 consecutive weeks as monotherapy. Serum fasting plasma glucose (FPG), Postprandial Plasma Glucose (PPG), Glycated Hemoglobin (HbA1c), Fasting Serum Insulin (FINS), Postprandial Serum Insulin (PINS), Triglycerol (TG), Cholesterol (CHO), Low-Density Lipoprotein (LDL-c), High-Density Lipoprotein Cholesterol (HDL-c), and Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), Body Mass Index (BMI) were determined before and after metformin treatment.

Results: There were no significant differences in the allelic frequencies of the PTPRδ rs17584499 C/T polymorphism between T2DM patients and healthy controls. After metformin treatment, the values of BMI, FPG, PPG, PINS, HbA1c, CHO, and TG in T2DM patients significantly decreased (P <0.01, respectively), while markedly increased FINS (P <0.001). Metformin significantly decreased the levels of PPG (P <0.05) and CHO (P <0.05) of patients with PTPRδ rs17584499 CT+TT genotypes compared of individuals with CC genotype.

Conclusion: PTPRδ rs17584499 C/T polymorphism may not be associated with the risk of T2DM. But it may affect the PPG, HbA1c, and CHO in Chinese T2DM patients with metformin monotherapy.

Keywords: Genetic polymorphism; Metformin; Protein tyrosine phosphates receptor type delta gene; Type 2 diabetes mellitus; Efficacy

Abbreviations: GWAs: Genome-Wide Association studies; SNPs: Single Nucleotide Polymorphisms; PCR: Polymerase Chain Reaction; T2DM: Type 2 Diabetes Mellitus; PTPRδ: Protein Tyrosine Phosphates Receptor Type Delta; OCTs: Organic Cation Transporters; MATEs: Multidrug and Toxin Extrusions Transporters; PTPs: Protein Tyrosine Phosphates; LAR-PTPs: Leukocyte Common Antigen-related Subfamily of PTPs; BMI: Body Mass Index; WHR: Waist to Hip Ratio; FPG: Fasting Plasma Glucose; PPG: Postprandial Plasma Glucose; HbA1c: Glycated Hemoglobin; FINS: Fasting Serum Insulin; PINS: Postprandial Serum Insulin; HOMA-IR: Homeostasis Model Assessment for Insulin Resistance; TC: Total Cholesterol; LDL-c: Low-density Lipoprotein-Cholesterol; HDL-c: High-density Lipoprotein-Cholesterol; DV: Differential Quotient (post-administration minus pre-administration); Pre:- Pre-administration; Post:- Post-administration

Introduction

Type 2 diabetes mellitus (T2DM) is a progressive and complex disorder that is difficult to treat effectively in the long term. The majorities of patients are overweight or obese, and will be unable to maintain long-term glycogenic control without oral antidiabetic agents [1]. Much effort has been devoted to exploring the pathogenesis of T2DM and finding the most effective drugs for T2DM, yet they were still remaining unknown. The disease is considered to be a polygenic disorder in which genetic variants confers a partial and additive effect. Genetic discoveries have provided the new targets for prevention, diagnosis and treatment of T2DM [2,3]. Until now, genome-wide association studies (GWAs) have identified about 40 susceptibility genes associated with T2DM in different population, these discoveries may give some new clues to explore the pathogenesis and the new targets for treatment T2DM [4-7]. Tsai et al. [8] recent reported that protein tyrosine phosphates receptor type delta gene (PTPRδ) as a novel susceptibility gene are significantly associated with the development of T2DM in Taiwan population by GWAs analysis. PTPRδ rs17584499 located at 9p24 [8]. Protein tyrosine phosphates (PTPs) are key regulators of the insulin receptor signal transduction pathway, expressed in the major human insulin target tissues or cells, such as liver, adipose tissue, skeletal muscle, and endothelial cells [9,10]. Human PTPRδ belongs to leukocyte common antigen-related subfamily of PTPs (LAR-PTPs). LAR-PTPs were a major subfamily of PTPs and suggested to be a negative regulator of the insulin receptor tyrosine kinase [11]. PTPRδ gene may play an important role in glucose homeostasis and insulin action.

Metformin is regarded as insulin sensitizing drug for T2DM patients with overweight or obesity [12]. Metformin reduced gluconeogenesis by increasing hepatic sensitivity to insulin and decreased the hepatic extraction of certain gluconeogenesis substrates.

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Received February 25, 2012; Accepted April 18, 2012; Published April 22, 2012


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(e.g. lactate) [13,14]. Metformin is negligibly bound to plasma proteins not metabolized in body. It is eliminated in urine via active renal tubular secretion [14]. Metformin is the first-line agent for T2DM patients. However, considerable individual differences in metformin efficacy are reported [15]. Numerous studies showed that Organic cation transporters (OCTs) and Multidrug and toxi extrusions transporters (MATEs) were major causes of the individual difference of metformin response rather than drug metabolizing enzymes and drug receptors. OCT1 (rs17584499) and rs1022 (C/T) polymorphisms could significantly influence pharmacokinetics of metformin in T2DM patients in different population [15-18]. Our previous studies showed distributive frequency of OCT1 859C/G polymorphism is very low in Chinese population (unpublished data). Toyama K et al. [17] reported that MATE1 variants could not influence the disposition of metformin in vivo in Asian. Thus, genetic polymorphisms of OCTs may be the crucial factors for the individual difference of metformin response in Chinese T2DM patients. Metformin efficacy may be affected by gene polymorphisms.

Up to now, there are no reports about the impacts of PTMRδ rs17584499 polymorphism on metformin therapeutic efficacy. PTMRδ participates in glucose homeostasis and insulin action. Metformin also reduces glucose levels by increasing insulin sensitivity. In this study we explore the association of PTMRδ genetic polymorphism with the development of T2DM and assess the effects of PTMRδ rs17584499 polymorphism on metformin efficacy in Chinese patients with T2DM.

Materials and Methods

Subjects

A total of 402 unrelated T2DM patients aged ranging from 24 to70 years (mean: 50.65 ± 10.03, 204 female and 198 male) and 171 healthy controls aged ranging from 23 to 72 years (mean: 46.14 ± 10.36, 67 female and 104 male) were enrolled from Xiangua hospital of Center South University, Changsha, Hunan Province, China and Liuyang Center hospital, Liuyang City, Hunan Province, China during January 2008 to August 2010. All of the T2DM patients were diagnosed according to the diagnosis criteria of the World Health Organization made in 1997 by fasting plasma glucose (FPG≥7.0 mmol/l) and/or postprandial plasma glucose (PPG≥7.0 mmol/l) and/or basal plasma glucose (PPG≥11.1 mmol/l). The criteria for controls were (1) no past diagnostic history of T2DM; (2) HbA1c was ranging from 3.4 to 6; (3) BMI ranging from 18.5-30 kg/m². Subjects with type 1 diabetes, gestational diabetes, and maturity-onset diabetes of the young (MODY), history of lactic acidosis, pregnant and lactating women or those with acute myocardial infarction, trauma, kidney and liver disease were excluded from this study. All of T2DM patients and healthy controls in this study were of Han Chinese population; these subjects were local residents in Hunan Province, China. Written informed consents were obtained from all participants before the start of this study. The study protocol was in accordance with the Helsinki Declaration II and was approved by the Ethics Committee of Xiangya School of Medicine, Central South University. A clinical study admission (the registration number: ChiCTR-CCC00000406) was approved by Chinese Clinical Trial Register. In present study, we selected 44 first-onset T2DM patients (33 male, 11 female) with the same OCT1 1022 CC, 848 CC, and OCT2 808 GG homozygous and different genotypes of PTMRδ rs17584499 C/T orally 500 mg metformin (ShuangHe, Beijing, China) daily for 12 consecutive weeks and had not administered any anti-diabetes drugs in last three month.

Clinical laboratory tests

After an overnight fast, blood samples for the determination of plasma glucose and insulin level were collected in the fasting state and at 2 h after breakfast; these parameters were measured at the end of weeks 0 and 12 after administration of metformin. Plasma concentrations of FPG, PPG, CHO, TG, and HDL-c were determined by use of an enzymatic colorimetric assay and lipoprotein electrophoresis, respectively. LDL-c concentration was calculated according to Friedewald formula [19]. Plasma insulin and HbA1c levels were measured by use of a radioimmunoassay kit (BNIBT, Beijing, China) and by high-performance liquid chromatography assay (HPLC), respectively. BMI was calculated as weight/(kg/height^2). The homeostasis model assessment for insulin resistance (HOMA-IR) value was calculated according to the following formula to estimate the insulin sensitivity. HOMA-IR = fasting serum insulin (mU/l) x fasting blood glucose (mmol/l)/22.5 [20].

Genotyping analysis

The Genomic DNA was extracted from the peripheral blood leukocytes using the Promega DNA purification kit (Promega, USA) according to the manufacturer protocol and then stored at -80°C until use. The primer pairs used in the amplification of PTMRδ rs17584499 C/T were forward primer: 5'-TGAGTCCTGACCTGACCCAAAG-3', reverse primer: 5'- CCAGGAATACGGAACATGAATAATAGC-3'. The primer pairs used for OCT1 1022C/T, 848C/T, and OCT2 808G/T polymorphisms were forward primer: 5'- TAGATGCTTCCCTGCG-3', reverse primer: 5'- TCACTTCCGTGAAACACTG-3'; forward primer: 5'- CTGCACTGACGACACACTG-3', reverse primer: 5'- GCAGGGCAACTCTCCATTG-3'; forward primer: 5'- AGGTGGATGATATGGACTG-3', reverse primer: 5'- GAGATTGGGCTTGTGTGAAA-3'. The polymerase chain reaction (PCR) amplification were carried out in a total volume of 25 µl containing 2.5 µl 10× PCR buffer, 2 µl dNTP, 1 µl of each primer, approximately 2 µl genomic DNA as a template and 0.2 µl Taq polymerase (above all reagents from Takara, Dalian, China). The PCR conditions for PTMRδ rs17584499 polymorphism were as follows: initial denaturizing at 95°C for 5 min, 35 cycles of denaturizing at 95°C for 30 s, annealing at 55.8°C for 30 s, and extension at 72°C for 30 s with a final extension of 5 min at 72°C. The PCR conditions of OCT1 1022C/T and 848C/T were the same as follows: first pre denaturating for 1 min at 94°C, then followed by denaturizing for 30 s at 94°C, 30 cycles of annealing for 1 min at 62°C and extending for 1 min at 72°C, at last extending for 7 min at 72°C. The PCR conditions of OCT2 808G/T were: first pre denaturating for 1 min at 94°C, then followed by denaturizing for 30 s at 94°C, 30 cycles of annealing for 1 min at 56.9°C and extending for 1 min at 72°C, at last extending for 7 min at 72°C. The Data fragments of the five SNPs were amplified on Eppendorf thermal cycler (Eppendorf AG, Germany). The fragments of PCR products were sequenced using the ABI 3700 automatic sequencer (ABI 3700, USA) according to the manufacturer’s protocols. The sequence data were analyzed by Chromos software (Version 1.62, Australia) to identify the genotypes.

Statistical analysis

All statistical analysis was carried out by SPSS software (version 13.0 for windows, Chicago, USA). Hardy-Weinberg equilibrium and Allelic frequencies in different groups were compared using Pearson-x² test. Baseline characteristics in T2DM patients and health controls were compared with two-sample t-test (normally distribution data analyzed) and Mann-Whitney U test (non normally distribution data analyzed). Baseline characteristics among genotypes were compared.
with One-way ANOVA. To estimate the effects of metformin on the metabolic parameters among the genotypes, the Paired-Samples T test were used. All data were presented as mean ± standard deviation (SD) and P<0.05 was considered statistically significant.

Results

Basic clinical characteristics of subjects

The basic clinical characteristics of 402 T2DM patients and 171 health controls were summarized in Table 1. There were significant differences in gender distribution (P=0.011), age (50.65 ± 10.03 vs 46.14 ± 10.36, years, P<0.001), BMI (24.84 ± 3.09 vs 22.99 ± 2.77, kg/m², P<0.001), waist measurement (86.85 ± 8.35 vs 83.78 ± 8.10, cm, P<0.001), hip measurement (95.44 ± 6.89 vs 92.13 ± 7.18, cm, P<0.001), FPG (9.33 ± 3.63 vs 5.07 ± 0.51, mmol/l, P<0.001), TG (3.06 ± 3.06 vs 1.53 ± 1.00, mmol/l, P<0.001), and LDL-C (3.11 ± 1.19 vs 2.51 ± 0.70, mmol/l, P<0.001) between T2DM patients and health controls. However, no significant differences in the levels of waist-to-hip ratio and HDL-c were observed in this study.

Genotyping and allelic frequencies

The genotypes of PTPRD rs17584499 C/T polymorphism were determined in 402 T2DM patients and 171 health controls. The allelic frequencies of PTPRD rs17584499 C/T in T2DM patients and health controls showed in Table 2. The T allele frequencies of PTPRD rs17584499 C/T polymorphism were 12.9% in T2DM patients and 9.4% in health controls. The C allele frequencies of PTPRD rs17584499 C/T were 87.1% in T2DM patients and 91.6% in health controls. No significant differences in allelic frequencies of PTPRD rs17584499 C/T between T2DM patients and healthy control. Genotyped frequencies of PTPRD rs17584499 polymorphism were in Hardy-Weinberg equilibrium (P>0.05).

Impacts of PTPRD rs17584499 polymorphism on therapeutic efficacy of metformin in patients with T2DM

To exclude the impacts of OCTs polymorphisms on metformin disposition in vivo, we selected 44 first-onset T2DM patients with the same OCT1 1022 CC, 848 CC, and OCT2 808 GG homozygous and different PTPRD rs17584499 genotypes undergo 12 weeks metformin monotherapy. After 12 weeks treatment, metformin significantly decreased the levels of BMI (24.27 ± 2.77 vs 25.54 ± 3.06, kg/m², P<0.001), FPG (6.29 ± 0.90 vs 9.13 ± 3.50, mmol/l, P<0.001), PPG (8.80 ± 1.90 vs 18.60 ± 6.26, mmol/l, P<0.001), PINS (34.76 ± 12.35 vs 49.61 ± 24.73, mU/L, P<0.001), HbA1c (6.74 ± 0.99 vs 8.66 ± 2.55, P<0.001), CHO (4.60 ± 0.86 vs 5.19 ± 1.50, mmol/l, P=0.01), and TG (1.65 ± 1.11 vs 2.98 ± 3.15, mmol/l, P<0.001), which markedly increased FINS (12.98 ± 4.80 vs 7.67 ± 6.27, mU/L, P<0.001) in patients (n=44) (Table 3). Patients with CT+TT genotype (n = 21) of PTPRD rs17584499 polymorphism significantly decreased the levels of PPG (-8.78 ± 5.72 vs -5.68 ± 4.38, mmol/l, P=0.049), HbA1c (-2.86 ± 2.68 vs -1.08 ± 2.02, P = 0.015), and CHO (-0.93 ± 1.14 vs -0.27 ± 1.63, mmol/l, P = 0.023) compared for patients with the CC genotype (n = 23) (Table 4, Figure 1).

Discussion

Our study is the preliminary work to investigate the influence of PTPRD rs17584499 C/T polymorphism on metformin therapeutic efficacy. In present study, we found that PTPRD rs17584499 C/T genetic polymorphism could influence the therapeutic efficacy of metformin in Chinese T2DM patients, patients with at least one T allele of PTPRD rs17584499 had better therapeutically efficacy of metformin.

PTPRδ gene is relatively high expressed in insulin target tissues and it could attenuate insulin action and acted as key regulators of the insulin receptor signaling pathway [9,10]. PTPRD gene belongs to LAR-PTPs family. LAR-PTPs (mainly containing LAR, PTPRD and PTPA) are structurally very similar and play the similar roles in the regulation of cell growth, proliferation, differentiation and metabolism [11]. Numerous studies reported that LAR-PTPs confirmed as the new targets of T2DM treatment elevated insulin receptor dephosphorylating activity in adipose tissue from obese subjects [21-23]. Metformin is the first agent for T2DM patients with obese or overweight. Metformin may suppress oxidation of fatty acids and reduce triglyceride levels in T2DM patients by insulin independent manner, the levels of BMI, CHO and LDL-c decreased after metformin treatment [24]. In our study, data showed that metformin could significantly decrease the levels of BMI, FPG, PPG, PINS, HbA1c, TG and CHO in T2DM patients after metformin treatment (n=44).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T2DM patients</th>
<th>Health control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex male</td>
<td>204 (50.7%)</td>
<td>67 (49.3%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>198 (39.2%)</td>
<td>104 (60.8%)</td>
<td>0.011*</td>
</tr>
<tr>
<td>Age</td>
<td>50.65 ± 10.03</td>
<td>46.14 ± 10.36</td>
<td>0.000***</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.84 ± 3.09</td>
<td>22.99 ± 2.77</td>
<td>0.000***</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>86.85 ± 8.35</td>
<td>83.78 ± 8.10</td>
<td>0.000***</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>95.44 ± 6.89</td>
<td>92.13 ± 7.18</td>
<td>0.000***</td>
</tr>
<tr>
<td>WHR</td>
<td>0.91 ± 0.06</td>
<td>0.91 ± 0.04</td>
<td>0.779</td>
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</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T2DM patients</th>
<th>Health controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>305 (75.9%)</td>
<td>139 (81.3%)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>90 (22.4%)</td>
<td>32 (18.7%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>7 (1.7%)</td>
<td>0 (0.0%)</td>
<td>0.123</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>T2DM patients</th>
<th>Health controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>700 (87.1%)</td>
<td>310 (90.6%)</td>
<td>0.087</td>
</tr>
<tr>
<td>T</td>
<td>104 (12.9%)</td>
<td>32 (9.4%)</td>
<td></td>
</tr>
</tbody>
</table>

P values are determined by Pearson χ² test

Table 2: Comparison of allelic frequencies of PTPRD rs17584499 C/T polymorphism between T2DM patients and healthy controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before</th>
<th>After</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.54 ± 3.06</td>
<td>24.27 ± 2.77</td>
<td>0.000***</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>9.13 ± 3.50</td>
<td>6.29 ± 0.90</td>
<td>0.000***</td>
</tr>
<tr>
<td>PPG (mmol/L)</td>
<td>18.60 ± 6.26</td>
<td>8.80 ± 1.90</td>
<td>0.000***</td>
</tr>
<tr>
<td>FINS (mU/L)</td>
<td>7.67 ± 6.27</td>
<td>12.98 ± 4.80</td>
<td>0.000***</td>
</tr>
<tr>
<td>PINS (mU/L)</td>
<td>49.61 ± 24.73</td>
<td>34.76 ± 12.35</td>
<td>0.000***</td>
</tr>
<tr>
<td>HOME-IR</td>
<td>3.48 ± 4.01</td>
<td>3.65 ± 1.42</td>
<td>0.777</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.66 ± 0.55</td>
<td>6.74 ± 0.99</td>
<td>0.000***</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.98 ± 3.15</td>
<td>1.65 ± 1.11</td>
<td>0.004**</td>
</tr>
<tr>
<td>CHO (mmol/L)</td>
<td>5.19 ± 1.50</td>
<td>4.60 ± 0.86</td>
<td>0.010**</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.98 ± 1.11</td>
<td>2.91 ± 0.68</td>
<td>0.556</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.21 ± 0.32</td>
<td>1.17 ± 0.25</td>
<td>0.248</td>
</tr>
</tbody>
</table>

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

Table 3: The basic clinical characteristics of all T2DM patients before and after metformin treatment (n=44).
The comparison of differential values (post-administration minus pre-administration) in T2DM patients with different PTPRδ rs17584499 C/T polymorphism before and after metformin treatment (n = 44).

Table 4: The comparisons of different values (postadministration minus preadministration) in T2DM patients with different PTPRδ rs17584499 C/T polymorphism after 12 weeks metformin treatment. *P < 0.05 for comparison of CC genotypes with CT+TT genotypes of PTPRδ rs17584499 polymorphism

A: dominant model *P < 0.05 for comparison of CC genotypes with CT+TT genotypes of PTPRδ rs17584499 polymorphism

PTPRδ rs17584499 polymorphism may be beneficial for T2DM patients who need treatment with metformin. The T allele of PTPRδ rs17584499 may also be beneficial for T2DM patients who need treatment with metformin. Further data analysis is necessary to be replicated in larger samples.

In summary, the genetic polymorphism of PTPRδ rs17584499 C/T was not associated with the susceptibility of T2DM. PTPRδ rs17584499 polymorphism could influence the therapeutically efficacy of metformin. Moreover, patients with at least one T allele of PTPRδ rs17584499 seem to be more sensitive to metformin treatment compared for individuals with the CC genotype. Therefore, we think that prior genotyping analysis for PTPRδ rs17584499 polymorphism may be beneficial for T2DM patients who need treatment with metformin. These findings need to be replicated in larger populations with a longer follow up visit. Our study also suggested that susceptibility genes of T2DM development need be detected in different populations may confer different risks and different drug response, which lead to a better understanding of the molecular pathogenesis of T2DM and provide the new targets for prevention, diagnosis and treatment in T2DM.

Acknowledgement
We thank the study participants. This work was supported by the National High-tech R&D Program of China (863 Program) (2009AA022703, 2009AA022704), National Natural Science Foundation of China (30673089, 30873154), Program for Changjiang Scholars and Innovative Research Team in University (IRT0946), Open Foundation of Innovative Platform in University of Hunan Province of China (10K078) and The Science and Technology Plan Key Grant of Hunan Province of China (2009TP0069-2). The Fundamental Research Funds for the Central Universities (201023100001).

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