Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

# Association between DPP4 gene polymorphism and serum lipid levels in Chinese type 2 diabetes individuals

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ARTICLE INFO

*Article history:*
Received 28 October 2015
Received in revised form 8 June 2016
Accepted 14 August 2016
Available online xxxx

*Keywords:*
DPP4
Polymorphism
Serum lipid levels
Type 2 diabetes

ABSTRACT

*Objective:* The goal of the genetic investigation was to identify the associations of serum lipid levels and DPP-4 variants in Chinese type 2 diabetes patients.
*Methods:* We detected four variants of the DPP4 gene in 190 Chinese individuals with type 2 diabetes and tested for an association with dyslipidemia in 82 selected samples. Data including basic information, HbA1c, FPG, serum lipid parameters were collected. Statistical analysis was performed by SPSS 13.0 through ANOVA and $\chi^2$ test.
*Results:* The genetic polymorphism of rs4664443, rs3788979, rs7608798 and rs1558957 in Chinese type 2 diabetes were consistent with Hardy-Weinberg equilibrium. The CT genotype of rs4664443 suffered from higher serum TG ($P = 0.013$), LDL ($P = 0.044$) and ApoB ($P = 0.006$) levels, whereas the TT genotype of rs7608798 exhibited a lower serum TG level ($P = 0.037$). For rs3788979, the serum TG level ($P = 0.034$) and BMI ($P = 0.04$) were significantly different among genotypes. Moreover, serum TG and TC levels and BMI showed a positive correlation with the number unfavorable alleles, and individuals with more than two unfavorable alleles had higher TG ($P = 0.004$), TC ($P = 0.011$), and BMI ($P = 0.044$) values.
*Conclusions:* This is the first study to investigate DPP4 allelic distributions and their association with dyslipidemia in Chinese type 2 diabetes patients, which may have clinical significance.


## 1. Introduction

Due to economic development, the aging population and urbanization, diabetes mellitus is one of the fastest growing global disorders (Chen et al., 2012). In 2010, approximately 285 million people worldwide were diagnosed with diabetes (Shaw et al., 2010), with 85–95% of the cases being type 2 diabetes (Khunti and Davies, 2010). Type 2 diabetes is always associated with a diversity of long-term complications that increase morbidity and mortality, such as dyslipidemia and cardiac dysfunction, with huge socioeconomic costs (Fredman et al., 2014; Penno et al., 2013). In 2007, there were 3.8 million people estimated to have died from diabetes-related causes (Mahmud et al., 2009), with approximately 50–70% of individuals with diabetes dying of cardiovascular disease (CVD) worldwide (Laakso, 1999).

Dyslipidemia, a common feature of type 2 diabetes, is and will continue to be the major driver of CVD among individuals with type 2 diabetes (Chehade et al., 2013; Mottillo et al., 2010). Plasma concentrations of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) are among the most important risk factors for CVD and are targets for therapeutic intervention (Fisman and Tenenbaum, 2014; Perez-Mendez et al., 2014). The plasma level of triacylglycerol-rich apolipoprotein B (ApoB), which reflects the sum of potentially atherogenic lipoprotein particles and is correlated with non-HDL levels (Barter et al., 2006), also has been proposed as a therapeutic target for lowering lipid concentrations.

Dipeptidyl peptidase IV (DPP4), a ubiquitous serine peptidase, cleaves N-terminal dipeptides from various substrates, such as GLP-1, PYY, and NPY, which are involved in controlling energy homeostasis (Tinoco et al., 2010). DPP4 is a novel adipokine that may impair insulin sensitivity in an autocrine and paracrine fashion (Lamers, 2011). Inhibition of DPP4 is not only a new therapy option for type 2 diabetes but also affects the metabolism of other substrates (Stephan et al., 2011), thereby altering lipid homeostasis. Genome-wide association studies (GWAS) have identified some important loci in DPP4 that are associated with serum lipid levels. Previous works have suggested that

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http://dx.doi.org/10.1016/j.npep.2016.08.005
0143-4179/

heterozygous for rs1558957 are at a higher risk of suffering from high plasma TG, LDL, HDL, and TC levels, whereas rs7608798 homozygotes are at a lower risk of elevated plasma TG levels among obese Caucasians (Bouchard et al., 2009). The rs4664443 locus was found to be tightly associated with ApoB in South Asians and Europeans with a BMI <25 kg/m$^2$ (Bailey et al., 2014).

Most of these findings, however, were with regard to those of Caucasian or European ancestry, and the prior studies merely investigated the association of single mutations and plasma lipid levels (Aghili et al., 2012; Bailey et al., 2014; Bouchard et al., 2009). Indeed, there are no studies on these risk alleles in Chinese ethnicity or type 2 diabetes groups (Rosenberg et al., 2010). Due to this lack of diversity in patient characteristics, a more comprehensive coverage of DPP4 gene polymorphisms is needed (Brugts et al., 2010).

In this study, we detected four important single-nucleotide polymorphisms (SNPs) of the DPP4 gene in Chinese type 2 diabetes individuals. We also integrated more patient-specific characteristics by using a multiple genetic factor analysis at DPP4 gene loci to verify whether DPP4 polymorphisms contribute to the inter-individual variability observed in serum lipid levels.

## 2. Materials and methods

### 2.1. Patient selection

The study protocol was approved by the Medical Ethics Committee of Shandong University School of Medicine. Informed consent was obtained from all participants. A total of 190 patients of Han nationality in Shandong Province, China, who were diagnosed with type 2 diabetes and aged between 18 and 75 were genotyped from July 2012 to June 2014. The definition of 'type 2 diabetes' was in line with the recent recommendations from American Diabetes Association (ADA) (American Diabetes Association, 2010). Approximately 3 ml of venous blood was collected from the patients and stored at −80 °C until the isolation of genomic DNA.

### 2.2. SNP selection and genotyping

Four SNPs of DPP4 were selected, including the reported mutations possibly associated with TG, TC, LDL, ApoB or CVD risks; rs4664443 (C/T), rs7608798 (T/C) and rs1558957 (C/T) and a new mutation rs3788979 (A/G) related to CV risk (Aghili et al., 2012). All four SNPs are located in the intron region.

Genomic DNA was isolated from 400 μl of blood using the Relax gene blood DNA system (Tiangen Biotech, Beijing, China) and stored at −20 °C. PCR reactions were carried out in a total 25 μl consisting of 5× PCR Dye plus Master Mix (GMbiolab, Taichung, Taiwan), 0.1 μM both forward and reverse primers, and 0.1 μg genomic DNA as template. The PCR parameters consisted of a pre-denaturation step for 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C for denaturation, 30 s at an appropriate temperature for annealing and 60 s at 72 °C for extension and ending with a 5 min re-extension at 72 °C. The PCRs were carried out using the Gene Pro PCR system TC-E (Bioer Technology, Hangzhou, China). The primers were designed using Primer 5.0 software. The details of the included primers are listed in Supplementary Table 1.

The PCR products were purified using the QIA quick PCR purification kit (Qiagen, Hilden, Germany). The PCR forward or reverse primer was used to perform sequencing reactions with Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using ABI Prism3730/XL DNA analyzer automated sequencers (Applied Biosystems, Foster City, CA, USA). The success rate for SNP calling was >95%. The sequencing results were then verified against the published sequence of DPP4 (GenBank accession no: NC_000002.12).

### 2.3. Collection of physiological and biochemical information

We reviewed the medical records of all 190 individuals. Physiological and biochemical parameters for each patient, including gender, age, height, weight, body mass index (BMI), mean glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), serum TG, TC, HDL-C, LDL-C and ApoB levels, were collected.

Because plasma lipid levels tend to be affected by diet, drug therapy and morbid state, subjects who met any of the following criteria were excluded: (i) taking lipid-lowering medications or anti-diabetic drugs during the past two months; (ii) consuming a high-fat diet or alcohol three days before the serum lipid test; (iii) having impaired renal or hepatic function or other severe clinical complications; (iv) having no comparable serum lipid profiles available or refusing to provide the missing data. The final analysis consisted of 82 participants.

### 2.4. Statistical analysis

The $\chi^2$ test was used to analyze gene frequency with Hardy-Weinberg equilibrium. Haplotypes were reconstructed using Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). Continuous data are presented as the mean ± standard error. We performed a multivariate linear regression analysis for each SNP adjusted for age and gender. A one-way analysis of variance (ANOVA) was used to analyze the trends in lipid covariates across the genotypes, and the homogeneity of variance was processed by logarithmic transformation. All statistics were performed using the SPSS package (ver.11, SPSS, Chicago, IL, USA); 95% confidence intervals were calculated for all observed allelic frequencies. A two-tailed P value of <0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Demographics

In total, 190 patients were genotyped in the study. The mean age of the patients was 50.7 years, and 62% were male. Their average FPG level was 8.32 mmol/l, and the mean HbA1c was 7.24%.

### 3.2. DPP4 genetic variants and haplotypes

The genotypes and allelic frequencies of the four mutations are shown in detail in Table 1. The genotypes of the four SNPs were consistent with Hardy-Weinberg equilibrium ($\chi^2 = 0.20$, P = 0.65 for rs4664443; $\chi^2 = 0.034$, P = 0.85 for rs1558957; $\chi^2 = 1.37$, P = 0.24 for rs7608798; $\chi^2 = 1.04$, P = 0.31 for rs3788979). There was no significant difference between gender groups according to the frequency of the four mutations (P > 0.05). Direct sequencing of all the samples revealed similarity with the published sequence for DPP4 in NCBI. The examples of the sequence maps showed in the Supplementary Figs. 1, 2, 3 and 4.

**Table 1**
Summary of allelic frequencies and genotypes for DPP-4.

| SNP | Genotypes | N (%) | Allele | Frequency |
|---|---|---|---|---|
| rs1558957 | C/C | 185(97.3) | C | 98.7% |
| | C/T | 5(2.63) | T | 1.31% |
| rs4664443 | C/C | 178(93.7) | C | 96.8% |
| | C/T | 12(6.32) | T | 3.16% |
| rs7608798 | C/C | 14(7.37) | C | 30.3% |
| | C/T | 87(45.8) | T | 69.7% |
| | T/T | 89(46.8) | | |
| rs3788979 | A/A | 54(28.4) | A | 55.0% |
| | A/G | 101(53.2) | G | 45.0% |
| | G/G | 35(18.4) | | |

Please cite this article as: Xing, X., et al., Association between DPP4 gene polymorphism and serum lipid levels in Chinese type 2 diabetes individuals, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2016.08.005