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Research paper

PPARD rs2016520 polymorphism is associated with metabolic traits in a large population of Chinese adults☆

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ABSTRACT

Aims: Polymorphism of rs2016520 in gene *PPARD* has been associated with lipid metabolism, obesity, metabolic syndrome and type 2 diabetes mellitus (T2DM). We aimed to study the association of rs2016520 with common metabolic traits in a large population of Han Chinese adults.
Methods: The polymorphism was genotyped in 1409 subjects using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS); all participants underwent standard clinical examination and a 75 g oral glucose tolerance test (OGTT); associations between the polymorphism and metabolic traits and indices of insulin resistance and insulin sensitivity were analyzed.
Results: There was no significant difference in genotypes between the normal glucose tolerance (NGT) and the prediabetes group ($\chi^2 = 3.17$, $P = 0.2$), except a nominal difference of allele frequency ($\chi^2 = 3.07$, $P = 0.07$). The G carrier presented lower fasting plasma glucose (FPG, $P = 0.03$), lower 2 h plasma glucose ($P_{dom} = 0.04$) and lower fasting insulin ($P = 0.02$), lower systolic blood pressure (SBP, $P = 0.03$), lower HOMA-IR ($P = 0.02$) and higher QUICKI ($P = 0.01$). Moreover, rs2016520 polymorphism was associated with FPG ($\beta = -0.09$, $P = 0.05$), it was also associated with indices of insulin resistance (HOMA-IR, $\beta = -0.06$, $P_{dom} = 0.02$; fasting insulin, $\beta = -0.04$, $P = 0.02$) and indices of insulin sensitivity (QUICKI, $\beta = -0.01$, $P = 0.004$). In addition, we observed that the allele G was also associated with lower SBP ($\beta = -1.29$, $P = 0.04$) and diastolic blood pressure (DBP, $\beta = -0.09$, $P = 0.01$). However, the minor allele G was not associated with risk of metabolic disorders including prediabetes, overweight, hypertension and metabolic syndrome.
Conclusions: Polymorphism of rs2016520 in gene PPARD was associated with benign metabolic traits in a large cohort of Chinese adults. The G allele may confer protection from type 2 diabetes and hypertension in Han Chinese.

1. Introduction

Insulin resistance is a fundamental etiopathogenic factor for type 2 diabetes mellitus (T2DM) and is also linked to a wide array of other pathophysiological derangements including hyperlipidemia, hypertension, metabolic syndrome, atherosclerosis and polycystic ovarian disease (Reaven, 1988). Euglycemic hyperinsulinemic clamp is the gold standard method for assessing insulin resistance and sensitivity (Polonsky et al., 1988; DeFronzo et al., 1979), however, this technique is not applicable impossible for large scale epidemiological studies. Thus relatively simple, non-invasive alternative techniques validated against the euglycemic clamp have been proposed. The homeostatic model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985) and quantitative insulin-sensitivity check index (QUICKI) (Katz et al., 2000) methods are commonly used for insulin resistance and insulin sensitivity, respectively. It has been demonstrated that HOMA-IR and QUICKI correlated well with directly measured insulin resistance and sensitivity.

More recently, genome-wide association studies (GWAS) provided a major increment to our knowledge of the genetics of T2DM. GWAS studies have identified approximately 60 loci associated with T2DM and related traits such as fasting insulin and glucose (Billings and Florez, 2010). Several studies have suggested that putative genetic polymorphisms may be involved in the process of insulin production and/or secretion (Morris et al., 2012). The large international Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) focused on the

Abbreviations: BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; FPG, fasting plasma glucose; HOMA-IR, homeostatic model for assessment of insulin resistance; HOMA-B, homeostatic model for assessment of B cell; Matsuda ISI, Matsuda insulin sensitivity index; QUICKI, quantitative insulin-sensitivity check index.

☆ Novelty Statement: We demonstrated the association between rs2016520 in the gene PPARD with metabolic profiles; the G allele of rs2016520 was associated with lower fasting plasma glucose, lower fasting insulin levels, lower blood pressure levels; the polymorphism was also associated with surrogate of insulin resistance and insulin sensitivity.

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genetic association study with metabolic traits, which directly results the relevance of understanding the determinants of the quantitative measures (Dupuis et al., 2010). Abnormal insulin action and secretion precede the development of T2DM and represent quantitative traits that can help identify the mechanisms conferring increased risk for the disease. Genetic variants have been proposed to influence regulation of glucose level at key cellular sites; thus better understanding of the genetic contribution to variability in glucose tolerance provides the opportunity to uncover genetic variation underlying these traits and to generate novel hypothesis that these loci may also contribute to T2DM susceptibility. Therefore, it is useful to investigate T2DM intermediate phenotypes in order to clarify the physiological mechanisms through which they exert their effects on disease etiology.

Peroxisome proliferator-activated receptors (PPAR) are a subgroup of the nuclear hormone receptor superfamily of ligand-activated transcription factors which play an important role in the pathogenesis of metabolic syndrome and T2DM. All three members of the PPAR nuclear receptor subfamily, PPARα, PPARβ/δ and PPARγ have been identified as the master regulators of glucose, fatty acid and lipoprotein metabolism, energy balance, cell proliferation and differentiation, inflammation, and atherosclerosis (Dong et al., 2015). PPARD also known as PPARβ/δ, is located on chromosome 6p21, a 85 kb region, it is widely expressed in the liver, kidneys, cardiac and skeletal muscle, adipose tissue, brain, colon and vasculature (Schmidt et al., 1992; Kliewer et al., 1994). Animal studies found that PPARδ knockout mice showed glucose intolerance on normal chow, and were prone to obesity on high-fat diet (Barak et al., 2002; Kostadinova et al., 2012). PPARδ activation in the liver also appears to decrease hepatic glucose output, thereby contributing to improved glucose tolerance and insulin sensitivity (Barak et al., 2002; Kostadinova et al., 2012). Meanwhile, treatment with PPARδ-specific agonist enhanced β-oxidation, decreased free fatty acid, and improved insulin sensitivity in mice and moderately obese men (Bojic et al., 2014; Greene et al., 2012).

PPARD polymorphism has emerged as a key role for the development of MetS and T2DM in recent years. Previous studies of the variation in PPARD in relation to features of the metabolic syndrome have resulted in conflicting results. Therefore the present study has systematically analyzed PPARD rs2016520 polymorphisms (also named +294T>C or −87T>C) with metabolic traits in a relatively large sample of Chinese middle-aged adults. We primarily hypothesized that rs2016520 polymorphisms influences insulin resistance, fasting serum lipid profiles, fasting glycaemia. In addition, we related genotype to prediabetes, overweight, hypertension and metabolic syndrome.

2. Subjects and methods

2.1. Study population

All participants were enrolled from a screening survey for Metabolic Syndrome in Yinchao community of Chengdu in China between September and November 2011. At enrollment, medical history, lifestyle behaviors, functional status and current medications were recorded by a questionnaire; anthropometrical measurements and 75-g OGTT were performed. We recruited 1409 subjects who underwent OGTT and with genotyping, which included 804 subjects with normal glucose tolerance (NGT, defined as fasting plasma glucose <5.6 mmol/L and 2-h glucose level post OGTT <7.8 mmol/L), 605 subjects with prediabetes, which included impaired fasting glucose (IFG, defined as plasma glucose between 5.6 and 6.9 mmol/L after an 8-h fasting) and/or impaired glucose tolerance (IGT, defined as plasma glucose between 7.8 and 11.0 mmol/L at 2 h glucose during standard OGTT) (Anon., 2012).

Participants with the following conditions were excluded from our study: a history of known diabetes; screen detected diabetes; other established endocrine diseases such as hyperthyroidism, hypothyroidism or Cushing syndrome; a history of cardiovascular and cerebrovascular event; hepatic failure or cirrhosis; current or prior history of malignancy; severe disability or mental disorder; pregnancy or breast feeding; those who were on lipid-lowering or glucose-lowering or anti-hypertension medications were excluded since these medications may influence levels of biomarkers.

This study was approved by the Medical Ethics Committee of West China Hospital of Sichuan University and conducted in accordance with the principles of the Declaration of Helsinki II.

2.2. Clinical examination and biochemical analysis

Individual height, weight, hip and waist circumferences were measured by trained physicians; waist circumference was measured in the standing position, midway between the iliac crest and the lower costal margin; hip circumference was measured at its widest part of the hip. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters (kg/m^2). Blood pressure was measured three times by using a mercury sphygmomanometer at 5-min intervals after 10-min rest in seated position, and the mean value was used.

Fasting venous blood was collected after 10 h overnight fasting, and participants were given a standard 75 g glucose solution, blood samples were then drawn at 30 min and 2 h after glucose challenge. Plasma glucose level was measured by a glucose-oxidase method adapted to an automated analyzer (Hitachi 704, Boehringer Mannheim). Serum insulin concentration was determined by electrochemiluminescence (cobas e411, Toche Company, Switzerland). Hemoglobin A1c (HbA1c) was measured by high performance liquid chromatography (HPLC, Bio-Rad D-10 hemoglobin A1C radiometer). Serum triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol) and low-density lipoprotein cholesterol (LDL-cholesterol) were measured by enzymatic methods with commercial reagent sets (Boehringer Mannheim).

2.3. Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by using the TIANamp blood DNA Midi Kit (RelaxGene Blood DNA System), according to the manufacturer's instructions. SNP rs2016520 in the gene *PPARD* was tested using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS, Mass Array; Sequenom, San Diego, CA, USA) (Gabriel et al., 2009). For genotyping quality control, 10% of samples were randomly genotyped twice for duplication accuracy, and no genotyping discrepancies were detected between the repeated samples. Genotype distribution is consistent with Hardy–Weinberg equilibrium.

2.4. Calculation

HOMA-IR was calculated as fasting insulin × fasting glucose / 22.5 (Wallace et al., 2004); HOMA-B was calculated as (20 × fasting insulin) / (fasting glucose − 3.5); Matsuda Insulin Sensitivity Index (Matsuda ISI) was calculated as [10,000 / √(fasting glucose × fasting insulin) × (mean $glucose_{0\text{-}30\text{-}120}$ × mean $insulin_{0\text{-}30\text{-}120}$)] (Matsuda and DeFronzo, 1999); QUICKI was calculated as 1 / [log(fasting insulin) × log(fasting glucose)].

2.5. Statistical analysis

In the additive model, we coded genotypes as 0, 1 or 2 (AA = 0, AG = 1, GG = 2) depending on the number of copies of the minor alleles; for the dominant model, homozygosity and heterozygosity with the minor allele were coded as 1 and the other was coded as 0 (AA = 0, AG + GG = 1); for the recessive model, homozygosity of the minor allele was code as 1 and the others were coded as 0 (AA + AG = 0, GG = 1); unless indicated, the association study was conducted under the additive model and P value was obtained from additive model. The data were presented as mean ± SD or median (interquartile),