Life Sciences 98 (2014) 55–59

Contents lists available at ScienceDirect

Life Sciences

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

Adiponectin and peroxisome proliferator-activated receptor-γ gene polymorphisms and gene-gene interactions with type 2 diabetes

Enling Ye [a,b], Hong Yang [a,b], Liangmiao Chen [a,b], Qingshou Chen [a,b], Mengli Sun [a,b], Zhenzhen Lin [a,b], Lechu Yu [a,b], Mengmeng Peng [a,b], Chi Zhang [b,c,*], Xuemian Lu [a,b,**]

[a] Department of Endocrinology, The Third Hospital Affiliate to Wenzhou Medical University, Ruian, Zhejiang 325200, China
[b] Ruian Center of the Chinese-American Research Institute for Diabetic Complications, The Third Affiliated Hospital of the Wenzhou Medical University, Wenzhou 325200, China
[c] Chinese-American Research Institute for Diabetic Complications, Wenzhou Medical University, Wenzhou 325035, China

ARTICLE INFO

Article history:
Received 31 October 2013
Accepted 31 December 2013

Keywords:
Adiponectin
Peroxisome proliferator-activated receptor
Gene polymorphism
Gene–gene interaction
Type 2 diabetes

ABSTRACT

Aims: To investigate whether gene polymorphisms of both adiponectin and peroxisome proliferator-activated receptor gamma (PPARγ) influence type 2 diabetes mellitus (T2DM) respectively in the Han people of the Wenzhou region of China and whether the interaction of gene polymorphism between adiponectin and PPARγ influences T2DM in the same subjects.
Main methods: This study included 198 patients with T2DM and 255 healthy individuals. Polymerase chain reaction–restriction fragment length polymorphism analyses were used to detect single nucleotide polymorphisms (SNPs). Logistic regression and multifactor dimensionality reduction (MDR) methods were used to analyze gene–gene interactions.
Key findings: The frequency distribution of adiponectin SNP11377 was not different ($p = 0.792$), but the frequency of CC, CG and GG genotypes showed the difference between two groups (T2DM: 57.1%, 33.3%, and 9.6%; control: 53.7%, 41.6%, and 4.7%, respectively; $p = 0.047$). Adiponectin SNP45, SNP276 and PPAR γ SNPp12a were equally distributed between the two groups ($p = 0.586$, 0.119, 0.437, respectively), and there were no significant differences in genotype frequencies between the two groups ($p = 0.751$, 0.144, 0.479, respectively). Linkage disequilibrium existed between SNP11377 and SNP45 ($p < 0.001$) and SNP45 and SNP276 ($p < 0.001$). Haplotype analyses showed no significant differences between the T2DM and control groups. According to the logistic regression and MDR gene–gene interaction analyses, SNP11377GG and SNP276GT interactions increased the risk of T2DM (odds ratio = 6.984, $p = 0.012$).
Significance: Adiponectin SNP11377 and SNP276 gene–gene interactions are associated with the increased risk of T2DM in this population.

Introduction

Adiponectin is an adipose tissue-derived protein hormone that modulates numerous metabolic processes, including glucose regulation and fatty acid oxidation (Diez and Iglesias, 2003). Adiponectin inhibits gluconeogenesis and liver glucose export, increases peripheral glucose usage, and reverses insulin resistance (Yamauchi et al., 2001). Adiponectin is related to metabolic syndromes, including type 2 diabetes mellitus (T2DM) (Ryo et al., 2004). Peroxisome proliferator-activated receptor-γ (PPARγ) plays a pivotal role in adipogenesis and glucose homeostasis (Chan et al., 2013). PPARγ mutations may lead to severe insulin resistance (Combs et al., 2002). Therefore, both adiponectin and PPARγ genes are critical for the development of T2DM.

The role of gene polymorphism of adiponectin or PPARγ in the development of T2DM has been investigated previously (Al-Azzam et al., 2013; Bermudez et al., 2013; Hara et al., 2012; Matharoo et al., 2013; Pei et al., 2013; Raza et al., 2012; Wang et al., 2013). However, different results were found in different populations. T2DM is a multifactorial disease regulated by genetic and environmental factors. Therefore, the gene polymorphism influence of adiponectin or PPARγ on the development of T2DM needs to be investigated within different patient populations. Currently, there is no systematic analysis of SNPs in these two genes in the Wenzhou region. In this study, we investigated whether gene polymorphisms of both adiponectin and peroxisome proliferator-activated receptor gamma (PPARγ) influence type 2 diabetes mellitus (T2DM) respectively in the Han people of the Wenzhou region of China and whether the interaction of gene polymorphism between adiponectin and PPARγ influences T2DM in the same subjects.

* Correspondence to: C. Zhang, Ruian Center of the Chinese-American Research Institute for Diabetic Complications, The Third Affiliated Hospital of the Wenzhou Medical University, University-town, Wenzhou, Zhejiang 325035, China. Tel.: +86 65866199 (work).
** Correspondence to: X. Lu, Department of Endocrinology, The Third Hospital Affiliate to Wenzhou Medical University, 108 Wansong Road, Ruian, Zhejiang 325200, China.
E-mail addresses: zhangchi515@126.com (C. Zhang), lu89118@medmail.com.cn (X. Lu).

0024-3205/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.12.232

Material and methods

This study was conducted at the Third Hospital Affiliated to Wenzhou Medical University. The study protocol was approved by the institutional review board of the Third Hospital Affiliated to Wenzhou Medical University. Written informed consent was obtained from all enrolled subjects prior to the study. All subjects involved in the study were formally noticed and the informed consents were obtained. Furthermore, we guaranteed that the privacy right of each subject was completely observed.

Study population

Patients of Han ethnicity in Wenzhou, diagnosed with T2DM from 2010 to 2011 according to the World Health Organization criteria (1999) (Alberti and Zimmet, 1998), were included in this study. A patient was included in the T2DM group if his/her baseline blood glucose was ≥11.1 mmol/l, fasting blood glucose (FBG) was ≥7.0 mmol/l, or 2-hour oral glucose tolerance test (OGTT) result was ≥11.1 mmol/l. In the absence of marked hyperglycemia, testing was repeated on a different day. Patients with high blood glucose due to acute infection, trauma, or other stress were excluded from the study. Patients were also excluded if they had familial type 1 diabetes, acute metabolic complications from diabetes mellitus, tumors, or liver or kidney disease. No patient was treated with insulin, thiazolidinedione, or fibrate prior to the study. A total of 198 patients (mean age 55.16 ± 11.79) with T2DM were included in this study. Control subjects were healthy (mean age 54.02 ± 10.40) and did not have diabetes or a family history of diabetes, tumors, tuberculosis, liver or kidney dysfunction, thyroid dysfunction, or cardiovascular disease. A total of 255 control subjects were included in this study.

Clinical and biochemical data

Clinical and biochemical data were collected from all subjects. Data included the duration of T2DM, patient age, drug use history, body mass index (BMI), waist-to-hip ratio (WHR), FBG, fasting blood insulin (FBI), homeostasis model assessment (HOMA-IR), glycated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively), and adiponectin levels. FBG, TC, TG, HDL-C and LDL-C were measured by automatic biochemical analyzer. FBI was measured by UniCel DxI 800 Immunoassay System (Beckman Coulter, America). HbA1c was measured by high pressure liquid chromatography. Adiponectin was measured by an enzyme-linked immuno-sorbent assay. The insulin resistance index of HOMA-IR was calculated by fasting blood insulin (mU/l) × fasting blood glucose (mmol/l) / 22.5.

Genetic polymorphism analyses

Genomic DNA was extracted from patient blood using DNeasy columns (Generay Biotech, Shanghai, China). Adiponectin SNP11377, SNP45, and SNP276 gene polymorphisms were determined by PCR. Template DNA (10–50 ng) was mixed with 1 μl forward and 1 μl reverse primers (10 μmol/l), 25 μl of 2× PCR Master Mix (Fermentas, Hanover, MD), and water in a total volume of 50 μl. The PCR reaction cycle included a pre-denaturation step for 4 min at 94 °C, followed by 30 cycles of 94 °C for 40 s, 59 °C for 30 s, and 72 °C for 40 s, and a final 5-minute incubation at 72 °C. The PPARγ SNPp12a PCR cycle included a 4-minute incubation at 94 °C, followed by 30 cycles of 94 °C for 40 s, a combined annealing/extension step for 70 s at 65 °C, and a final 5-minute incubation at 72 °C. PCR products were digested using restriction enzymes and separated by 5% polyacrylamide gel electrophoresis. Primers were synthesized by Generay Biotech. Restriction enzymes were purchased from Fermentas. Primer sequences and restriction enzymes are shown in Table 1.

Table 2
Comparison of clinical characteristics between T2DM patients and control subjects.

Clinical characteristic	T2DM patients	Control subjects	*p*-Value
Age[a]	55.16 ± 11.79	54.02 ± 10.40	0.279
Sex[d] (male/female)	118/80	144/111	0.504
bmi[a]	24.4386 ± 2.54	23.1573 ± 2.92	0.000
WHR[c]	0.95 [0.92, 0.98]	0.88 [0.82, 0.92]	0.000
HbA1c[c]	7.3 [6.6, 8.3]	5.3 [5.1, 5.5]	0.000
FBG[c] (mmol/l)	7.08 [6.10, 8.90]	4.96 [4.75, 5.20]	0.000
FBI[b] (mU/l)	6.07 ± 1.59	7.53 ± 1.95	0.807
HOMA-IR[b]	1.98 ± 1.40	1.15 ± 0.49	0.000
Adiponectin[c] (mg/l)	5.58 [5.07, 6.68]	6.89 [6.23, 8.13]	0.000
TG[b] (mmol/l)	2.11 ± 1.23	1.76 ± 0.99	0.000
TC[a] (mmol/l)	4.75 ± 1.02	4.93 ± 0.97	0.520
HDL-C[b] (mmol/l)	1.21 ± 0.29	1.31 ± 0.29	0.000
LDL-C[a] (mmol/l)	2.77 ± 0.82	2.90 ± 0.80	0.076

[a] Quantitation of normal distribution, *t*-tests were used for the analyses. The results are shown as mean ± standard deviation.
[b] Quantitation of normal distribution (after log-transformation), *t*-test was used for the analyses, and the results are shown as mean ± standard deviation.
[c] Quantitation of skewed distribution, Wilcoxon Rank sum tests were used. The results are shown as median and quartiles.
[d] Categorical data, Chi square test was used.

Statistical analyses

Statistical analyses were performed using SPSS 16.0 software. Chi-squared tests were used for categorical data. One-way ANOVA and *t*-tests were used for quantitative data (or log-transformed data) with normal distributions. Nonparametric tests were used for measured data with skewed distributions. Hardy–Weinberg tests were used to determine if the population represented typical allele and genotype frequencies. Univariant logistical regression (LR) was used to analyze the associations between genes and diseases. SHEsis software was used for analyzing haplotype and linkage disequilibrium (LD). LR and multifactor dimensionality reduction (MDR) were used for analyses of gene–gene interactions. Two-sided analyses with $p < 0.05$ were considered statistically significant.

Table 1
Primer sequences and enzymes used in SNP analyses.

SNP	Reference SNP	Primers	Enzymes	Amplicon length (bp)	Digested products (bp)
SNP11377 C>G	rs266729	F: 5′-ACTTGCCCTGCCTCTGTCTG-3′ R: 5′-GCCTGGAGAACTGGAAGCTG-3′	HhaI	251	137, 114
SNP45 T>G	rs2241766	F: 5′-GAAGTAGACTCTGCTGAGATGG-3′ R: 5′-TATCAGTGTAGGAGGTCTGTGATG-3′	SmaI	372	219, 153
SNP276 G>T	rs1501299	F: 5′-GGCCTCTTTCATCACAGACC-3′ R: 5′-AGATGCAGCAAAGCCAAAGT-3′	Mva1269I	196	148, 48
SNPp12a C>G	rs1801282	F: 5′-GCCAATTCAAGCCCAGTC-3′ R: 5′-GATATGTTTGCAGACAGTGTATC AGTGAAGGAATCGCTTTCCG-3′	Bsh1236I	267	224, 43

Note: F = forward primer, R = reverse primer.