Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

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

APM1 gene variants −11377C/G and 4545G/C are associated respectively with obesity and with non-obesity in Chinese type 2 diabetes

Xizhen Wang [a], Sizhong Zhang [b,c], Yuanzhong Chen [d], Hekun Liu [a,*], Cailian Lan [a], Xuexiang Chen [a], Shuguang Chi [a], Suyun Chen [a], Wei Zhang [b,c]

[a] Department of Cell Biology and Genetics, and Center of Neuroscience, Fujian Medical University, 88 Jiaotong Road, Fuzhou 350004, Fujian, China

[b] Department of Medical Genetics, West China Hospital, Sichuan University, Chengdu 610041, China

[c] Division of Human Morbid Genomics, State Key Laboratory of Biotherapy of Human Diseases, Chengdu 610041, China

[d] Department of Hematology, Union Hospital, Fujian Medical University, Fuzhou 350004, China

ARTICLE INFO

Article history:
Received 21 October 2008
Received in revised form
26 February 2009
Accepted 2 March 2009
Published on line 8 April 2009

Keywords:
Adiponectin
Type 2 diabetes
Obesity
Diabesity
Single nucleotide polymorphisms

ABSTRACT

In order to explore the different genetic backgrounds of non-obese and obese type 2 diabetes, we tried to genotype six SNPs (−11391G/A, −11377C/G, −10068G/A, G54V, Y111H and 4545G/C) in the adipose most abundant gene transcript-1 (APM1) gene in 338 type 2 diabetes (T2D) patients and 460 non-diabetic subjects by PCR–RFLP. Among these mutations, the 4545G/C mutation (rs1063539) contributed to the genetic risk of T2D in the non-obese group (OR = 2.34, 95%CI: 1.31–4.21, $P = 0.004$), and 57% of the risk is related to this polymorphism. On the contrary, −11377C/G (rs266729) was associated with type 2 diabetes in the obese group only (OR = 2.45, 95%CI: 1.13–5.31, $P = 0.02$), and 59% risk of diabesity could be attributed to that. All the associations above were adjusted for age and gender in unconditional logistic regression. Besides, the −11377G/4545C haplotype was merely related to obese diabetes (OR = 2.12, 95%CI: 1.08–4.14, $P = 0.03$). In addition, the obese diabetic group had significantly higher levels of triglyceride and insulin, better beta-cell function but lower glucose levels than the non-obese group (all P values <0.01). This study suggests that the genetic susceptibility is different between type 2 diabetes with and without obesity in Chinese Han population.

1. Introduction

Type 2 diabetes (T2D) involves a complex interaction between genetic variants and environmental factors, and obesity can increase the risk of developing diabetes [1]. As the results of obesity epidemic, adipose tissue has become the prime-time focus of biomedical research and it has become clear that adipose tissue is not only an energy storehouse but also an active endocrine organ secreting a variety of proteins that regulate glucose levels, lipid metabolism, and energy homeostasis [2–4]. One of these proteins that have attracted a lot of attention recently is adiponectin.

Adiponectin (also called APM1, ACRP30, ADIPOQ, and GBP28) is an abundant adipocyte-secreted protein in plasma. It can regulate glucose levels, insulin action and lipid metabolism [5,6]. The circulating adiponectin was significantly reduced in patients with type 2 diabetes and obesity [7,8]. Prospective studies showed that subjects with high adiponectin levels were

* *Corresponding author*. Tel.: +86 131 10630336; fax: +86 591 22862666.
E-mail address: fjlhk@163.com (H. Liu).

0168-8227/$ – see front matter
doi:10.1016/j.diabres.2009.03.004

protected against type 2 diabetes [9]. Moreover, administration of recombinant adiponectin decreased glucose and insulin resistance in mice of obesity or diabetes [6,10]. The plasma level of adiponectin is partly influenced by genetic factors which account for about 40–70% [11]. The human adiponectin gene is mapped to the 3q27 region where the metabolic syndrome and T2D loci were reported [12–14].

Some studies have found that the single nucleotide polymorphisms (SNPs) of this gene might increase the risk of T2D [15–17], whereas few reported the relationship between its SNPs and type 2 diabetes combined with obesity. As far as we know, there are only two teams who reported these associations with obese T2D. One found that *APM1* −11391G/−11377G haplotype was associated with type 2 diabetes in obese group, and that exon 3 variant Y111H was related to type 2 diabetes only in non-obese group [18]. The other detected an association with obese type 2 diabetes in *APM1* −11377C/G but not in −11391G/A [19]. These two teams' results suggest that different genetic backgrounds could increase T2D susceptibility according to the presence or absence of obesity.

We agree with the hypothesis that the susceptibility SNPs of *APM1* gene might be different between obese diabetes (diabesity) and non-obese diabetes because not all diabetic patients are corpulent. In order to address this issue, we recruited 798 individuals (338 type 2 diabetic patients and 460 non-diabetic subjects) in China and selected six SNPs throughout the *APM1* gene. The population in this study totally differs from those reported in the two studies above, and the six variants include −11391G/A, −11377C/G, −10068G/A, G54V, Y111H and 4545G/C. These polymorphisms were chosen because they might influence the gene expression or change the protein function.

2. Materials and methods

2.1. Subjects

In this study, a total of 798 subjects were recruited, consisting of 338 type 2 diabetic subjects (mean age 57.8 ± 11.3 years, male/female: 196/142) and 460 non-diabetic subjects (mean age 58.7 ± 9.1 years, male/female: 290/170). In order to avoid the possibility of bias resulting from population stratification, all subjects were unrelated Chinese Han population in Sichuan province and selected from the West China Hospital, Sichuan University during the years of 2002–2004, as well, there was no statistical significance in age and sex ratio between these two groups. The type 2 diabetic patients were diagnosed according to the 1999 American Diabetes Association criteria and were subdivided into two groups (obese and non-obese group) on the basis of the Asia Pacific obesity criteria (1999). The non-diabetic subjects (controls) had a negative history of type 2 diabetes or any other metabolic diseases. The study was approved by the Internal Ethical Review Board of the West China Hospital, Sichuan University, and the informed consent forms were obtained from all subjects studied.

2.2. Methods

Blood samples were collected in the morning after subjects had fasted for 12 h. Fasting serum concentrations of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using an automatic chemistry analyzer (Olympus AU5400, Japan) or an enzymatic kit (Roche Diagnostics GmbH, Basel, Switzerland). Fasting plasma glucose (FPG) was measured by a glucoseoxidase or hexokinase reaction, and fasting serum insulin (Fins) was assayed by means of electro-chemiluminescence immunoassay (ECLIA, Roche Diagnostics, Rotkreuz, Switzerland). The homeostasis model assessment (HOMA) indices for insulin resistance (HOMA-IR) and for beta-cell function (HBCI) were subsequently calculated by using the following formulas: HOMA-IR = Fins (mU/L) × FPG (mmol/L)/22.5 and HBCI = 20 × Fins (mU/L)/[FPG (mmol/L) − 3.5], respectively.

2.3. SNP genotyping

DNA was extracted by salting-out from 5 mL ACD (anticoagulant citrate dextrose) peripheral blood. We selected six SNPs (−11391G/A [promoter], −11377C/G [promoter], −10068G/A [promoter], G54V [exon 2], Y111H [exon 3] and 4545G/C [exon 3, 3′-UTR]) throughout the *APM1* gene. All the SNPs were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). Primers for each SNP were designed using Primer Premier 5 software (Premier, Canada). They and their restriction endonuclease were shown in Table 1. Firstly, the target sequences containing the SNPs were amplified by PCRs in a thermocycler (GeneAmp9600). The reaction was performed in a 25 μL system and its amplification procedures were as follows: predenaturation at 94 °C for 5 min, 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 56–63 °C for 30 s and extension at 72 °C for 30 s, then the last extension at 72 °C for 10 min. Secondly, the PCR products were overnight digested with special restriction enzymes (MBI, Fermentas) at 37 °C in 15 μL systems. After that, their fragments of different lengths could be separated by an electrophoresis on 3% agarose gel stained with ethidium bromide and the genotype of each sample could be determined by direct observation on an ultraviolet transilluminator (BIO-RAD Gel Doc 2000). In order to verify the genotypes, 15% samples were selected at random to be sequenced directly. And the results showed that 100% genotypes were consistent with their previous ones.

2.4. Statistical analysis

The allelic distribution of each SNP was tested for the Hardy–Weinberg equilibrium. Most calculations and statistical analyses were performed using the SPSS for Windows software (version 13.0; Chicago, IL). The description of continuous variables was expressed as medians (M) and quartile ranges (QR) because of their abnormality. A χ^2 test was used to evaluate whether there were differences in allele or genotype frequencies. Two independent samples were compared using a Mann–Whitney *U*-test and three samples using a Kruskai–Wallis *H* test. Unconditional logistic regression was for excluding some confounding factors such as age and gender. To evaluate the association degree between a polymorphism and type 2 diabetes, we calculated odds ratio (OR), 95% confidence interval (95%CI) and attribute risk proportion (ARP).