Gene 530 (2013) 158–163

Contents lists available at ScienceDirect

Gene

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

Short Communication

Association of the +45T>G adiponectin gene polymorphism with insulin resistance in non-diabetic Saudi women

Amal Mohammed Husein Mackawy *

Medical Biochemistry, Medical Lab department, AMS College, Qassim University, Saudi Arabia

ARTICLE INFO

Article history:
Accepted 1 July 2013
Available online 17 August 2013

Keywords:
Adiponectin
Adiponectin gene polymorphism
Insulin resistance
Type 2 diabetes mellitus

ABSTRACT

Background: The human adiponectin gene variations are associated with obesity, insulin resistance, and diabetes. However, these associations have not been fully examined in a non-diabetic population in Saudi Arabia. We aimed to investigate the association of 45T>G single nucleotide polymorphism (SNP) in the adiponectin gene with total adiponectin levels, insulin resistance (IR), fasting blood glucose (FBG) and other markers of obesity in non-diabetic Saudi females.

Methods: One hundred non diabetic Saudi females were enrolled in this study. They were further divided according to their body mass index (BMI) into two groups. Group I, 46 non diabetic subjects with normal body weight and group II, 54 overweight and obese females. Adiponectin 45T/G polymorphism was detected by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). Serum adiponectin was measured by ELISA.

Results: Obese women exhibited a higher distribution of TG/GG genotype compared with non-obese women. SNP +45T>G genotypes were associated with higher FBG, insulin levels and HOMA–IR with lower total adiponectin levels in obese Saudi women. Otherwise the all estimated variables revealed non-significant differences among the non-obese genotypes. The observed differences in insulin resistance markers were very significant among women with a higher body weight but not among normal body weight women, thus suggesting that SNP +45T>G effects on insulin sensitivity may depend upon body weight and body fat status.

Conclusion: SNP +45T>G of adiponectin gene has a significant role in the development of insulin resistance in Saudi women possibly through an interaction with increase body weight and hypoadiponectinemia.

1. Introduction

Adiponectin is a protein secreted from adipocytes released in the circulation of human healthy subjects at relatively high levels (Halvatsiotis et al., 2010). Animal models and human studies support an important role for adiponectin in the pathophysiology of metabolic syndrome and most of its individual components, namely insulin resistance (IR), obesity, and dyslipidemia (Sheng and Yang, 2008; Whitehead et al., 2006). Blood levels of adiponectin are reduced in obese persons compared with lean individuals (Oh et al., 2007), prospective studies have consistently found a decreased risk for type 2 diabetes mellitus (T2DM) with increasing levels of total adiponectin (Pérez-Martínez et al., 2008; Yamauchi et al., 2002). Adiponectin exerts its insulin-sensitizing effects in the liver by suppressing gluconeogenesis and in the skeletal muscle by enhancing fatty acid oxidation (Pérez-Martínez et al., 2008).

It was represented that the ratio of high molecular weight (HMW) to total adiponectin is related to risk for T2DM independent of total adiponectin, suggesting an important role of the relative proportion of HMW adiponectin in diabetes pathogenesis (Heidemann et al., 2008). Adiponectin gene is localized on chromosome 3q27within the region which was identified as susceptibility locus for type 2 diabetes and metabolic syndrome (Vionnet et al., 2000a). Several adiponectin gene (*ADIPOQ*) single nucleotide polymorphisms (SNPs) have been shown to influence adiponectin levels and have been associated with risk for obesity, IR, T2DM, and cardiovascular disease (CVD) (Menzaghi et al., 2002; Qi et al., 2006; Stumvoll et al., 2002; Tankó et al., 2005; Ukkola et al., 2003; Vasseur et al., 2002). Two of the most commonly studied

Abbreviations: dNTP, deoxyribonucleoside triphosphate; ELISA, enzyme-linked immunosorbent assay; EtdBr, ethidium bromide; SNP, single nucleotide polymorphism; IR, insulin resistance; FBG, fasting blood glucose; BMI, body mass index; PCR–RFLP, polymerase chain reaction, restriction fragment length polymorphism; HOMA–IR, homeostasis model of assessment, insulin resistance; T2DM, type 2 diabetes mellitus; HMW, high molecular weight; *ADIPOQ*, adiponectin gene; CVD, cardiovascular disease; BW, body weight; SD, standard deviation; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Taq, Thermus aquaticus; bp, base pair; ANOVA, analysis of variance; $\chi 2$, Chi-square test; CI, confidence intervals; ORs, Odd ratios; FastDigest®Sma, fast digestive Sma restriction enzyme; FI, fasting insulin; kDa, kilo Dalton; BF, body fat; Gly, glycine; G, guanine; T, thymidine; LD, linkage disequilibrium; mRNA, messenger RNA; dNTP-, deoxynucleotide triphosphate; A, adenine; C, cytosine.

* Tel.: +20 966508129407 (mobile).
E-mail address: amalmackawy@yahoo.com.

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.07.003

SNPs at the *ADIPOQ* locus are a silent T to G substitution in exon 2 (+45T>G, rs2241766) and a G to T substitution in intron 2 (+276G > T, rs1501299) (Jang et al., 2008; Yang and Chuang, 2006).

Genetic associations of SNP in exon 2 (45T/G) of adiponectin gene with type 2 diabetes and adiponectin level were reported in Japanese population and with IR in some Caucasian populations (Italy, Germany) (Krętowski et al., 2005).

However, little is known regarding the effect of +45T>G SNP on the circulating adiponectin and IR in healthy Saudi women.

Aim of the work was to investigate the association of 45T>G (SNP) in the adiponectin gene with total adiponectin levels, IR, FBG and other markers of obesity in non-diabetic Saudi females.

2. Materials and methods

The present study was carried out at Medical Laboratory Department, Applied Medical Science, Qassim University. One hundred subjects without a known history of diabetes, CVD, or cancer were included in this study. The subjects were recruited among the patients and the employees of the public hospitals in Qassim region. After giving written informed consent to participate, subjects provided a fasting blood sample, and underwent anthropometric and body composition measurements. Fasting blood glucose (FBG) levels of >126 mg/dl, cortisol treatment, and lipid-lowering medication were criteria for exclusion from the analysis.

The subjects were further divided into two groups according to their body weight (BW) and body mass index (BMI) measurements:

Group I: 46 women with normal BW and with mean ± SD of BMI of 23.02 ± 1.9 kg/m^2 with a mean ± SD of age was 44.94 ± 5.54 year.

Group II: 54 overweight to obese subjects with mean ± SD of BMI of 31.31 ± 2.90, their mean ± SD of age was 45.87 ± 6.20 years.

2.1. For all the subjects the following were done

a Full history and clinical examination.

b Anthropometric measurements:Body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared (Diabetes Care, 2000).
Waist circumference (cm) was measured in the middle between the 12th rib and the iliac crest.

c Laboratory investigations:

1 Estimation of fasting blood glucose level by enzymatic method (Trinder, 1969).

2 Measurement of fasting insulin by ELISA (Temple et al., 1992).

3 Determination of insulin resistance by homeostasis model assessment (HOMA) index calculated by the following formula (Matthews et al., 1985):
HOMA index = fasting insulin (μU/ml) × fasting plasma glucose (mg/dl)/405.

4 Total lipid profile levels:
- Triglyceride was determined enzymatically (Bucolo and David, 1973).
- Total cholesterol was estimated by enzymatic method (Allain et al., 1974).
- Estimation of high density lipoprotein cholesterol (HDL-C) Assmann et al., 1983).
- LDL-C concentration was calculated by the Friedewald formula (Friedewald et al., 1972).

5 Determination of plasma adiponectin levels by ELISA (Arita et al., 1999).

6 Determination of adiponectin gene +45T>G SNP by PCR-based restriction fragment length polymorphism (RFLP) (Melistas et al., 2009).

2.2. Sampling

Under complete aseptic conditions, 5 ml of venous blood were collected after 12 hour fasting in sterile EDTA containing tubes and divided into tubes:

One tube of whole blood was collected for DNA extraction and adiponectin gene detection and kept immediately at −20 °C. Plasma specimen of the other tube was obtained by centrifugation at low speed centrifugation at 2500 ×*g*; 15 min for estimating the adiponectin levels and other research investigations.

2.3. DNA analysis

PCR-RFLP method was used to determine the distribution of allele and genotype frequencies of the SNP45 T/G polymorphism in exon 2 of the adiponectin gene (Fig. 1). The DNA was isolated and purified by genomic DNA purification kit in which standard DNA isolation from 500 μl whole blood in standard 45 min protocol, including RNase treatment. The PCR was performed on 30 ng DNA in 20 mL containing 10 mmol/L Tris HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, pH 8.3, 0.2 mmol/L dNTP, 0.03 U/ml Taq polymerase and 0.4 mmol/L forward and reverse primers: forward primers 5′-GCA GCTCCT AGA AGTAGA CTC TGC TG-3′ and the reverse primers 5′-GCA GGT CTG TGATGA AAG AGG CC-3′. for 35 cycles (30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C). The PCR fragments (372 bp) were digested using the restriction enzyme FastDigest®SmaI (Fermentas International Inc., Canada).

The digested samples were separated by electrophoresis on 3% agarose gel stained with ethidium bromide and visualized on a UV transilluminator. The resulted three genotypes were identified as: (i) wild type TT (372 bp); heterozygous TG (restriction fragments 370, 209 and 160 bp); and homozygous mutant GG (restriction fragments 209 and 163 bp).

3. Statistical analysis

The statistical data were calculated for mean and standard deviation (SD). Analysis of variance F test (ANOVA) was used to compare the results of all examined cases in all studied genotypes within each other. The differences between mean value for each element were tested by student's "*t*" test. Frequency of genotypes and alleles of 45T>G of adiponectin gene was compared by Chi-square test (χ^2) which used for qualitative variables analysis. Odd ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression analysis. Differences in anthropometric and biological indices in all subjects with different genotypes were tested F-test and the significance level was set at 0.05 or less. All data analysis was performed using SPSS 16.0.

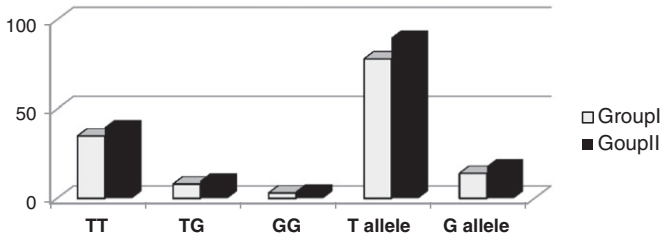

Fig. 1. Allele and genotype frequencies for adiponectin 45T>G polymorphism in all studied groups.