Gene 493 (2012) 142–147

Contents lists available at SciVerse ScienceDirect

Gene

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

Short Communication

Adiponectin gene polymorphisms (T45G and G276T), adiponectin levels and risk for metabolic diseases in an Arab population

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ARTICLE INFO

Article history:
Accepted 21 November 2011
Available online 1 December 2011

Received by A.J. van Wijnen

Keywords:
Adiponectin
Adiponectin gene polymorphisms
Saudi
Diabetes mellitus

ABSTRACT

In this study we examined the association of adiponectin gene variants with circulating adiponectin, and known metabolic diseases in 298 healthy controls and 297 Saudi subjects with type 2 diabetes mellitus (T2DM). Anthropometric and biochemical parameters were measured by standard procedures. Genotyping of T45G and G276T single nucleotide polymorphisms of adiponectin gene was carried out by PCR-RFLP analysis. No significant differences in the genotype distribution of T45G and G276T polymorphism were found between control and diabetic subjects. Neither SNP conferred an association with T2DM, obesity, hypertension or dyslipidemia. Despite a marked decrease in patients as opposed to controls, adiponectin levels were not different according to genotypes of T45G and G276T polymorphisms in control and patients. Thus, neither adiponectin SNPs independently conferred increased T2DM risk nor in other metabolic conditions considered such as obesity, hypertension or dyslipidemia. These findings support the existence of population based differences in the association of adiponectin gene variants with metabolic phenotypes and emphasize the importance of studying multiple polymorphisms, sufficient enough to identify the adiponectin gene as a genetic marker for several non-chronic communicable diseases.

1. Introduction

Adiponectin is a collagen-like protein hormone produced in adipose tissue and is believed to have anti-inflammatory and insulin sensitizing properties (Maeda et al., 1996; Kadowaki et al., 2006). Accordingly, reduced plasma levels of adiponectin are reported to be associated with an increased risk in developing insulin resistance, type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome, and even osteoarthritis (Kadowaki et al., 2006; Matsuzawa et al., 2004; Weyer et al., 2001; Daimon et al., 2003; Hu et al., 2011). The protective effect of adiponectin against these metabolic phenotypes may involve the suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in the muscle and the stimulation of insulin secretion (Rabe et al., 2008). These effects are suggested to be mediated by the activation of signaling pathways of adenosine monophosphate activated protein kinase and the peroxisome proliferator activated receptor-alpha (PPAR-α) (Rabe et al., 2008). Adiponectin expression is affected by weight loss, PPAR-γ receptors, inflammatory and angiogenic factors (Yang et al., 2001; Maeda et al., 2001; Hajer et al., 2008; Bruun et al., 2003). Consistent with the association at protein levels, genetic studies have also demonstrated a link between adiponectin gene and the metabolic phenotypes. Several single nucleotide polymorphisms (SNPs) are described in the adiponectin gene and were found to be associated with obesity (Stumvoll et al., 2002; Bouatia-Naji et al., 2006), type 2 diabetes mellitus (Hara et al., 2002; Hivert et al., 2008; Tso et al., 2006; Schwarz et al., 2006; Szopa et al., 2009) and insulin resistance (Stumvoll et al., 2002; Jang et al., 2008; Pérez-Martínez et al., 2008; Rasmussen-Torvik et al., 2009; Melistas et al., 2009). Although it should be noted that other studies have reported a lack of such an association; with conflicting reports observed, at times, within the same ethnicity (Lee et al., 2005; Vasseur et al., 2002; Populaire et al., 2003; Gu et al., 2004; Vozarova de Courten et al., 2005). Moreover these discrepancies were also found within the same population (Jang et al., 2008; Lee et al., 2005; Hwang et al., 2010; Li et al., 2011).

There is a high incidence of T2DM within the Saudi population as well as other metabolic diseases; attributed mainly to a considerable

Abbrevation: BMI, body mass index; DNA, deoxyribonucleic acid; HDL-Cholesterol, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; PCR, polymerase chain reaction; PPAR, peroxisome proliferator activated receptor; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SPSS, statistical Package for the Social Sciences; T2DM, type 2 Diabetes Mellitus.

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doi:10.1016/j.gene.2011.11.045

change in the dietary habits and sedentary life style (Al-Daghri et al., 2011a; Al-Daghri et al., 2010; Al-Daghri, 2010). Studies on circulating adiponectin in this population was also observed to be highly heritable (Al-Daghri et al., 2011b), and is associated with telomere length, a biomarker for aging (Al-Daghri et al., 2011c). However, no genetic studies to date have examined whether any of the reported adiponectin gene variants noted in other studies are associated with diabetic risk in a Saudi population. Thus, here we study the distribution of two common and widely studied adiponectin SNPs, T45G and G276T, their association with T2DM risk, circulating adiponectin, insulin resistance and anthropometric parameters in a Saudi population with and without T2DM.

2. Materials and methods

2.1. Subjects

This cross sectional study included 297 T2DM patients and 298 control subjects of Saudi Arabian descent. Studies were conducted in accordance with the guidelines set by the ethics committee of the Research Center, College of Science, King Saud University, Riyadh. Informed consents were obtained from all the subjects prior to their inclusion into the study. A standard questionnaire was obtained from all the subjects collecting the information on medical history, use of past and current medications, and demography. T2DM was defined as fasting plasma glucose levels in excess of 126 mg/dl (7.0 mmol/l) and a history of using anti-diabetic medication. Patients with any medical condition or patients on medication known to interfere with glucose metabolism, or suffering from chronic kidney disease were excluded from the study. Control subjects were apparently healthy, non-T2DM individuals free of any medical complications and were attending the primary care centers for routine health check-ups. Control subjects with history of T2DM, fasting plasma glucose levels measuring ≥126 mg/dL (≥7.0 mmol/l) or HbA1C > 5.8%, parental history of T2DM or history of using anti diabetic medication were excluded from the study.

2.2. Anthropometric and clinical measurements

Anthropometric measurements were obtained by trained personnel of health care centers. Height and body weight were measured without shoes and the study subjects wearing light clothes. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg. Waist circumference was measured to the nearest 0.5 cm at the levels between the midpoint of the lowest rib and iliac crest parallel to the floor in a standing position, while the hip circumference was measured to the nearest 0.5 cm at maximum extension of the buttocks. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Subjects with BMI 30 kg/m^2 were categorized as obese group. Blood pressure of the subjects in a sitting position was measured taking the mean of the two reading collected at an interval of 30 min. Hypertension was defined as mean systolic blood pressure of 140 mm Hg and/or a diastolic blood pressure of 90 mm Hg.

2.3. Biochemical parameters

Biochemical parameters including, HDL-cholesterol, triglycerides, total cholesterol, and fasting plasma glucose were measured by automated clinical chemistry analyzer (KoneLab) using the commercially available kits. Insulin was assayed by a solid phase enzyme amplified sensitivity immunoassay (Medgenix INS-ELISA, Biosource, Belgium). Plasma adiponectin levels were measured by radioimmunoassay following the manufacturer's instructions (Millipore, Billerica, MA, USA), with an intra- and inter-assay variation of 5.6–15.0% and a minimum detectable concentration of 145.5 pg/ml. Insulin resistance was measured by homeostasis model assessment (HOMA-IR), calculated using the formula; insulin (μU/ml) × glucose (mmol/l) / 22.5. Dyslipidemia (low levels of HDL-cholesterol) was defined as HDL-cholesterol levels <1.03 mmol/l for men and <1.29 mmol/l for women.

2.4. Genotyping

Two SNPs, a T > G substitution at +45 in exon 2 (T45G) and a G > T substitution at +276 in intron 2 (G276T) of adiponectin gene were arbitrarily chosen for genotyping on the bases of literature review and a higher allele frequency in the SNP data base. Genotyping was carried out by PCR amplification of peripheral blood genomic DNA extracted using Blood genomic prep mini spin kit (GE Health Care, Buckinghamshire, UK) followed by restriction enzyme digestion as was used in previous study (Al-Daghri et al., 2011b). For T45G polymorphism analysis, DNA was amplified using the forward primer, 5′- GAA GTA GAC TCT GCT GAG ATG G -3′ and the reverse primer, 5′- TAT CAG TGT AGG AGG TCT GTG ATG -3′. The amplified products were digested with restriction enzyme, *Sma I* (Fermentas, Germany) and the genotypes were ascertained by agarose gel electrophoresis. For G276T polymorphism, DNA was PCR amplified using the forward primer, 5′- GGC CTC TTT CAT CAC AGA CC -3′ and the reverse primer, 5′- AGA TGC AGC AAA GCC AAA GT -3′. The amplified products were digested with restriction enzyme, *Mva 1269I* (Fermentas, Germany). PCR product size for 45T/G (wild type allele) was 372 bp [Fragment size: 219 and 153 bp for homozygous mutant allele; 372, 219 and 153 bp for heterozygote allele] and 196 bp (homozygous mutant allele) for 276G/T [Fragment size: 148 and 48 bp for wild type allele; 196, 148 and 48 bp for heterozygote allele].

2.5. Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS 11.5 SPSS Inc., Chicago and USA.) and SAS 9.1 for Windows. All non-Gaussian variables were either log or square root transformed. Differences between variables were computed using *t* test and ANOVA. Post-Hoc tests, such as Bonferroni or Dunnett's *T* test were used to compare multiple groups. Allele frequency difference between controls and T2DM subjects was determined by Chi-square test, where $p < 0.05$ was considered to be statistically significant.

3. Results

The general characteristics of control and T2DM subjects are presented in Table 1. T2DM subjects were significantly older than controls. Anthropometric measures including BMI, waist circumference, and sagittal abdominal diameter were also significantly higher in T2DM patients compared with control subjects. Among the insulin resistance parameters, T2DM subjects had increased levels of fasting glucose, insulin and HOMA-IR. Additionally, triglycerides, LDL-cholesterol, systolic and diastolic blood pressures were markedly elevated in diabetic patients, while HDL-cholesterol is decreased in comparison with control subjects.

3.1. Association of adiponectin gene SNPs with T2DM and other metabolic abnormalities

The distribution of T45G and G276T polymorphisms of adiponectin gene are provided in Table 1. The genotype distribution of both T45G and G276T SNPs were in agreement with the Hardy–Weinberg equilibrium. We observed that the frequency of TT, TG and GG genotypes of T45G SNP was not statistically different between control and patients (minor allele frequency; 0.16 vs. 0.14 respectively, $p = 0.5$). Similarly, the frequency of GG, GT and TT genotypes of G276T SNP was comparable among the control and diabetic subjects (minor