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Association of adiponectin gene polymorphisms with an elevated risk of diabetic peripheral neuropathy in type 2 diabetes patients

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ABSTRACT

Objective: In this study, we examined the association between two adiponectin (*ADPN*) gene polymorphisms, +45 T/G and +276G/T, and susceptibility to diabetic peripheral neuropathy (DPN) in type 2 diabetes mellitus (T2DM) patients.
Methods: A total of 180 T2DM patients were enrolled in this study and assigned to two groups: DPN group (n = 90) and non-DPN (NDPN) group (n = 90). In addition, 90 healthy subjects were chosen as healthy normal control (NC). The plasma level of ADPN was quantified by ELISA method and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used for genotype analysis of the two *ADPN* polymorphisms, +45 T/G (rs2241766) and +276G/T (rs1501299), in all the study subjects. Statistical analysis of data was performed with SPSS version 20.0 software.
Results: Serum levels of ADPN were markedly reduced in the DPN group compared to NDPN and NC groups (all $P < 0.05$). The frequencies of TT, TG and GG genotypes and the T and G alleles of T45G and G276T polymorphisms in DPN group were significantly different than the NDPN group (all $P < 0.05$). Notably, T45G and G276T polymorphisms were associated with significantly reduced plasma levels of ADPN in DPN and NDPN groups, compared to the NC group ($P < 0.001$). Significant difference in ADPN plasma levels were also observed between TT, TG and GG genotypes of T45G and G276T polymorphisms. Our results indicate that the T allele in *+45 T/G* and *+276G/T* polymorphisms is correlated with an elevated risk of DPN in T2DM patients. Haplotype analysis showed that GG and GT haplotypes showed a negative relationship with DPN, while TG haplotype positively correlated with risk of DPN in T2DM patients (all $P < 0.05$).
Conclusion: Our results show that T45G and G276T polymorphisms of *ADPN* are associated with a significantly elevated risk of DPN in T2DM patients, likely by down-regulating ADPN serum level.

1. Introduction

Peripheral neuropathy is a frequent complication in type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) patients (Davies et al., 2006). Diabetic peripheral neuropathy (DPN) is a serious global health problem because diabetic patients sustain extensive nerve damage and peripheral nerve dysfunction, without any overt symptoms, and DPN is often diagnosed in these patients by excluding other potential causes (He et al., 2014; Kolla et al., 2009). In western countries, DPN exhibits high incidence rates in diabetics at 60%–90% each year, with no gender differences. The incidence of distal sensory neuropathy is 4% in first the five years following the diabetes onset, but is as high as 20% within the next twenty years (Allen et al., 2014; Raafat & Samy, 2014). The fatality rate of DPN is 44% within 2.5 years and 56% in 5 years, with half the deaths resulting from renal failure and the other half attributed to sudden respiratory circulation arrest and hypoglycemia (Ponirakis et al., 2014; Razazian et al., 2014). The underlying factors in DPN progression include hyperglycemia, metabolic disorders, vascular injury, neurotrophic factor deficiency, abnormal cytokine release, oxidative stress and immune factors, with hyperglycemia as the most prominent factor leading to DPN (Basol et al., 2013; Funnell, 2014; Ziegler et al., 2014). Not surprisingly, almost 50% of T2DM patients are diagnosed with DPN and chronically suffer agonizing pain caused by peripheral nerve damage. The diabetes

Conflict of interest: none.

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control and complications trial showed that rigid glycemic control effectively decreased or halted the progression of DPN and 35% patients successfully achieved rigid glycemic control within ten years (Deng et al., 2014; Jensen et al., 2014; Tang et al., 2012). In this context, adiponectin (ADPN) is a hormone secreted by fat cells and plays an important role in DPN because ADPN regulates lipid and carbohydrate metabolism (Jeong et al., 2012; Otsuka et al., 2006; Tuttolomondo et al., 2010).

ADPN circulates at high concentrations in healthy human plasma and participates in anti-inflammation, insulin sensitivity and cardio-protective mechanisms (Ando et al., 2013; Jeong et al., 2012; Siitonen et al., 2011). ADPN protein is encoded by the *ADPN* gene, which is mapped to chromosome 3q27, a susceptibility locus for metabolic syndrome and diabetes. The human *ADPN* spans 17 kb and consists of three exons and two introns (Hu et al., 1996; Scherer et al., 1995). ADPN is also the major adipokine in plasma, constituting approximately 0.01% of the total plasma protein (Sandy An et al., 2013). ADPN decreases insulin resistance by promoting glucose uptake in skeletal muscles, enhances fatty-acid oxidation in the liver and skeletal muscles, reduces triglyceride levels and inhibits gluconeogenesis and glucose output (Abdelgadir et al., 2013; Yadav et al., 2013). Importantly, plasma ADPN levels negatively correlate with insulin sensitivity and reduced plasma ADPN level can lead to insulin resistance and accelerate diabetes progression (Abdelgadir et al., 2013; Han et al., 2011; Sandy An et al., 2013). Single-nucleotide polymorphisms (SNPs) in *ADPN* gene are critical to human physiology because plasma levels of ADPN are dramatically influenced by these polymorphisms, among which +45 T/G in exon 2 and +276G/T in intron 2 increase insulin resistance and body weight by reducing *ADPN* expression levels (Al-Daghri et al., 2011; Han et al., 2011). Curiously, the +45 T/G variant is associated with increased morbidity in T2DM patients from China and Japan and the +276G/T variant is linked with higher morbidity inT2DM patients from Poland and Japan (Esteghamati et al., 2012; Mtiraoui et al., 2012). Previous studies presented a strong evidence of a high contribution of heredity factors in altering ADPN plasma levels, with the influence by genetic factors ranging between 30% and 70%, and these factors are tightly associated with elevated risk of diabetes (Chung et al., 2011; Murea et al., 2012). Although a few previous studies examined the association between *ADPN* polymorphisms and diabetes (Al-Daghri et al., 2011; Udomsinprasert et al., 2012), the limited data available and the contradictory results from these studies prompted us to undertake a systematic analysis of two *ADPN* polymorphisms (+45 T/G and +276G/T variants) for their affect on the plasma ADPN levels and risk of DPN in T2DM patients. In this study, our aim was to develop reliable tools to identify T2DM patients at high-risk for DPN.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Institutional Review Board of First Affiliated Hospital of Jilin University. The written informed consent was obtained from each eligible patient and the entire study was performed based on the Declaration of Helsinki.

2.2. Subjects and experiment group

The study was conducted as a case–control design. A total of 180 T2DM patients were enrolled at the First Affiliated Hospital of Jilin University between May 2012 and August 2013. All T2DM patients were diagnosed based on the 1999 WHO criteria: fasting plasma glucose (FPG) ≥ 7.0 mmol/L or 2-h PG ≥ 11.1 mmol/L (Gabir et al., 2000). Inclusion criteria for DPN patients were: clinical manifestation with acroparesthesia or motor nerve involvement; reduced degree of deep and superficial sensation; reduced sensory nerve conduction velocity (SCV) and motor nerve conduction velocity (MNCV). Eligible T2DM patients were assigned into two groups: DPN group and non-DPN (NDPN) group. DPN group included 90 patients (46 males and 44 females), with a mean age of 54.1 ± 5.6 years, and NDPN group consisted of 90 patients (50 males and 40 females), with a mean age of 54.9 ± 5.1 years. In addition, ninety healthy subjects (40 males and 50 females), with a mean age of 53.5 ± 5.0 years, were chosen as healthy normal control (NC).

2.3. Baseline data collection

Data related to baseline characteristics of the study subjects were collected and recorded, including gender, age, body mass index (BMI), diabetes duration and waist-to-hip ratio (WHR). Waist circumference (waistline) was measured at the midpoint between iliac crest and costal margin at the mid-axillary line and hipline was measured from left greater trochanter marker and right greater trochanter marker. Glycosylated hemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (HPLC, Bio-Rad Laboratories, Hercules, CA, USA). Fasting plasma glucose (FPG) was measured by glucose oxidase test (using assay kit from Shanghai Kehua Bio-Engineering Co., Ltd). Total cholesterol (TC) and triglyceride (TG) were measured by standard enzyme-based colorimetric assays and the assay kits for TC and TG were obtained from Yantai Ausbio Biochemical Engineering Co., Ltd. High density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by direct detection methods (using assays kit from Daiichi Pure Chemicals Co., Ltd.).

2.4. ADPN level

After overnight fasting, forearm venous blood samples (2 × 5 ml) were collected through venipuncture from all T2DM patients and healthy controls. The blood was centrifuged at 2700 rpm for 10 min at room temperature and the supernatant was collected and placed in EDTA containing tubes and stored at −80 °C until further use. Total plasma ADPN concentration was measured using Enzyme-linked immunosorbent assay (ELISA) with components from ADPN detection kit (Innogent Company, Shenzhen).

2.5. Detection of ADPN gene +45 T/G and +276G/T polymorphisms

Genomic DNA from whole blood samples was extracted using whole blood genomic DNA extraction kit (Shanghai Sangon, SK8224), according to manufacturer's instructions. Laboratory personnel were blinded to the case–control status of the subjects. *ADPN* +45 T/G (rs2241766) and +276G/T (rs1501299) were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Primer sequences for *ADPN* +45 T/G (rs2241766) and +276G/T (rs1501299) are shown in Table 1. PCR amplification was performed in a total volume of 20 μl reaction mixture containing: genomic DNA (4.0 μl), Taq DNA polymerase (5 U/μl) (Shanghai Sangon, SK2492), 10× PCR buffer (2.0 μl), 10 mmol/L dNTP Mix (0.6 μl), 25 mmol/L $MgCl_2$ (1.8 μl), upstream primer and downstream primer (per 0.5 μl) (20 μmol/L, Shanghai Sangon), and double distilled water (10.3 μl). PCR conditions were: predenaturing (94 °C for 2 min), denaturing (94 °C for 40 s), annealing (60.5 °C for 60 s),

Table 1
Primer sequences for *ADPN* +45 T/G (rs2241766) and +276G/T (rs1501299).

Primer	Sequences
G276T	F: 5'-CTCCTACACTGATATAAACTATATGAAT-3 R: 5'-AATGTACTGGGAATAGGGATGA-3'
+45T/G	F: 5'-CTCCCTGTGTCTAGGCCTTAC-3' R: 5'-TAGAAGTAGACTCTGCTGAGATG-3'

F: forward; R: reverse.