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Genetic association analysis of the adiponectin polymorphisms in type 2 diabetes with and without complications

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Abstract

Background: Adiponectin gene polymorphisms are associated with adiponectin plasma levels, BMI, insulin sensitivity and type2 diabetes. This study was carried out to examine the possible association of adiponectin gene polymorphisms at positions +45T/G and –11391G/A and type2 diabetes in an Iranian population.
Methods: Type 2 diabetic patients (N=244) were recruited from diabetes clinic in Rafsanjan, South-east of Iran. Healthy control subjects (N=99) were recruited from the same area. DNA was extracted using salting out method
Results: No significant association was found between adiponectin gene polymorphisms +45T/G and –11391G/A and diabetes in our study. In addition, no significant association was found between these polymorphisms and diabetic retinopathy, nephropathy and neuropathy. Linkage disequilibrium (LD) analysis showed no significant LD between these two polymorphisms also no significant differences for LD was found between cases and controls .
Conclusion: Our findings confirm previous data reporting the lack of association between +45T/G polymorphism in adiponectin gene and development of diabetic neuropathy, retinopathy and nephropathy. Moreover, our data showed no association between -11391 polymorphism and type 2 diabetes .

Keywords: Adiponectin, Polymorphism, Diabetes, Complications

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Introduction

Adiponectin is an adipokine secreted exclusively by adipose tissue and has gained much attention due to its close association with insulin sensitivity (1). Plasma adiponectin levels have been reported to be reduced in patients with obesity, type2 diabetes, and coronary artery disease (1-3). Single nucleotide polymorphisms (SNPs) of the adiponectin gene have been associated with BMI, insulin sensitivity and type 2 diabetes in previous studies (1,4). In present study, we examined the association between adiponectin gene polymorphisms at positions +45T/G and -11391 G/A with type 2 diabetes and also with diabetic complications such as neuropathy, nephropathy, retinopathy in an Iranian population .

Methods

Subjects' characteristics

The study group comprised of type 2 diabetic patients (N=244) who were recruited from diabetes clinic in the Ali-ibn-Abitaleb hospital, Rafsanjan University, South-east of Iran. Healthy control subjects (N=99) were recruited from the same area. All subjects selected for this study were from Persian origin. Ethnicity variation is rare in this area and subjects with other ethnic backgrounds were not entered in this study.

After giving informed consent and providing a personal and demographic data, 3-5 cc of venous blood were collected in EDTA tubes, stored at -20 ºC for DNA extraction. The study was approved by the Ethics committee of Tehran University of Medical Sciences.

Diagnostic criteria

Patients with diabetes were diagnosed according to American Diabetes Association Criteria. Diabetic retinopathy was diagnosed by an expert ophthalmologist based on ophthalmoscopic examination. Diabetic nephropathy was defined as microalbuminuria of more than 30mg/24h in two to three samples with exclusion of other conditions that can cause proteinuria. Neuropathy was defined by symptoms or signs according to Diabetes Control and Complication Trial criteria (5). Patients with neuropathic foot ulcers were defined as neuropathy .

DNA extraction and Genotyping

DNA was extracted from anticoagulated blood collected in EDTA using salting out method.

Molecular analysis of +45T/G adiponectin gene polymorphism was performed based on standard assay described by Schaffler et al. (6). We have developed an assay for genotyping -11391 polymorphism using PCR-RFLP assay. Following primers were designed: Forward primer, CATC AGAA TGTG TGGC TTGC and Reverse primer, AGAA GCAG CCTG GAGA ACTG. MspI restriction endonuclease digested the PCR product yielding DNA fragments of 137 and 26 base pairs when G allele was present and leaving a 163 bps of undigested product when an allele was present. The products of the digest were then visualized on a 3.5% agarose gel stained with ethidium bromide .

Statistical analysis

Strength of association between different groups and alleles or genotypes of Adiponectin polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined 0.05 using contingency tables by either Chi-square or Fisher exact analysis. All analyses were carried out using the STATA (v.8) software .

Linkage disequilibrium and haplotype analysis

Estimated haplotype frequencies and testing for linkage disequilibrium between pairs of polymorphisms in the cases and controls were calculated using the EHPLUS program, which provides log likelihood, Chi-square and the number of degrees of freedom. To test for heterogeneity in haplotype frequencies between cases and controls, the likelihood ratio test is used.

Results

Male/Female ratio was 70/174 in type 2 diabetic patients and 58/41 in controls; mean age was 53±10 years in type 2 diabetic patients and 53±15 years in controls and mean BMI was 26±4 kg/m2 in diabetic patients and 23±4 kg/m2 in controls. Among our patients 45 cases were diagnosed with retinopathy (20 with proliferative and 25 with non-

proliferative diabetic retinopathy), Diabetic neuropathy was present in 158 cases and proteinuria was present in 22 patients.

No significant difference was found for adiponectin +45T/G and -11391G/A polymorphisms between cases and controls and an equal distribution for allele and genotype frequencies were found (Table 1). When we compared allele and genotype frequencies for adiponectin gene polymorphisms between patients with and without diabetic neuropathy, nephropathy or retinopathy no significant differences were found (Table 2)

Pair wise haplotype estimation for adiponectin +45T/G and -11391G/A polymorphisms in type 2 diabetes patients and controls showed no significant LD between these two polymorphisms ($\chi 2$ 4.6 and 2.1; respectively) in our population. Although an increase in the frequency of haplotype (T-G) was observed in patients (80%) compared to controls (74%) no significant difference in LD was observed using log likelihood analysis .

Table1. Allele and genotype frequencies of Adiponectin gene polymorphisms in Type 2 diabetes

Gene	**Controls**	**Diabetes**
+45 T/G	**(N=99)**	**(N=241)**
Genotype		
TT	64(64.6%)	171(71%)
TG	27(28.3%)	63(26.1%)
GG	8(8.1%)	7(2.9%)
Allele		
T	281(81.2%)	405(84%)
G	65(18.7%)	77(15.9%)
-11391 G/A	**(N=99)**	**(N=243)**
Genotype		
GG	92(92.9%)	227(93.4%)
GA	7(7.1%)	15(6.2%)
AA	0(0%)	1(0.4%)
Allele		
G	191 (96.4%)	469(96.5%)
A	7 (0.03%)	17(0.03%)

Table2. Genotype frequency of adiponectin polymorphisms in diabetic patients with and without neuropathy, nephropathy and retinopathy

Diabetes complication	**Genotype n (frequency)**			**P-value**
+45 T/G	TT	TG	GG	
Neuropathy	116(73%)	37(23%)	6(4%)	0.09
No Neuropathy	55(64%)	30(35%)	1(1%)	
Nephropathy	20(41%)	8(29%)	0(0%)	0.6
No Nephropathy	142(69%)	56(27%)	7(4%)	
Retinopathy	35(78%)	8(18%)	2(4%)	0.3
No Retinopathy	129(68%)	54(29%)	5(3%)	
11391G/A	GG	GA	AA	
Neuropathy	142(93%)	10(6%)	1(1%)	0.7
No Neuropathy	81(95%)	4(5%)	0(0%)	
Nephropathy	20(91%)	1(9%)	1(0%)	0.1
No Nephropathy	192(94%)	13(6%)	0(0%)	
Retinopathy	40(91%)	4(9%)	0(0%)	0.4
No Retinopathy	170(93%)	11(6%)	1(1%)	

Discussion

In this study no association was found between the adiponectin polymorphisms and diabetes in an Iranian population. Also no associations were found between these polymorphisms and diabetic neuropathy, retinopathy and nephropathy. However this should be confirmed in a larger number of samples.

The polymorphism in exon 2 of the human adiponectin gene is a synonymous mutation (Gly-Gly). However previous studies have shown an association between the G allele of this polymorphism and elevated adiponectin plasma level (5). Previous data demonstrated that +45T/G polymorphism in adiponectin gene is not associated with the development of

diabetic neuropathy, retinopathy and nephropathy (7,8). This was confirmed in this study. Polymorphism at position -11391G/A was shown to be associated with the reduced insulin sensitivity in obese subjects in previous reports (9). In another report -11391 G/A mutation was associated with adiponectin levels and with type 2 diabetes in French Caucasian population (10). But no association has been observed between -11391 G/A polymorphism and type 2 diabetes in Iranian population. We also performed LD analysis between +45T/G and -11391G/A polymorphisms and no differences for LD have been observed between cases and controls. This indicates that our population might be different from French population for adiponectin gene polymorphisms allele and genotype frequencies however this needs to be confirmed using more samples. Future studies on a larger population and also on the plasma adiponectin level in patients with diabetes and its association with adiponectin polymorphism will be needed to further confirm the effect of adiponectin gene polymorphism on type 2 diabetes in Iranian population .

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