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Original Article

Promoter resistin gene polymorphism in patients with type 2 diabetes and its influence on concerned metabolic phenotypes

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Abstract

Introduction: Recently, it has been suggested that resistin, as an adipokine, links obesity with insulin resistance; moreover, the resistin gene polymorphisms, particularly in promoter region, is associated with serum resistin level and susceptibility to diabetes. This study investigates the association between resistin polymorphism at -420C/G and type2 diabetes (T2DM) in Iran.

Methods: This cross-sectional study was conducted on 47 type 2 diabetic patients and 66 non-diabetic controls. Blood sample was obtained from the subjects and after DNA extraction, the SNP analysis was performed via PCR-RFLP method. Then, the association between polymorphism in -420C/G and several clinical and biochemical characteristics including age of onset, fasting glucose and HbA1c were investigated.

Results: The frequency of CC genotype among diabetic patients (48.9%) as compared with healthy subjects (24.2%) was twofold. The relative risk for -420 CC diabetic patients were 2.99(95% CI: 1.34-6.68, P= 0.009). The C allele frequency was higher among the diabetic patients compared with healthy controls, although this difference was not significant (P=0.13). Also, diabetic patients with CC genotype had the lowest age of onset and the highest fasting glucose and HbA1c among all studied patients (P≥0.05).

Conclusion: The results show that polymorphisms of promoter resistin gene are associated with type2 diabetes; nonetheless, CC genotype compared to GG and CG increases susceptibility to T2DM.

Keywords: Polymorphism, Resistin, Type 2 diabetes

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Introduction

The poor pancreatic beta cell function on a background of insulin resistance resulted in type 2 diabetes (T2DM) (1). Although, there is a strong correlation between insulin resistance and obesity, the precise mechanisms by which elevated adiposity can lead to insulin resistance are unidentified (2). However, the secretion of adipokines from adipocytes has been recognized as a possible mechanism and resistin, as one of such adipokines, might contribute to pathogenesis of T2DM (3, 4).

In mice, resistin is secreted from adipocytes and antagonizes insulin action on hepatic glucose homeostasis in both in vitro and in vivo conditions. Serum levels of resistin are increased in diet-induced or genetic obese mice. Increased resistin secretion was linked to impaired glucose tolerance and insulin action, and administration of anti-resistin antibodies improved blood glucose and insulin sensitivity in obese diabetic mice. For that reason, insulin resistance inducing function of resistin as an adipocyte-secreted cytokine appears to be recognized in murine models (3, 5-7).

However, both positive and negative associations had been reported in previously performed human studies on the role of resistin in insulin signaling pathway and glucose metabolism. Therefore, the study on genetic variation of RETN (the gene coding resistin production in human), has became the target of several investigations about identification of involved mechanisms in pathogenesis of T2DM.

Until now, several single nucleotide polymorphisms (SNPs) have been described in the RETN gene. It has also revealed that among several SNPs described in the RETN, the promoter SNPs could clarify the role of resistin in disease development, insulin resistance and obesity. RETN -420C/G (rs1862513), as one of the most commonly studied polymorphisms in the promoter region, was reported to be associated with the regulation of RETN expression and resistin concentration in plasma (8-10). Several studies attributed the RETN -420C/G polymorphism to obesity, insulin sensitivity, and type2 diabetes. On the other hand, there are some studies with inconsistent results (11-16).

The aim of the present study was to investigate the association between RETN- 420C/G polymorphism and T2DM in an Iranian population.

Methods

Study population

Forty seven patients with type 2 diabetes and 66 non-diabetic controls were studied. Diabetic patients were selected from subjects referred to diabetes clinic of the Endocrinology and Metabolism Research Center (EMRC) of the Tehran University of Medical Sciences (TUMS). The controls were randomly selected from participants of osteoporosis project study in Tehran. Type 2 diabetic patients were diagnosed according to World Health Organization (WHO) criteria (fasting blood glucose ≥126 mg/dL and/or 2-h glucose levels ≥200 mg/dL during oral glucose tolerance test) and/or who were on medication for diabetes (17). Control subjects had normal fasting glucose according to the aforementioned criteria and subjects with a family history of diabetes were excluded from study. Body Mass Index (BMI) was used as an obesity index.

The study protocol was approved by the research ethics committee of the Endocrinology and Metabolism Research Center (EMRC) and the ethics committee of the Iranian Ministry of Health and Medical Education and informed written consent was obtained from participants.

Laboratory measurements

The peripheral blood was taken after 10-12 hours fasting. Serum following to centrifuge was aliquoted and stored at -80 ºC. All samples were run in the same assay. All measurements were performed in the EMRC laboratory of Shariati hospital. HbA1C was measured using HPLC exchange ion method (DS5 England), FBG by GOD/PAP method, triglyceride (TG) by GPO-PAP method, total cholesterol by Enzymatic Endpoint method, direct high-density lipoprotein-cholesterol by Enzymatic

clearance assay, all were done by using Randox laboratories kit (Hitachi 902). Serum hsCRP, a well-known marker of inflammation, was determined by immunoturbidometric assay (High sensitivity assay, by Hitachi 902).
OGTT was performed according to the World Health Organization standard protocol (17). After overnight fasting, the subjects were given a standard glucose solution of 75 gr glucose in 250 ml of water. Blood samples were taken after 120 minutes to measure plasma glucose concentrations by utilizing the GOD/PAP and Randox method laboratory kits.

Detection of -420C/G

Genomic DNA was extracted from whole blood, using FlexiGen Kit (QIAGEN Inc. Valencia, CA), according its protocol.
The -420C/G (rs 1862513) polymorphism in the 5′ flanking region of the RTN gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The region of interest was amplified by the PCR using the forward primer 5′-TGT CAT TCT CAC CCA GAG ACA- 3′ and the reverse primer 5′ -TGG GCT CAG CTA ACC AAA TC- 3′. The PCR was performed in a total volume of 20 containing 200ng genomic DNA, 0.5 pM of each primer, 0.2 mM dNTP, 2 mM MgCl2, 2µl of 10 X buffer and 1 U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania).
PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The resulting fragment was 534 bp in length. The PCR products were digested with 1 U BbsI (Fermentas, Vilnius, Lithuania) for 16 h at 37˚c using the recommended buffer. Then the digestion products were separated by 2.5% agarose gel electrophoresis stained with ethidium bromide and were visualized under ultraviolet.
Digestion of the PCR product produced two fragments, with lengths of 327 and 207 bp in the presence of the C homozygote, and three fragments (327,207 and 534 bp) for the heterozygote (CG), while G homozygotes remained uncleaved.

Statistical analysis

Numerical variables were reported as the mean ± SD and categorical variables were presented as percentage. All of the statistical analyses were performed using the SPSS version 15 software. Comparisons of quantitative variable between cases and controls were carried out using student's T-test. We used chi-square to compare the qualitative variables and ANOVA (Analysis Of Variance) to compare the quantitative variable in different genotypes. P-values less than 0.05 were considered to be statistically significant.

Results

This study consisted of 113 subjects which 79 (70%) were male. T2DM was presented in 42% (n=47) of all participants. The clinical characteristics of subjects are shown in table 1.
There were no significant differences in terms of age distribution and BMI between the cases and controls, but diabetic patients had significantly higher FBG, HbA1C and cholesterol levels than the control subjects.
Table 2 shows the genotype and allele frequencies for the RETN -420C/G among the diabetic and healthy subjects.
Genotype distribution for the RETN -420C/G among the diabetic and healthy subjects was different (P=0.009). We also noted that the GG genotype carriers had more than twofold risk for developing T2DM compared with the CC carriers.
The Odds ratio (OR) for diabetic patients with CC genotype was 3.78 (95% CI: 1.65-8.66) and the relative risk for patients with CC genotype was 2.99 (95% CI: 1.34-6.68, P= 0.009).
The C allele frequency was higher among the diabetic patients as compared with healthy subjects which was not significantly significant (P=0.13).
Table 3 shows the age of onset, Hb1AC and FBG according to RETN -420C/G genotypes in diabetic patients. Because of the lower

frequency of diabetic patients with GG genotype and different distribution of CC genotype in diabetic patients, the G allele carriers (CG and GG) were considered as a single group.

Our data showed that in patients with CC genotype, age of onset were lower compared with the G allele carriers. Furthermore, CC homozygotes had higher FBG and Hb1AC levels than carriers of the G allele, but no statistically significant association was found between RETN -420C/G polymorphism and noted parameters.

Table1. Clinical characteristics of study participants

Variables	Control subjects (n=66)	Diabetic patients (n=47)
Age(years)	58.7±8.7	58.5±9.4
BMI(kg/m^2)	27.7±5.0	28.8±6.2
FBG(mg/dl)	86±18	129±53
Total cholesterol(mg/dl)	171±71	218±39
Triglyceride(mg/dl)	148±52	166±94
hsCRP(mg/dl)	2±1	2±1
HbA1c (%)	5.36±0.71	6.88±1.73
HDL-cholesterol(mg/dl)	47±12	43±8

In cross – sectional study, comparisons of quantitative variable between cases and controls were carried out using student's T-test.
All variables are presented as mean±SD.
* P-values were significant (<0.05)

Table 2. Allele and genotype frequencies of RETN -420C/G polymorphism in control subjects and type 2 diabetic patients

ETN -420C/G	Control subjects (n=66)	Diabetic subjects (n=47)	Relative risk(95%CI)
CC	16(24.2%)	23(48.9%)	2.99 (1.34-6.68)
CG	41(62.1%)	16(34%)	0.31 (0.14-0.68)
CG	9(13.6%)	8(17%)	1.29 (0.46-3.66)
C-allele	73(55%)	62(66%)	1.56 (0.9-2.7)

In cross – sectional study, we used chi-square to compare the qualitative variables.
* P-values were significant (<0.05).
Percent of subjects is given in parentheses.

Table3. Age of onset, fasting glucose and HbA1c according to genotype in diabetic patients

Variables	CC (N=23)	GG+CG (N=24)
Age of onset(years)	50.56±5.6	54.5±7.77
FBG(mg/dl)	124±64	108±40
HbA1c (%)	7.41±2.03	7.07±1.57

In cross-sectional study , Comparisons of quantitative variable between CC genotype and GG+CG genotype were carried out using student's T-test. *P-values were not significant (>0.05)
All variables are presented as mean±SD.

Discussion

Recently, resistin has been reported as an important adipokine, may link obesity with insulin resistance and diabetes. Several of single nucleotide polymorphisms were associated with obesity, insulin resistance and diabetes.

Regarding influence of resistin on insulin signaling pathway and contribution of genetic variants of promoter region of RETN to expression level of resistin, study of promoter SNPs in RETN, can lead to recognition of role of resistin on pathogenesis of diabetes.

The RETN SNP -420C/G, is one of the most common studied polymorphism in the promoter region, and genotype distribution in this region is associated with regulation of resistin gene expression (18, 19).

This study was conducted as the first cross-study analysis of RETN in Iran. Among the diabetic patients, the frequency of CC genotype was twofold, in comparison to healthy subjects. Also, diabetic patients with CC genotype had higher fasting blood glucose and HbA1C in comparison with other genotypes, but these differences were not statistically significant.

Our results are in accordance with the findings of Ukkola et al. on Finnish non-diabetic and hypertensive subjects. Ukkola et al. revealed that subjects with CC genotype showed higher fasting blood glucose, HbA1C and LDL levels in comparison to other genotypes. Also, they showed that the subjects with CC genotype had the highest insulin resistance and hypertriglyceridaemia (20).

Also, Wang et al. reported the CC genotype of SNP -420C/G was associated with reduced insulin resistance in Caucasians (21). The results of these two investigation and our study provide evidence that the C allele in -420C/G resistin gene was associated with concerned phenotypes of obesity and diabetes.

In contrast, the results of other studies confirmed the GG genotype in -420C/G resistin gene was associated with high glucose concentration, insulin resistance and susceptibility to diabetes.

The findings of discrete studies in China (22), Japan (23, 24) and Quebec (11) showed that -420C allele is associated with high blood glucose and susceptibility to type2 diabetes. Despite this, the studies of Engert et al. in Scandinavia (11) and Norata et al. (25) in Italy showed no significant association between -420 genotypes and insulin resistance and diabetes.

In the present study, the patients with CC genotype had lower age of onset of T2DM in comparison with G carriers, but these differences were not statistically significant. In contrast, the findings of Ochi et al. in Japan showed the age of disease onset in patients with CC genotype was lower as compared with G carriers (26).

As previously noted, there is inconsistency between the results of different studies about involvement of genetic variations of resistin gene in promoter region in pathogenesis of insulin resistance and diabetes.

The contradictory findings in this study and others may attribute to several factors including the study design, sample size, ethnicity differences and age distribution of subjects. However, the interaction between resistin gene and other involved genes in etiology of diabetes and environmental factors could explain this discrepancy.

In conclusion, our results showed that CC genotype as compared with CG and GG genotypes was associated with increased risk of T2DM.

However, there were no significant differences between genotypes in -420 position of RETN with respect to fasting blood glucose, HbA1C and age of onset in diabetic patients. Thus, further studies with larger sample size are warranted to clarify the role of resistin gene in etiology of diabetes.

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