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Genetic Association of ADIPOQ Gene Variant (rs822396) with Type 2 Diabetes in Iranian Patients

Behnaz Hezarkhani, M.Sc.[1], Seyedeh Parisa Chavoshi Tarzjani, M.Sc [2], Zahra Sadeghi, M.Sc [2], Seyedeh Mohadese Shahzadeh Fazeli, M.D. [3], Zahra Mirzayee, B.Sc. [4] , Seyed Abolhassan Shahzadeh Fazeli, Ph.D.[5]

1- Departments of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran

2- Department of Genetic, Faculty of Biological Science, Tehran North Branch, Islamic Azad University, Tehran, Iran

3- Department of Medical, Alborz Medical University, Karaj, Iran

4- Fazeli-Sanati Genetics Laboratory, Tehran, Iran

5-Professor, Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran (Corresponding author: Email: shfazeli@yahoo.com)

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Abstract

Background: Genetic and environmental factors influence serum adiponectin and may contribute to the risk of metabolic syndrome and type 2 diabetes (T2D). There are many studies conducted to investigate the association between different polymorphisms of the *ADIPOQ* gene and T2D risk in all around the world. Therefore, the present study was conducted to investigate the association between adiponectin gene *(ADIPOQ)* polymorphism (rs822396) and the risk of T2D, which has not been studied yet in the Iranian patients.

Methods: Selected SNP was genotyped in 40 T2D patients and 40 controls by PCR amplification and direct Sanger sequencing. Fisher's exact test and Chi2 test were used to estimate the risk of T2D associated with the selected SNP, and genotypic and allelic distributions were compared between the case and control groups.

Results: It was revealed that there were no significant differences in the distribution of genotypes and allele frequencies of rs822396 between Iranian patients with T2D and controls.

Conclusion: According to the results, -3971 A/G polymorphism is unlikely to be involved in the susceptibility to or the severity of T2D in Iranian patients. Further large prospective studies are required to confirm these findings.

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Introduction

Type 2 diabetes mellitus (T2DM) is the predominant type of diabetes mellitus (DM), which is a complex metabolic disorder characterized by fasting or postprandial hyperglycemia with hereditary and environmental factors (1,2). T2DM comprises 90% of patients with DM in the world (3). Recent studies have reported that 9.5% of the US population has diabetes (4) and in South Asians, T2DM develops at younger ages (5). More than 100 loci have been found to be associated with T2D (6).

The major adipocyte secretory protein is adiponectin which is one of several adipocytes. This protein plays important roles in insulin sensitivity (7). Changes in adipose tissues cause alterations in metabolic and endocrine functions and secreting a variety of proteins that influence metabolism (8), including adiponectin, which are associated with T2DM (9). Adiponectin is an abundant secretory protein in plasma (0.01% of total protein) (2,10). One of the most important genetic factors determining the adiponectin level is the adiponectin gene (*ADIPOQ*) (11). The gene coding for adiponectin, *ADIPOQ* (also known as APM1, ACRP30 or GBP28), is located on chromosome 3q27, which consists of three exons and two introns (approximately 16 kb) (1-3, 12,13).

Adiponectin provides a critical link between visceral fat accumulation and insulin resistance (11,14). Circulating adiponectin levels are decreased in parallel with reduced insulin sensitivity, causing insulin resistance and T2DM (15). Recently, genome-wide association studies (GWASs) have indicated that genetic variations influence adiponectin levels, in addition to visceral fat accumulation (16).

The *ADIPOQ* gene is shown to be very polymorphic (3). A number of *ADIPOQ* single nucleotide polymorphisms (SNPs) and low circulating levels of adiponectin are significantly correlated with obesity, insulin resistance, and T2DM (12,17). In different populations, it has been suggested that *ADIPOQ* is one of the susceptibility genes for T2DM (2,18).

With this background, the present study was performed to investigate the genetic association of the *ADIPOQ* gene polymorphism (rs822396) with the risk of T2D in Iranian population.

Materials and Methods

In this case-control study, a total of 40 patients with T2D and 40 healthy individuals were included. Informed consent was obtained from all participants before sample collection. All the patients and controls were Iranian.

Whole blood was collected by venipuncture in tubes containing EDTA. Genomic DNA was extracted from peripheral blood using the NIGEB kit (Karaj, Iran). The concentration and purity of DNA samples were determined by spectrophotometric analysis.

The *ADIPOQ* gene polymorphisms (-3971A/G) were genotyped using DNA sequencing following PCR amplification. Table 1 shows the primer sequence used for detecting the selected SNP in the *ADIPOQ* gene.

The conditions for amplification were as follows: Initial melting step at 95˚C for 5 minutes, followed by 30 cycles of denaturation at 95˚C for 50 seconds, annealing at 60˚C for 50 seconds, extension at 72˚C for 50 seconds, and a final elongation step at 72˚C for 5 minutes.

Table 1. Forward and reverse primers characteristics

	Primer
ADIPOQ-F	TACAATCAGAGTCCGTTCTTGGTC
ADIPOQ-R	AGAAATGGGAAGAATCTGTGAGGC

The -3971A/G polymorphism in the *ADIPOQ* gene was performed by direct Sanger sequencing of the PCR products (Figure 1).

The allele and genotype frequencies amongst cases and controls were compared by Chi-square test and P-value. Statistical analyses were performed using SPSS software (SPSS Inc., Version 21, Chicago, IL, USA). The odds ratio (OR) with 95% confidence intervals (CI) were calculated to

evaluate the strength of the association between examined *ADIPOQ* polymorphism and T2D.

Results

-3971A/G (rs822396) polymorphism in patients with T2D (n=40) and controls (n=40) were genotyped. The mean age of patients with T2D and control group at the time of collection was 58.5 and 48.5 years, respectively. Patients with T2D consisted of 18 (45.0%) men and 22 (55.0%) women and control group consisted of 22 (55.0%) men and 18 (45.0%) women. All participants had Iranian nationality.

The amplified PCR products of the *ADIPOQ* gene were observed on 2% agarose gel electrophoresis (Figure 2), and then, were subjected to direct Sanger sequencing to determine polymorphisms, afterwards, were analyzed for genotypes using the FinchTV software (Figure 1).

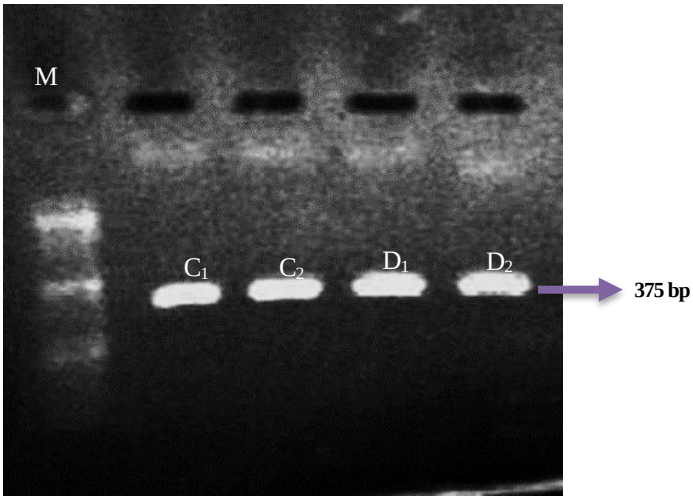

Figure 2. PCR amplification of *ADIPOQ* gene. Lane M represents DNA ladder (1500 bp); lane D_1 and D_2 represent patients with T2D; lane C_1 and C2 represent healthy control individuals.

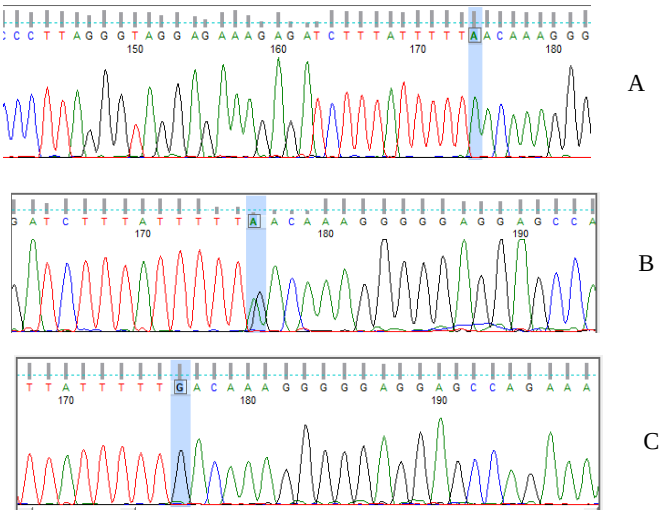

Figure 1. Sequencing results of the homozygous and heterozygous mutations of the *ADIPOQ* gene.

A) Homozygous AA. B) Heterozygous AG. C) Homozygous GG.

The genotypes and allele frequencies of *ADIPOQ* gene polymorphism are shown in Table 2. Genotypic frequencies of *ADIPOQ* gene polymorphism (AA/AG/GG) were observed at 47.5, 50.0, and 2.5% in patients with T2D and at 60.5, 35.0, and 5.0% in healthy controls, respectively.

The statistical analysis confirmed the non-significant association between T2D and rs822396 A/G polymorphism of the *ADIPOQ* gene (P=0.43) (Table 3).

Analysis showed that the most common genotype between case and control groups was AA genotype. The AG genotypes frequency of *ADIPOQ* gene polymorphism in the cases was higher than the controls, and the difference was not statistically significant (P=0.25) (Table 3). Allele A had a higher distribution in the case and control groups (A-allele= 75%, G-allele= 25%) so allele A considered as reference allele for statistical analyses (Table 2). The differences between patients and controls in allele and genotype frequencies of -3971 A/G were not significant (Table 3).

Table 2. Genotype and allelic frequencies of *ADIPOQ* gene polymorphisms in patients with T2D and controls

			Group		
			Case	**Control**	**Total**
Genotype		Count	19	24	43
	AA	% within Genotype	47.5%	60%	53.75%
		Count	20	14	34
	AG	% within Genotype	50%	35%	42.5%
		Count	1	2	3
	GG	% within Genotype	2.5%	5%	3.75%
Allele	**A**	Count	58	62	120
		% within Group	72.5%	77.5%	75%
	G	Count	22	18	40
		% within Group	27.5%	22.5%	25%

Table 3. Statistical analysis of rs822396 polymorphisms in patients with T2D and controls.

		P-value		C.I (95%)	OR
Genotype	**AA**	0.433[a]	Reference	-	1
	AG		0.202[b]	0.726-4.484	1.805
	GG		1.0[a]	0.053-7.502	0.632
Allele	**G**				
	A		0.465[b]	0.637-2.680	1.307
Sex			0.372[b]	0.278-1.615	0.67
Age			0.000018[c]	1.060-1.169	1.113

*OR, odds ratio; CI, confidence interval. a: Fisher's exact test, b: Chi2 test, c: Binary logistic regression

-3971A/G SNP had been never seen between Iranian while in the present study, it was observed in 3.75% of individuals.

Statistical analysis showed that there was no significant association between -3971A/G polymorphism (rs822396) and in Iranian patients with T2D (P>0.05). There was also no significant difference between sex and -3971A/G gene polymorphism in Iranian patients with T2D (P>0.05), but an association was observed between mentioned SNP and age (Table 3).

Discussion

Adiponectin is specifically secreted from adipose tissue, and its plasma levels are decreased during visceral fat accumulation. Decreasing adiponectin levels are suggested to be associated with the development of insulin resistance, obesity, and T2D (15). GWASs have shown that SNPs in the *ADIPOQ* gene have a significant association with adiponectin levels (11). In different populations, the -3971A/G polymorphism and its association with T2D have been studied excluding the Iranian population. According to this information, in the present study, it was focused on the study of the *ADIPOQ* gene.

In the present study, one SNP of the *ADIPOQ* gene was genotyped in a case-control study involving 40 patients with and 40 non-T2D controls to determine the contribution of the genetic risk of *ADIPOQ* gene variants towards the development of T2D in Iranian patients which has not been studied yet. It was demonstrated that -3971 A/G (rs822396) polymorphism in intron of *ADIPOQ* was not significantly associated with the risk of T2D in the Iranian patients, which is consistent with the results of a study by Chaikhiandee et al. (2016) in the Thai population (2). Kang et al. (2012) reported that there was no significant relationship between *ADIPOQ* gene polymorphism (rs822396) and diabetes (19).

In contrast, a previous study by Mtiraoui et al. (2012) showed an association between rs822396 A/G SNP and T2DM under the dominant models only, in Tunisian Arabs (20). Another study showed that -3971A/G rs822396 SNP association with T2DM was mediated through obesity in the south Indian population (12). In addition, Tso et al. (2006), did not find an association between -3971A/G rs822396 polymorphism and predicting glycaemic status in southern Chinese people (21).

And in US African-Americans and Whites, other SNPs in the *ADIPOQ* gene were found to be strongly associated with serum adiponectin concentrations (22).

The difference between the results of this study and other studies, may be due to ethnic variations and diversity in geographical and environmental conditions of the studied populations.

Also, there are studies which investigated the association of other SNPs in the *ADIPOQ* gene in Iranian population. The rs224766 polymorphism of *ADIPOQ* genes may be considered as genetic risk factors for coronary artery disease (CAD) in patients with T2D in Tehran Heart Center, Iran (23).

In Jahrom, a city in the southeast of Shiraz, Iran, no association was found between rs266729 polymorphism in adiponectin promoter gene and T2DM (24). A significant difference was found in the genotype frequency of 45T/G, but no significant differences were found in allele or genotype frequencies of 795A/G SNPs between Iranian patients with diabetes and healthy controls (25).

In the recent study, it was found that *ADIPOQ*-3971 A/G gene polymorphism was not associated with susceptibility to

T2D in Iranian patients with diabetes. It is a preliminary study which presents data for future comprehensive study for making a clinical conclusion.

In summary, the findings of the present case-control study suggest that rs822396 polymorphism in -3971A/G position of *ADIPOQ* gene was not associated with the development of T2D in the Iranian patients.

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Conflicts of interests

The authors declare that they have no conflict of interests.

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