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Original article

# Investigation of association rs266729 G/C ADIPOQ gene polymorphism with type two diabetes mellitus risk in Turkish population

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**Summary.** One of the most important health problem is the diabetes in our century. There are some specific risk factors for diabetes for example genetic factors, obesity, immobility and life style. Some risk factors can be change but some of them unchangeable like genetic factors. Detection of plasma levels of some hormones and proteins can be useful. One of the important hormone which is the very useful predictor of early detection of diabetes is adiponectin. ADIPOQ gene contains 3 exons and 2 introns sequence, specifically promoter region is highly polimorphic and two important SNPs were identified. These SNPs are rs17360539 and rs266729 and it was determined that this two SNPs is very important for the association with risk of type 2 diabetes mellitus. In this study, we have investigated whether rs266729 ADIPOQ gene polymorphism is associated with risk of type 2 diabetes mellitus in Turkish population. In this study we have collected peripheral blood samples from 50 type 2 diabetes mellitus patients and 50 healthy control individuals. Genomic DNA was isolated from blood samples and ADIPOQ gene polymorphism was investigated by polymerase chain reaction restriction fragment length polymorphism assay (PCR-RFLP). In our results, we have not observed any statistically significant association between ADIPOQ gene polymorphism and risk of T2DM.

**Key words:** type two diabetes mellitus, ADIPOQ gene, polymorphism

## Introduction

One of the most important health problem is the diabetes in our century. According to International Diabetes Federation data, there are more than 300 million people who diagnosed diabetes in the worldwide and 90-95 % of these cases are type 2 diabetes mellitus (1). There are some specific risk factors for diabetes for example genetic factors, obesity, immobility and life style. Some risk factors can be change but some of them unchangeable like genetic factors. Scientist try to find some molecular markers which can be used prediction of diabetes. Because of this, genome wide association studies (GWAS) and investigation of single nucleotide polymorphisms is very important.

Detection of plasma levels of some hormones and proteins can be useful. One of the important hormone which is the very useful predictor of early detection of diabetes is adiponectin (2).

Adipokines which are very heterogen and special members of a group of proteins. They modulate very important metabolic activities in human body such as regulation of insulin sensitivity in periferal tissues, regulation of energy expenditure and appetite, control of glucose and lipid methabolism, angiyogenesis and blood pressure (3-4).

Adipokine is a member of this protein (hormone) group and adipokine is coding by ADIPOQ gene which is set in chromosome 3q27. Adiponectine also called as adipocyte complement related protein 30

(ACRP30), or gelatin-binding protein 28 (GBP28). This hormone mainly secreted from adipocytes and composed of 244 aminoacids (5-8).

It was determined that, adiponectine suppress hepatic glukoneogenesis, increase glucose uptake and fatty acid oxidation in muscle cells which improves insulin sensitivity and decreasing the risk of type 2 diabetes mellitus (9-10).

ADIPOQ gene contains 3 exons and 2 introns sequences, specifically promoter region is highly polimorphic and two important SNPs were identified. These SNPs are rs17360539 and rs266729 and it was determined that this two SNPs is very important for the association with risk of type 2 diabetes mellitus (11).

Many studies have shown that this chromosome region is associated with some metabolic disorders for example obesity and diabetes. But some studies are not validated these results and showed some conflicting results. Because of this, it's necessary to validate these studies with bigger sample sizes (12).

In these study, we have investigated whether rs266729 ADIPOQ gene polymorphism is associated risk of type 2 diabetes mellitus in Turkish population.

## Materials and Methods

*Participants*

The study was approved by the ethics committee of Firat University Medical Faculty (ethics committee date/number 16.02.2016 / 04-05). A total of 50 patients with T2DM were consecutively recruited who met the criteria of World Health Organization and followed up in the Internal Medicine Department of the Firat University Hospital in Turkey. Age matched healthy volunteers consist of 50 individuals were randomly selected. Fasting plasma glucose < 6.1 mmol/L no medications which affect the glucose and lipid metabolism, and absence of systemic diseases and no family history for T2DM at first degree relatives were used as selection criteria for control group.

*Genotyping analysis*

Blood samples were collected from all the participants into the blood tubes which containing ethylene diamine tetra acetate (EDTA). DNA was extracted with commercially available genomic DNA isolation kit (Promega Corporation, Madison, WI) according to the manufacturer's recommendations. Concentrations of DNA samples was measured by Nanodrop UV spectrophotometer (UV-Visible NanoDrop 1000, Thermo Fisher Scientific Inc.) and all of the concentrations were adjusted to 50 ng $\mu L^{-1}$ and all DNA samples were stored at -20°C until analysis of the ADIPOQ polymorphism. The rs266729 single nucleotide polymorphism was genotyped by PCR restriction fragment length polymorphism (RFLP) with the following primers: 5'-GGT GGA CTT GAC TTT ACT GG-3' (forward) and 5'-TAG AAG CAG CCTGGA GAA-3' (reverse). The PCR conditions were: initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C for 30 seconds, 60°C for 40 seconds, 72°C for 40 seconds, a final extension of 72°C for 5 minutes. The PCR products were digested at 37°C for 16 hours with 5.0 U of HhaI restriction enzyme (Promega). After enzymatic digestion, PCR products were loaded in the 3% of agarose gel and visualized by SYBR Safe staining.

*Statistical analysis*

Statistical analyses were performed with SnpStats (web tools) (13). The genotype distribution was tested for Hardy-Weinberg equilibrium with chi-square (2) test in T2DM patients and controls. The student t-test was used to compare differences in the clinical characteristics between the T2DM and non-diabetic control groups, $p< 0.05$ was considered to be statistically significant. The distributions of rs266729 ADIPOQ gene polymorphism between T2DM patients and control groups were compared using the Fisher's exact test. $p< 0.05$ was considered significant.

## Results

We have studied rs266729 ADIPOQ gene polymorphism on T2DM patients in Turkish families. Clinical characteristics of subjects are summarized in Table 1. The statistical analysis showed that BMI (kg/m2), Creatinine (mg/dL), Urea (mg/dL), diastolic blood pressure (mmHg), systolic blood pressure (mmHg) levels of T2DM patients were significantly

higher than control group (p0.05) (Table 1). Totally, 50 subjects with T2DM and 50 healthy controls were enrolled in our study. In this study, the genotype distributions of all groups were found consistent with Hardy-Weinberg equilibrium. Genotypes and alleles frequencies of the rs266729 polymorphism of ADIPOQ gene in T2DM patients and controls are shown in the Table 2 and Table 3. In the T2D, GG genotype was found in 6 patients (12%), CG genotype in 20 (40%) patients, CC genotype in 28 (56%) patient and significant differences were noted in comparison with the frequencies in the genotype subjects ($p < 0.05$, Table 2). The frequency of the G allele in control group was 0.29, while C allele frequency was 0.71, and significant differences were noted in comparison with the frequencies between G and C alleles in control groups ($p < 0.05$, Table 3).

Restriction enzyme digestion agarose gel electrophoresis results were shown in Figure 1. After digestion with HhaI restriction enzyme, 334 bp PCR product do not digested and formed 334 bp single band in CC genotype (wild type). CG genotype formed 334 bp + 212 bp + 122 bp three band and GG genotype formed 212 bp + 122 bp two band.

## Discussion

In this preliminary study, we have compared type 2 diabetes group and healthy control group according to

**Table 1.** Demographic data of patients and control samples.

| Clinical characteristics | Patient | Control | p value |
|---|---|---|---|
| Sex (n) (Male/Female) | 20/30 | 13/37 | 0.614 |
| Age (years) | 58.20 ± 13.06 | 33.32 ± 12.88 | 0.754 |
| Duration of T2D (years) | 11.10 ± 7.78 | | |
| BMI (kg/m$^2$) | 29.66 ± 6.60 | 25.67 ± 6.38 | 0.018 |
| AST (U/L) | 23.36 ± 8.71 | 21.62 ± 4.64 | 0.962 |
| ALT (U/L) | 23.54 ± 9.11 | 20.72 ± 8.94 | 0.761 |
| Creatinine (mg/dL) | 0.83 ± 0.42 | 0.64 ± 0.15 | 0.041 |
| Urea (mg/dL) | 39.16 ± 18.06 | 24.98 ± 7.44 | 0.014 |
| Diastolic blood pressure (mmHg) | 70.50 ± 8.40 | 68.10 ± 8.10 | 0.023 |
| Sistolic blood pressure (mmHg) | 111.90 ± 11.64 | 105.90 ± 14.55 | 0.037 |

**Table 2.** Genotype frequencies of rs266729 ADIPOQ gene polymorphysm

**SNP1 genotype frequencies (n=100)**

| | All subjects | | STATUS=0-Control | | STATUS=1-Case | |
|---|---|---|---|---|---|---|
| Genotype | Count | Proportion | Count | Proportion | Count | Proportion |
| C/C | 55 | 0.55 | 27 | 0.54 | 28 | 0.56 |
| C/G | 37 | 0.37 | 17 | 0.34 | 20 | 0.4 |
| G/G | 8 | 0.08 | 6 | 0.12 | 2 | 0.04 |

**Table 3.** Allele frequencies of rs266729 ADIPOQ gene polymorphysm

**SNP1 allele frequencies (n=100)**

| | All subjects | | STATUS=0-Control | | STATUS=1-Case | |
|---|---|---|---|---|---|---|
| Allele | Count | Proportion | Count | Proportion | Count | Proportion |
| C | 147 | 0.74 | 71 | 0.71 | 76 | 0.76 |
| G | 53 | 0.26 | 29 | 0.29 | 24 | 0.24 |

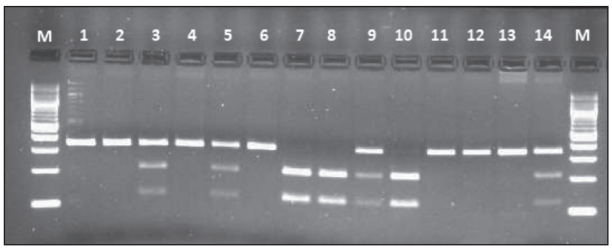


**Figure 1.** *HbaI* restriction enzyme digestion results of ADIPOQ gene PCR products. M: Marker, Lines 1-2-4-6-11-12-13 shows 334 bp CC genotypes, Lines 3-5-9-14 shows 334 bp-212 bp and 122 bp CG genotypes, Lines 7-8-10 shows 212 bp and 122 bp GG genotypes.

rs266729 single nucleotide polymorphysm situation via restriction fragment lenght polymorphism (RFLP) assay. After than we have analyzed our results with SnpStats which is a open source web tool for analyzing SNP data.

According to our analysis results, our sample population was consistent with Hardy-Weinberg equlibrium. But we have not detected any association with rs266729 SNP and risk of type 2 diabetes mellitus. In this SNP, CC genotype reflects ancestral allele and GG genotype reflects polimorphic allele. In our results, we have detected 2 % of GG genotype in the case group while 6 % of GG genotype detected in the control group. This results were shown that there is no any association between rs266729 ADIPOQ gene polymorphism and risk of diabetes.

Our results are inconsistent with results of some studies for example, Alkhateeb et. al., 2013 is investigated this SNP in Jordanian Arab population. They have used same PCR-RFLP technic and investigated 420 type 2 diabetes patients and 230 healthy control subjects for this SNP. According to their results, there is a strong association with the rs266729 ADIPOQ gene polymorphism and risk of type 2 diabetes (14).

Another study have been performed in Tunisian population via Mtiraoui et. al. 2012, and they have investigated 13 promoter polymorphism in the 917 type 2 patients and 748 healthy control subjects. They also performed Haploview analysis for describing linkage disequilibrium and their results have shown that there is an association between rs266729 ADIPOQ polymorphism and risk of type 2 diabetes.

In addition to this studies found association with rs266729 gene polymorphism and risk of type 2 diabetes in the some other ethnic populations. For example, Vasseur et. al. 2002 investigated this association in French Caucasians population and they also found very strong association. Another study is Harvest et. al. 2004. This study investigated this polymorphism in Swedish Caucasians population and they also found an association with this SNP and risk of type 2 diabetes mellitus (15).

But some other studies have found like our results. For example, Lin et. al. investigated this polymorphism in the Taiwanian population. They have used 137 case and 110 control subjects and investigated adiponectin serum levels and insulin resistance. According their results, they have not found any statistical significant relationship between this polymorphism and type 2 diabetes risk (16).

In another study Gable et. al. investigated that association between rs266729 gene polymorphism and risk of type 2 diabetes and cardiovascular diseases. Their results were shown that there is no statistically significant association between rs266729 gene polymorphism and type 2 diabetes. And they declare that ADIPOQ serum levels are not a good serum biomarker for prediction of cardiavascular risk and type 2 diabetes mellitus (17, 18).

In conclusion, we have investigated association with rs266729 ADIPOQ gene polymorphism and type 2 diabetes mellitus risk in Turkish population. We have not found any statistical significant association between this polymorphism and diabetes risk. Limitation of our study is small sample size, and this is a preliminary study and further functional studies are required for validation of the role of rs266729 ADIPOQ gene polymorphism and typ 2 diabetes risk in Turkish population.

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