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# ASSOCIATION OF T45G POLYMORPHISM OF ADIPONECTIN GENE WITH POLYCYSTIC OVARY SYNDROME IN IRAQI WOMEN

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## ABSTRACT

Adiponectin is a 244 – amino acid-long polypeptide, that is exclusively secreted by a dipocytes and acts as a hormone with anti-inflammatory and insulin sensitizing properties, the adiponectin gene contains 3 exons spans 16 kb on chromosome 3q27.45T/G polymorphism in exon 2 of the adiponectin gene which is associated with insulin resistance. The aim of the present study is to study the association of T45G polymorphisms of adiponectin gene with the risk of polycystic ovary syndrome. Fifty patients and Twenty five controls were enrolled in this study. Significance difference was found between genetic variation of adiponectin gene T45G SNP and the increased risk of PCOS in women Iraqi population. The distribution of T45G polymorphisms of adiponectin gene in PCOS patients was higher than in controls.

**KEYWORD:** Adiponectin, Polycystic ovary syndrome, 45T/G polymorphism.

## INTRODUCTION

Polycystic ovary syndrome is a multifaceted metabolic disease in women of reproductive age.[1] PCOS has a strong genetic component[2], and it is often associated with obesity and insulin resistance (IR).[3] Thus, identification of the susceptibility genes may offer better understanding of the molecular mechanisms underlying pathogenesis of PCOS and other related metabolic disorders.

Adiponectin is the most abundant adipocytokine and accounts for 0.01% of total plasma protein.[4] Adiponectin may have insulin-sensitizing and putative anti-atherosclerotic properties.[5] Both IR and adiponectin are important parameters in the development of

PCOS.[6] Therefore, it is necessary to investigate whether the genetic influence of adiponectin plays a role in the pathogenesis of PCOS. The adiponectin gene is encoded by ADIPOQ (adipocyte C1q and collagen domain containing), also known as adipose most abundant gene transcript1 (APM1), gelatin binding protein of 28 kDa (GBP28) or adipocyte complement-related protein of 30 kDa (Acrp30). This gene is located on chromosome 3q27. Genome-wide scan and linkage studies in this chromosomal region have revealed a susceptibility locus for obesity, type 2diabetes, and coronary heart disease.[7] In the recent years, several genetic association studies with metabolic disorders including obesity, type 2 diabetes, and PCOS have been conducted.[8,24] Interestingly, single nucleotide polymorphisms (SNPs) + 45 T/G in exon 2 of the ADIPOQ gene respectively, was found to be strongly associated with type 2 diabetes, obesity, and IR.[9,10] This one polymorphisms are also found to be associated with PCOS among European Caucasian women.[11,12] However, information about association of adiponectin genetic polymorphisms with PCOS in Iraqi women is still limited. In the present study, we have evaluated the genetic association between this polymorphism and PCOS in Iraqi women, which may provide evidence for better understanding of the susceptibility of the ADIPOQ gene variation in the development of PCOS.

## Subjects and methods

This study was conducted during the period from October 2014 to May 2015 in the University of Baghdad/Institute of Genetic Engineering & Biotechnology for post Graduate Studies. This study includes fifty patients with polycystic ovary syndrome with age range 16-45 years and Twenty five controls. The patients were selected from high institute for infertility diagnosis and assisted reproductive technologies, Al-Nahrain University. The patients with PCOS were diagnosed based on the presence of two out of three criteria of Rotterdam European Society for Human Reproduction and Embryology (ESHRE) including oligo- and/or anovulation, and clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries.

### Anthropometric measurements

Anthropometric measurements were done on the same person. Height and body weight were measured without shoes. Body mass index (BMI) was calculated as weight/height 2 kg/m2).

### Genotyping

T45G SNP T>G substitution at +45 in exon 2 (T45G) of adiponectin gene was chosen. Genotyping was carried out by PCR amplification of peripheral blood genomic DNA

extracted using Blood genomic mini spin kit (BioNeer)(Figure1) followed by restriction enzyme digestion as was used in previous study.[13] For T45G polymorphism analysis, DNA was amplified using the forward primer, 5′- GAAGTAGACTCTGCTGAGATGG- 3′ and the reverse primer, 5′- TATCAGTGTAGGAGGTCTGTGATG-3′. PCR was performed in a 25µl total volume, Primer forward 0.6µl (10PM), Primer reverse 0.6µl (10 PM), Template DNA 3 µl, (3- 6µg µl /) and 12.5 master mix. A total of 35 PCR cycles with denaturation at 94$^{o}$c for 25 sec, annealing for 40 sec at 61 $^{o}$C and extension at 72 $^{o}$C for 30 sec. were conducted. An initial DNA denaturation at 95$^{o}$C was carried out for 3 minutes and final extension at 72 $^{o}$C were carried out for 3 minutes each. 10 µl of amplified products was mixed with 0.4 *Sma*1 enzyme, 2ul of enzyme buffer and 7.6 free nucleases deionized distilled water then incubate for 3 hours in 25 $^{o}$C.[14] All enzyme digestion mixture was loaded to the well in 2% agarose gel stained with 0.5 µg /ml ethidium bromide, at 100 V for 15 min then 50 Volt for 50 min in 1 X TBE buffer. Then visualized under UV light using ultraviolet transilluminater A DNA ladder (100-1000) pb was used and the gel was photographed by a digital camera. The absence of polymorphism GG homozygote yielded the 372 bp uncut fragment only, the presence of polymorphism TG heterozygote yielded the 372, 216 and 156 bp fragments (Figure 2).

**Statistical Analysis:** The Statistical Analysis System- SAS (2012) was used to detect the effect of different factors in the studied of parameters. The least significant difference –LSD test was used for significantly comparing the means in this study.

## RESULTS AND DISCUSSION

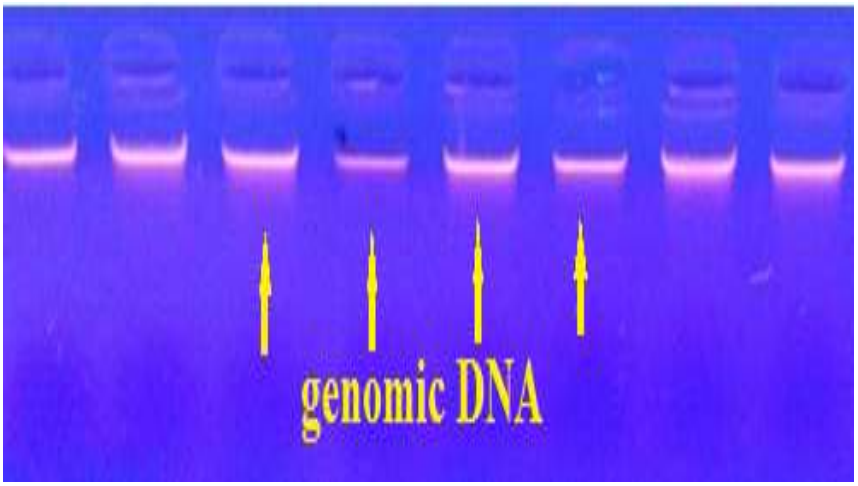


**Figure (1): Chromosomal DNA bands on 1.5 % agarose gel at 7 volt/cm for 60 min. DNA samples were extracted from some PCOS women.**

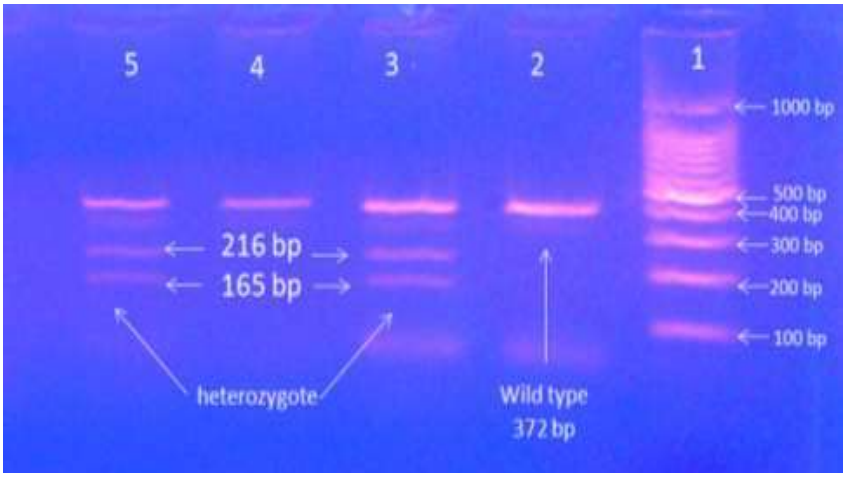


**Figure (2) PCR product digested with *Sma*1electrophoresis on 2% Agarose..Lane1: DNA ladder (100-1000). Lane2, 4: Wild type 372 bp TT genotype Lane 3, 5: heterozygote TG heterozygote 372, 216 and 156 bp. The (RFLP) products wererun on 2% agarose gel at 5 volt/cm$^2$ for 1hour.visulized under U.V light after staining with Ethidium Bromide.**

In the present study the association of T45G polymorphisms of adiponectin gene with the risk of PCOS was examined. The results of this study support the association between genetic variation of adiponectin gene T45G SNP and the increased risk of PCOS in Iraqi women. In this study there is a significant difference between + 45 T/G Polymorphism and PCOS. The distribution of T45G polymorphisms of adiponectin gene in PCOS patients higher than in controls (figure 3).

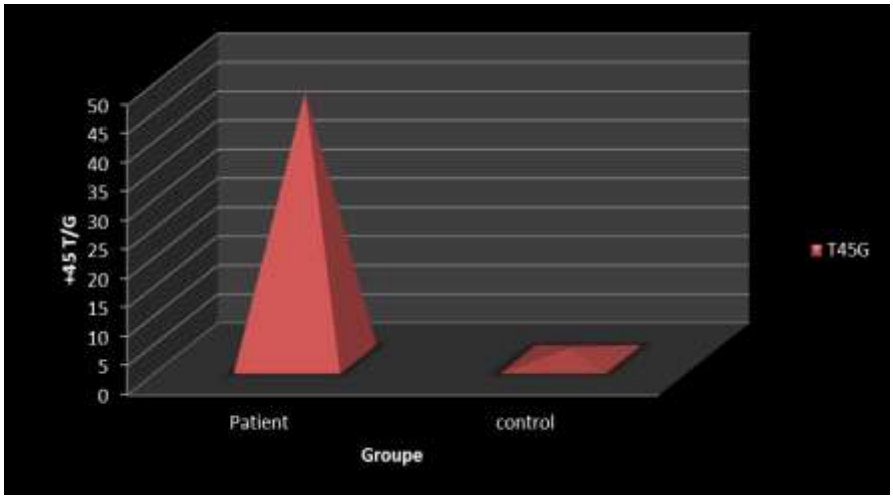


**Figure (3) Comparison between patients and control group:+45 T/G polymorphism. Furthermore individuals with the TG genotype in the patient group were significantly higher than those with the TT genotype (table1).**

**Table1: Comparison between TT and TG in patient and control**

| Mutation | No. and % | | P- value |
|---|---|---|---|
| | Patient 50 | Control 25 | |
| TT | 23 (46.00%) | 2 (8.00%) | 0.0026 ** |
| TG | 27 (54.00%) | 23 (92.00%) | 0.0019 ** |
| ** (P<0.01), NS: Non-significant. | | | |

The genetic variations in the adiponectin gene can affect the circulating adiponectin level and stimulation of adiponectin receptor that may affect the activity of adiponectin. This has been associated with insulin resistance, which is a risk factor for many chronic diseases. Insulin resistance is a prominent feature of PCOS independent obesity, insulin resistance with compensatory hyperinsulinemia are prominent features of the syndrome possibly genetically determined. The polymorphism of T45G SNP has been significantly related to the risk of developing PCOS in the studied population. Genome wide scans have mapped a susceptibility locus for type 2 diabetes, obesity and PCOS to the ADIPOQ gene.[15-16] The same result was obtained by other studies.[12-17- 18-19]

The difference in the results between different studies can be explained by that the genetics underlying of PCOS is multifactorial and complex in nature.[20] Multiple genetic and environmental factors contribute to individual risk of developing PCOS. Some have only a marginal and modest effect when considered individually, and the combination of these factors is responsible for disease risk.[21] Results of this study revealed that the differences between the genotypes and the BMI were not significant. These results have been obtained by other studies.[22-23]

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