# The Relationship Between SREBF-1 Polymorphism with Insulin Resistance in Women Polycystic Ovary Syndrome


## Abstract

It was reported a significant relation between 54G/C SREBF1 with diabetes. It may be a relation between SREBF-1 polymorphism with insulin resistant in women PCOS but this relation is unknown. This study was conducted to investigate the relationship between SREBF-1 polymorphism with insulin resistant in women polycystic ovary syndrome. In this study, 200 subjects were studied including 100 PCOS subjects (Case group) and 100 healthy subjects (control group). Blood samples were taken, demographic characteristics were requested and genomic DNA was extracted. To determine the mutations of 54G/C SREBF1, RFLP-PCR method was used. Following laboratory investigation, the data were investigated in SPSS software. The mutant frequency and its rate were determined by using SPSS software. The mutant allels rate was determined by analysis of variance. The genotypic frequency of GG and CC was significantly higher in patients than in the control group, while the frequency of heterozygosity was significantly higher in the control group than in the PCOS group. Allelic frequency was 78.00 for G allele and 22.00 for C allele in the patient group. In the healthy group, it was 75% for G allele and 25% for C allele. The results showed that homozygote genotypes had higher sensitivity for higher glucose and insulin resistant. In conclusion, 54G/C polymorphism of SREBF-1 had significant role in PCOS and also a closed relation with glucose and insulin resistant. It could be stated that G/C genotype frequency could be considered as biomarker for detection of PCOS.



## Keywords

Insulin Resistant [Mesh Link??];
PCOS [Mesh Link??];
Polymorphism [Mesh Link??];
SREBF-1 [Mesh Link??]

## Introduction

Polycystic ovary syndrome (PCOS) is a set of symptoms and diseases in which women have high amounts of androgens or male hormones [1]. This disease has many symptoms, including painful menstruation or no menstrual symptoms, acne, hairy body, skin disorders, mood problems, type 2 diabetes, heart failure, infertility, and endometriosis cancer [2, 3]. Polycystic ovary syndrome disease is an autosomal dominant genetic disease with a high penetrance coefficient, but its occurrence varies in different women and there is a probability that 50% of the children born will have symptoms of the disease [4]. The negative and extensive effects of this syndrome on the physiology and metabolism of the body lead to a metabolic disorder with visible abnormalities such as high blood insulin, insulin resistance, abdominal obesity and disorders related to blood fat, which in the long term, its prevalence rate increases [5, 6]. Insulin resistance as an important abnormality in patients with polycystic ovaries is due to the connection between inflammatory and insulin signaling pathways [7, 8]. Insulin resistance may increase steroidogenesis and LH release in individuals with polycystic ovaries through hyperinsulinemia [9]. Studies have shown that hyperinsulinemia caused by insulin resistance and hyperandrogenism have mutual causes [10]. Insulin resistance, as an important abnormality in patients with polycystic ovary, is due to the connection between inflammatory and insulin signaling pathways [7].

PCOS is a polygenic disease that is affected by several genes, but the genes involved in this disease have not been precisely identified, but the results of some studies indicate that different polymorphisms of the SREBF1 gene, which lead to the production of a protein linked to Regulation of sterol gene expression can play an important role in this disease [11]. The protein bound to the regulatory component of sterol gene expression is a transcription factor that binds to the gene sequence of the regulatory component of sterol gene expression [12]. In mammals, this protein is expressed by genes called transcription factor genes connected to the regulatory components of the sterol gene [13]. Inactive SERBs proteins bind to the nuclear envelope and endoplasmic reticulum, and in cells with low serum levels of sterol, enter the water-soluble N-terminal domain cleft inside the cell nucleus [14]. In fact, in this case, the activated SREBPs are connected to a specific region of the genome sequence and by affecting the upstream region of the said gene, which is responsible for the production of the enzyme involved in sterol production [15]. Today, several polymorphisms have been identified and introduced in relation to this gene, of which the C/G 54 polymorphism is one of the most important. It may be a relation between SREBF-1 polymorphism with insulin resistant in women PCOS but this relation is unknown. This study was conducted to investigate the relationship between SREBF-1 polymorphism with insulin resistant in women polycystic ovary syndrome.

## Materials and Methods

This case-control study was conducted on women with PCOS referring to clinical centers in Iran during 2021-2023 years. To determine the appropriate number of samples for this study, the sample volume calculation software was used by using the coefficients e, r and z in the calculation formula N=Z 2 R/E 2, with a confidence interval of 95%. 150 patients with polycystic ovary syndrome were selected during the diagnosis. The total number of samples was 150 people for control and 150 people for Case. First, a consent letter was obtained from the subjects, and then venous blood sampling was performed, 5mL of blood was taken from each subject and poured into vials containing EDTA as an anticoagulant, and then the vials It was gently shaken to mix and prevent the formation of blood clots. Finally, the samples were stored in the freezer. Demographic data were collected from patients. Blood samples were used to investigate polymorphism status in women with PCOS. The rest was used to investigate insulin resistant with the help of specific kits. Blood glucose between 140mg/dL and 199mg/dL is pre-diabetes and blood glucose level of 200mg/dL or higher is considered diabetes. To investigate insulin resistant, HOMA index was used and a value higher than 2.38 was considered as insulin resistant.

To investigate polymorphism, PCR was used. The primers were including 54(G/C) polymorphism of SREBF-1, F: TGAGGCTCCTGTGCTACTTTGCC and 54(G/C) polymorphism of SREBF-1, R: GGACAGAGCTGGGAGGTGAGAAG. Final volume was 34 µL, including, Beta PCR mix (30µL), 1µL of forward primer, 1µL of reverse primer, 0.5µL of DNA Polymerase Taq and 1.50µL of DNA Pattern. After performing the PCR reactions, the resulting products were electrophoresed on 0.2% agarose gel, then stained and imaged. After ensuring the functionality of the PCR products, the products of SREBF-1 polymorphic site 54(G/C) were cut using XmnI enzyme and the mutation frequency was

analyzed using the expected fragments. In the case of G nucleotide, the enzyme acts and cuts, and the sizes of 165 and 211 base pairs of nucleotides were observed. Therefore, the size of fragments in the C/C mode is 376 nucleotide base pairs, in the G/C mode, the size of the fragments was 211, 165 and 376. The size of fragments in the GG state will be 165 and 211 base pairs of nucleotides.
Kruskal-Wallis test was used to compare between groups. Analysis of variance method was used to compare the mean number of mutant alleles in the studied population. After completing the laboratory work, the expected and observed frequencies were calculated using the Hardy-Weinberg law, and entered into the SPSS software.

## Findings

*Demographic data*

The results showed that the mean age in the control group and the polycystic ovary syndrome group was 38 years. These results show that the mean age in both groups was similar. The minimum and maximum age in the control group was 28 and 44 years, respectively. However, in the minimum syndrome group, it was 29 years and the maximum was 45 years. The results showed that the mean body mass index in the control and syndrome groups was 28.10 and 27.56, respectively, with a standard deviation of 4.09 and 5.04. The minimum and maximum data in the control group was 21.20 and 37.65, while in the syndrome group it was 24.17 and 38.18, respectively. According to the deviation of the obtained criteria, it can be stated that there is no significant difference between the groups.

*PCR results*

Table 1 shows the results for allelic frequency in different groups. The genotypic frequency of GG and CC was significantly higher in patients than in the control group, while the frequency of heterozygosity was significantly higher in the control group than in the PCOS group. Allelic frequency was 78.00 for G allele and 22.00 for C allele in the patient group. In the healthy group, it was 75% for G allele and 25% for C allele.

**Table 1.** Genotypic frequency of 54(G/C) SREBF-1 polymorphism in healthy group (n=100) and patient group (n=100)

| Genotypes | Patient | Control | P-value | OR | C.I. 95.00% |
|---|---|---|---|---|---|
| **GG** | 62% | 54% | 0.002 | 0.97 | 0.55-1.52 |
| **GC** | 32% | 43% | 0.001 | 1.05 | 0.69-2.10 |
| **CC** | 5% | 3% | 0.001 | 0.69 | 0.35-1.35 |

*Glucose and insulin resistant*

We did not observe any insulin resistant and high glucose in healthy subjects. The results showed that homozygote genotypes had higher sensitivity for higher glucose. Thus, GG and CC are risky factors for higher glucose and GC is a protective genotype (Table 2). The results showed similar results for insulin resistant (Table 3). Insulin resistant was significantly higher in GG and CC compared with CC genotype.

**Table 2.** The relation between blood glucose and frequency of 54(G/C) SREBF-1 polymorphism in patient group (n=100)

| Genotypes | N | % | P-value |
|---|---|---|---|
| **GG** | 45 | 72% | |
| **GC** | 10 | 30% | 0.001 |
| **CC** | 2 | 40% | |

**Table 3.** The relation between insulin resistant and frequency of 54(G/C) SREBF-1 polymorphism in patient group (n=100)

| Genotypes | N | % | P-value |
|---|---|---|---|
| **GG** | 46 | 74.2% | |
| **GC** | 8 | 25% | 0.001 |
| **CC** | 4 | 80% | |

## Discussion

Polycystic ovary syndrome is a reproductive disorder that is characterized by various symptoms. The cause of the disease is not well known. Several hypotheses have been proposing to explain the cause of this disease. The genetic disorder that supports polycystic ovary syndrome is that different members of the same family may have differences in this respect, but about half of the girls in the same family are evaluated for serum testosterone concentration. However, the presence of

abnormal genes in women with polycystic ovary syndrome has been suggested, and attempts have been made to find the polymorphisms that cause it. The main genes involved in steroidogenesis, effects of steroid hormones, regulation of gonadotropin release, insulin secretion and action, and metabolism of adipose tissue are usually extensively evaluated [16, 17]. The results of some studies indicate that different polymorphisms of the SREBF1 gene, which leads to the production of a protein connected to the regulatory component of sterol gene expression, can play an important role in this disease. The protein bound to the regulatory component of sterol gene expression is a transcription factor that binds to the gene sequence of the regulatory component of sterol gene expression. In mammals, this protein is expressed by genes called transcription factor genes connected to the regulatory components of the sterol gene [18]. Inactive SERBs proteins bind to the nuclear envelope and endoplasmic reticulum, and in cells with low serum levels of sterol, to the water-soluble N-terminal domain cleft located inside the cell nucleus. The results of this study showed that there is a relationship between SREBF1 gene polymorphism and SNP 54G/C with polycystic ovary syndrome patients. The findings of the present study may be useful for early disease diagnosis and genetic treatment of patients with polycystic ovary syndrome. Studies have identified several genes related to polycystic ovary syndrome, especially those involved in metabolism, inflammation, and insulin signaling [19, 20]. According to the search, only one study in the world has discussed the relationship between polycystic ovary syndrome and SREBF1 gene polymorphism, and their results are in agreement with the findings of the present study. Li *et al.* [21] investigated the frequency of the polymorphism and its role in PCOS disease and concluded a significant relationship between the disease and 54C/G polymorphism. In fact, if the frequency of this polymorphism is high in people, especially women, the possibility of diseases such as polycystic ovary syndrome increases. In another study, Lee *et al.* [22] showed that the expression of SREBP-1 is increased in endometrial cancer cells and hyperplasia. Although other studies have not addressed the 54C/G polymorphism of the SREBP-1 gene and its relationship with polycystic ovary syndrome, a relationship between other polymorphisms and polycystic ovary syndrome has been reported. Ha *et al.* [23] investigated the pathogenesis of polycystic ovary syndrome and the environmental and genetic factors involved in its development and showed that different gene polymorphisms are involved in causing this disease and can lead to different pathological conditions. Chung *et al.* [24] investigated the relationship between genetic indices and the disease and evaluated the obtained data using meta-analysis. They concluded a significant relationship between polymorphism of the mentioned gene and polycystic ovary syndrome and these genetic indices are one of the determining factors in suffering from such diseases.

The results showed a significant relation between high glucose and insulin resistant with polymorphism. The role of SREBF-1 in type 2 diabetes mellitus has been reported, but its relationship with polycystic ovary syndrome as a diabetes-related disease has not yet been elucidated [25, 26]. Transgenic analysis of mice interferes with the role of SREBF-1 in the expression of genes involved in the metabolism of fatty acids and glucose [27]. In another study, the relationship between SREBF-1, obesity and type 2 diabetes was reported in English and French populations [28]. Overall, the results of this study accept that there is a difference between the genotypes of both populations and this shows that there is a significant difference between the SREBF-1 polymorphisms.

## Conclusions

In conclusion, 54 G/C polymorphism of SREBF-1 had significant role in PCOS and also a closed relation with glucose and insulin resistant. It could be stated that G/C genotype frequency could be considered as biomarker for detection of PCOS.

**Conflict of Interest:** The authors report no conflicts of interest in this work.

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