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*RESEARCH ARTICLE*

# Association Between *TP53* Gene Polymorphism and Obesity

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***Association Between TP53 Gene Polymorphism and Obesity***

***SUMMARY***

*Obesity is a chronic disorder with increasing prevalence worldwide and occurs when energy intake is greater than energy expenditure. Obesity is one of the factors that cause oxidative stress and arises from an imbalance between the reactive oxygen species (ROS) and the cell's antioxidant defense system. Increasing ROS in obesity, influencing the hypothalamic neurons, affects hunger and satiety control, so correspondingly on body weight control. When ROS amount increases, through DNA, protein and lipid oxidation, cell damage, necrosis, and apoptosis take place. Tumor protein p53, the guardian of the genome, is responsible for the regulation of genes involved in apoptosis as well as energy generating metabolic pathways. In our study, we investigated the TP53 (Arg72Pro) polymorphism in 151 patients diagnosed with obesity. TP53 mutation (rs1042522) was determined by real-time PCR. In 8 patients, the TP53 mutation was identified as carrying heterozygous (Arg72Pro) and in 143 patients carrying homozygous (wild type) (Arg72Arg). No individual with a homozygous mutant (Pro72Pro) genotype was found in the studied group. Associations between TP53 genotypes and clinical obesity parameters such as body mass index, thyroid stimulating hormon, glucose, postprandial blood sugar, triglyceride and cholesterol levels were compared statistically. According to the results of statistical analysis, it was observed that TP53 polymorphism was associated with insulin level. Genotype frequencies were also compared with previous studies performed in control populations and found to be different. This study shows that there may be a relationship between TP53(Arg72Pro) polymorphism and obesity.*

***Key Words:*** *Obesity, Oxidative stress, TP53, Polymorphism.*

***TP53 Gen Polimorfizmi ve Obezite Arasındaki İlişki***

***ÖZ***

*Obezite, alınan enerjinin, harcanan enerjiden fazla olmasından kaynaklanan, tüm dünyada prevalansı endişe verici şekilde artan kronik bir hastalıktır. Obeziteye neden olan etkenlerden biri olan oksidatif stres, reaktif oksijen türleri (ROT) ile hücrenin antioksidan savunma sistemi arasındaki dengesizlikten ortaya çıkar. Obezitede artış gösteren ROT'lar hipotalamik nöronlar üzerinde etkili olarak, açlık ve tokluğun kontrolünde ve buna bağlı olarak vücut ağırlığının kontrolünde etkili olurlar. ROT arttığında, DNA, protein ve lipitlerin oksidasyonu yoluyla hücre zedelenmesi, nekroz ve apopitoz oluşur. Genomun koruyucusu olan tümör proteini p53, enerji üreten metabolik yolların yanı sıra apoptozda yer alan genlerin düzenlenmesinden sorumludur. Çalışmamızda obezite tanısı almış 151 hastada TP53 (Arg72Pro) polimorfizmi araştırıldı. TP53 mutasyonu (rs1042522), gerçek zamanlı PCR ile belirlendi. 8 hastada TP53 mutasyonu heterozigot taşıyan (Arg72Pro) ve 143 hastada homozigot taşıyan (yabanıl tip) (Arg72Arg) olarak tanımlandı. Çalışılan grupta homozigot mutant (Pro72Pro) genotipine sahip birey bulunamadı. TP53 genotipleri ile vücut kitle indeksi, tiroit stimüle edici hormon, glukoz, tokluk kan şekeri, trigliserit ve kolesterol düzeyleri gibi klinik obezite parametreleri arasındaki ilişkiler istatistiksel olarak karşılaştırıldı. İstatistiksel analiz sonuçlarına göre TP53 polimorfizminin insülin düzeyi ile ilişkili olduğu gözlendi. Ayrıca genotip frekansları kontrol popülasyonlarında gerçekleştirilen önceki çalışmalarla karşılaştırıldı ve farklı olduğu bulundu. Bu çalışma, TP53 (Arg72Pro) polimorfizmi ile obezite arasında ilişki olabileceğini göstermektedir.*

***Anahtar Kelimeler:*** *Obezite, Oksidatif stres, TP53, Polimorfizm.*

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## INTRODUCTION

The prevalence and incidence of obesity, a public health problem that has gained importance recently, increases day by day. Obesity badly affects life expectancy (Mensah, 2004). Obesity both reduces the quality of life and shortens its duration. Because of that, diet, exercise and medical treatments have been applied, and the lack of success has become the focus of researches on hormones, mediators and genes that may be the source of surgical interventions (Bertakis&Azari, 2005). Obesity is accepted as an increasing disease in the world and in our country, which occurs as a result of the interaction of genetic and environmental factors. Obesity is the result of the body's fat mass to lean mass, and the body weight is higher than the expected level according to the height fit. Body Mass Index (BMI) is classified as 25-29,9 kg/$m^2$ overweight, 30-34,9 kg/$m^2$ obese, over morbidity obesity according to the World Health Organization (WHO) (Aydemir, 2006). BMI can be easily calculated by dividing body weight in kilograms by the square of the neck in meters (Body weight / height$^2$) and its unit is kg / $m^2$. BMI can be easily calculated by dividing body weight in kilograms by the square of the neck in meters (Body weight/height$^2$) and its unit is kg/$m^2$. Obesity is increasingly becoming an epidemic problem. The MONICA study carried out by WHO in 6 different regions of Asia, Africa and Europe and lasting for 12 years, it was reported that an increase in the prevalence of obesity between 10 and 30% in 10 years (Silventoinen, 2004). According to WHO, it is estimated that there are over 1.9 billion overweight and 650 million obese adults worldwide in 2016. The risk of cardiometabolic disease increases significantly in obese and overweight patients. Obesity, even if carbohydrate metabolism is normal, endothelial dysfunction, dyslipidemia, hypertension (HT) and vascular inflammation may develop due to insulin resistance and increased adipokines. All these pathogenetic changes contribute to the development of atherosclerosis. The atherosclerotic process becomes more severe and accelerated with the decline of carbohydrate metabolism. The frequency of cardiometabolic diseases and other systemic problems increases in proportion to the severity and duration of obesity. These accompanying diseases with obesity increase the risk of developing complications and causes some difficulties in the process of regulation of treatment (Artham, 2009). The International Cancer Research Agency announced the relationship between obesity and many types of cancer in 2002. Particularly noteworthy cancers are colon, postmenopausal breast, endometrial, kidney and esophageal cancers. A cohort study containing 900,000 cases in the USA showed the contribution of obesity to 11% in postmenopausal colon cancer, 9% in breast cancer, 39% in endometrial cancer, 25% in kidney cancer, and 37% in esophageal cancer. It has also been shown that the risk of cancer increases as the degree of obesity increases (Calle et al, 2004; Birmingham, 2009). The mechanism of the relationship associated with obesity is multifactorial. Increasing insulin activates the IGF-1 pathway, causing an increase in cancer cells. Also, adipocytokines are thought to play a role in the mechanism. Colon, prostate and breast cancer leptin levels positive with endometrial, breast, colon and prostate because there is a negative correlation between cancer. Also, those associated with obesity are hypoxia, genetic predisposition and increased inflammation are also accused factors in the obesity-cancer relationship. Obese cases should be followed up in terms of cancer risk besides metabolic diseases and they should be supported about this issue (Hursting, 2010; Basen-Engquist, 2011).

Although oxygen is essential for human life, it is produced during normal metabolism. Some types of reactive oxygen have the potential to intense harm to the body (Diplock, 1998). Reactive oxygen species (ROS), mostly formed by free radicals, with normal oxygen molecules are oxygen forms with higher chemical reactivity (Nawar, 1996). It is well known that ROS increases obesity. ROS act on hypothalamic neurons effective in controlling hunger and satiety and, consequently, body weight. The increase of

ROS also causes cell damage, necrosis and apoptosis through the oxidation of DNA, proteins and lipids (Buyukuslu & Yigitbasi, 2015). The unpaired electrons in free radicals give them huge reactivity that damage protein, lipid, DNA and nucleotides. This harm promotes aging to these components of the body and causes degenerative diseases such as, cardiovascular diseases, various types of cancers, cataracts, weakened immunity and nervous system disorders. (Diplock, 1998). Oxygen metabolism in living cells, environmental pollutants, various factors such as radiation, pesticides, various medical treatments, and contaminated waters are inevitably led to the formation of free radicals: single oxygen ($O_2$), superoxide anion ($O_2$), hydroxy (OH), peroxy (ROO) and alkoxy (RO) radicals (Kaur&Kapoor, 2001). Different natural defense systems in the body keep free radicals under control against the damages of reactive oxygen species. These systems are found in different cells and prevent oxidation caused by free radicals. The substances having the ability to capture and stabilize free radicals are so-called "antioxidants" (Nawar, 1996; Diplock, 1998). Enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase, vitamins such as vitamin C and vitamin E, and compounds such as uric acid, bilirubin and polyphenols are well known antioxidants and generally responsible for limiting free radicals damaging cellular components such as DNA, proteins and lipids (Diplock, 1998; Elliot, 1999; Ou, 2002). Oxidative DNA damage is important in the pathogenesis of many diseases, especially carcinogenesis. It is known to play a role. High reactivity having hydroxyl radicals on oxidative stress and intracellular structures as in lipids and proteins as it is said, H atom to double bonds in DNA bases by adding or from the C-H bonds of 2-deoxyribose and H atom from methyl groups in thymine structure it reacts with the DNA molecule (Breen, 1995). The thymine peroxyl radicals formed are reduced and oxidation products such as hydroxy hydroperoxides, thymine glycol, 5-hydroxy methyl uracil, 5-formyl uracil and 5-hydroxy 5-methyl hydantoin. Hydroxyl radicals (OH-) interact at the 8th position in the guanine molecule, leading to oxidation. Undergoing as a result of oxidative damage of DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG) has formed. Besides, $Cu^{+2}$ ions have a high affinity for DNA, especially connected guanine bases. By interacting with $H_2$ and $O_2$, they contribute to DNA damage. DNA adduct 8-OHdG is the most known marker of oxidative DNA damage. (Helbock, 1999). Oxidative stress occurs as a result of the disruption between the formation of ROS and the inactivation of these products by the antioxidant defense system. Adipose tissue is one of the main sources for the formation of ROS, and fat accumulation is closely related to increased oxidative stress through NADPH oxidase activation.

P53 is a transcription factor that regulates the cell cycle (Ngo et al. 2010). A central role in the p53 cell cycle is an important tumor suppressor that plays, it acts as a transcription factor. DNA damage, hypoxia, oxidative stress, oncogene cellular activation, such as telomere erosion after the stress signals p53 is activated. TP53 targets genes in cellular aging, angiogenesis and it also plays a role in autophagy (Vousden, 2009; Bieging, 2014). P53 is a powerful tumor suppressor. P53 protein in more than 50% of cancers inactivated due to TP53 gene mutation state. TP53 gene mutation its prevalence is different in various cancers (Leroy, 2014). P53 is shown to be responsible for poor prognosis in most studies (Sheikh, 2003). The *TP53* gene encoding p53 localized in chromosome 17p13.1 is the most common target of genetic change in human tumors. A little over 50% of tumors carry a mutation in this gene. Almost every cancer, including lung, colon, and breast carcinomas, which are the three leading causes of cancer-related deaths, have a homozygous loss in *TP53* gene activity (Baselga & Norton, 2002). Physiological p53 protein has a role in stopping the cell cycle due to DNA damage and apoptosis, and mutation in the *TP53* gene is the most common single gene mutation in human cancers (Cross et al. 1995). Publications are reporting that the presence of mutant p53 protein is associated with poor prognosis in many cancers such as lung, breast, prostate and blad-

der cancers (Quinlan et al. 1992; Moul, 1999). It is known that p53, which plays a suppressor role, has an important role in preventing cancer formation mechanisms (Oguztuzun, 2016).

Obesity accompanying morbidities decreases lifetime and quality. In the USA, 11869 nurses were followed up for 13 years, and an increase in cardiovascular and cancer-related deaths was detected in the fatter group. Overweight women, even within normal limits, have a higher risk of coronary heart disease than those who are not overweight (Kopelman & Stock, 2000). In 8800 men who have been followed up for twenty-six years, the mortality rate due to all causes was 2 times higher in obese and 3.3 times higher due to coronary heart disease (Kırım S., 2005). In a prospective research study in nine hundred thousand men and women who examined cancer-related deaths during the 16-year follow-up period, a positive correlation was found between overweight and mortality due to many types of cancer (Calle et al. 2003). Although it is known that genetic factors are effective in both disorders, the information obtained through intense studies to date still cannot fully explain their genetic basis.

Therefore, in this study it is aimed to reveal the polymorphic condition in the *TP53* gene in obese patients. Besides, it was aimed compare the clinical data of the patients whose *TP53* polymorphic states were revealed in obesity.

**MATERIALS AND METHODS**

The study group was consisted of 151 obese patiens who underwent bariatric surgery in Ankara Keçiören Training and Research Hospital General Surgery Service in 2017. Ethics committee approval of this study was provided by the decision of the Ethics Committee of Keçiören Education and Research Hospital with the decision numbered 2012-KAEK-15/1160.

Blood samples were collected from patients participating in the study after their informed consents. The diagnosis, all information available for the operation of obesity, and appropriateness of the blood samples collected were made at the same hospital service. The patients consisted of 23 men and 128 women. The average age of the patients was 39, the average BMI of the patients was 46.5 kg/m2, the average of the TSH levels was 2.5, the average of the insulin levels was 19.5, the average of glucose levels in the blood was 111, the average of postprandial blood sugar levels was 137.6, the mean of triglycerides was 176, and the cholesterol level averages was 215.6.

DNA isolation from blood samples collected was performed by adhering to the PROMEGA® blood DNA isolation kit protocol. *TP53* genetoyping was performed by real time PCR LightCycler® 480 device. Primers and probes specified in the method of Talseth et al. (2006) were used to determine the genetic polymorphism (rs1042522) in the *TP53* gene (Arg72Pro) encoding the p53 protein. Sequences of primers and probes are shown in Table 1. In order to confirm the efficiency of the primers used in the study and the base size of the studied gene region, the primers were controlled by a 1.5% electrophoresis gel study after conventional PCR. Base sizes were compared for *TP53* using the National Center for Biotechnology Information's (NCBI) international database. During the real time PCR stage, the testing phase of the study was carried out by using FastStart Essential DNA Probes Master (06402682001), Lightcycler 480 Multiwell Plate 96 (04729692001) and Lightsnip *TP53* probe (07330782001) (Roche Applied Science). Compopnents used in the PCR stage are as follows; 12.5µL from 2x OneTaq Quick LoadMaster Mix solution, 1µL from Forward primer (10pM), 1µL from Reverse primer (10pM), 2.5µL from cDNA (50ng/µL), 2.5µL probe, and DNAse/RNAse free water calculated for in a 25 µL total volume. PCR conditions were performed as follows; Initial Denaturation is 5 minutes at 95°C, 50 cycles of denaturation at 95°C for 10 seconds, Annealing at 58°C for 20 seconds and Extension at 72°C for 20 seconds. 1 cycle, 5 seconds at Acquisition 95°C and 1 minute at 55°C. 10 seconds at Final Extension 40°C.

**Table 1.** Primers and fluorescent probes used in the study.

| *Primer/Probe* | *Sequence* |
|---|---|
| Forward primer | 5'-CCAGATGAAGCTCCCAGAATGC-3' |
| Reverse primer | 5'-GCCGCCGGTGTAGGA-3' |
| Wildtype probe | 5'-VIC-TCCCCGCGTGGCC-3' |
| Mutant probe | 5'-FAM-CTCCCCCCGTGGCC-3' |

The definition of *TP53* R72P polymorphism using the allelic discrimination method was as follows; Samples with a peak at 58°C were interpreted as wild type (G/G) and samples with a peak at 66°C were interpreted as mutant type (C/C). Samples with both degrees of the peak in each same sample were evaluated as (G/C) Heterozygous. One representative sample from each of the three genotypes was included as the reaction control for each "PCR run" as an internal control. (Figure 1).

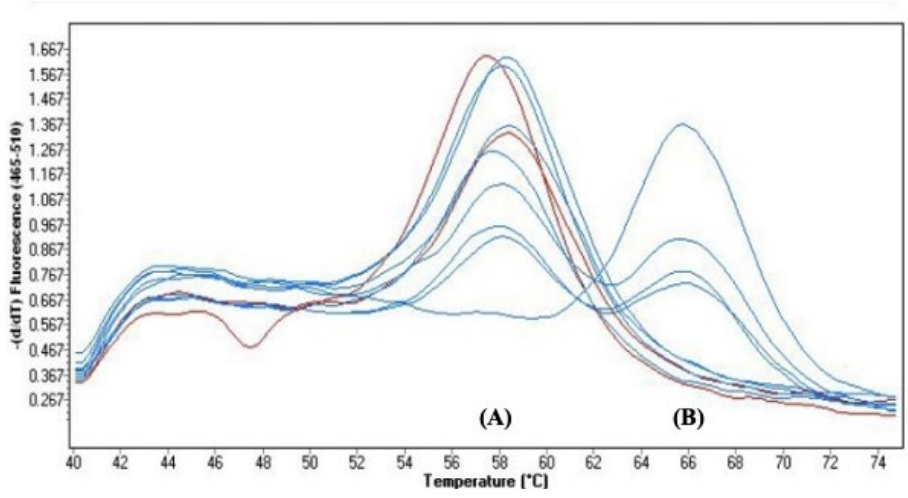


**Figure 1.** Allelic discrimination of *TP53* (Arg72Pro) polymorphism.

Identification of *TP53* (Arg72Pro) polymorphism by allelic discrimination Overview of a PCR reaction performed using the method. (A): General view of Wild Type (Arg/Arg) samples peaking at 58°C. (B): View of Mutant (Pro/Pro) internal control with a peak at 66°C. (A&B): General view of Heterozygous (Arg/Pro) samples with a peak at both degrees.

Clinical data of patients from whom samples were obtained were also compared with cross statistical data and interpreted by comparing their significance with real time PCR data. Statistical Package for the Social Sciences (SPSS) package software ANOVA analysis were used to compare the clinical data of the patients with the cross-data of the results.

## RESULTS AND DISCUSSION

In our study, *TP53* genetic polymorphism in patients diagnosed with obesity were investigated and their relationship to the disease was interpreted. Genotype distributions of 151 obese patients were determined as *TP53* mutation carrying heterozygous (Arg-72Pro) in 8 patients and homozygous (wild type) carrying (Arg72Arg) in 143 patients. No individual with a homozygous mutant (Pro72Pro) genotype was found in the studied group. The clinical parameters and genotypes of *TP53* mutation, heterozygous and homozygous obese patients were compared. As seen in Table 2; after statistical analysis, it is seen that the polymorphism in *TP53* is related to the insulin parameter. It

has been observed that the insulin level is higher in heterozygous individuals than in homozygous individuals. In obese patients, no statistically significant relationships were found between BMI, TSH, glucose, postprandial blood sugar, triglyceride and cholesterol levels. In obese individuals, significant changes occur in *TP53* polymorphism. While these changes may occur as an adaptive response, the findings suggest that the oxidative stress observed in obesity may be one of the possible mechanisms underlying this change. Besides, it is supported that *TP53* variant genotype can contribute to insulin changes. Accordingly, the insulin level made a difference in heterozygous and homozygous obese individuals. The level of insulin was higher in heterozygous individuals than in homozygote individuals. But other parameters were not related. This result is in line with the study of Bonfigli et al., (2013) who found that (Arg72Pro) polymorphism was associated with insulin resistance in type 2 diabetic subjects.

**Table 2.** Correlation between blood parameters and *TP53* polymorphisms.

| | *Variance Analysis* | | *ANOVA* | |
|---|---|---|---|---|
| *TP53* | *Levene Statistic* | *Sig.* | *F* | *Sig.* |
| BMI | 0.821 | 0.366 | 0.021 | 0.886 |
| TSH | 1.358 | 0.246 | 1.753 | 0.187 |
| Insulin | 67.629 | **0.0001** | 14.311 | **0.0001** |
| Glucose | 0.334 | 0.564 | 0.120 | 0.730 |
| Blood sugar (postprandial) | 0.590 | 0.444 | 0.330 | 0.567 |
| Trigliserid | 0.330 | 0.566 | 0.004 | 0.949 |
| Cholesterol | 0.147 | 0.702 | 0.011 | 0.916 |

When we compare the results of our study with previous studies conducted for (Arg72Pro) polymorphism, we observed that there are significant differences between genotype frequencies of obese individuals in our study group and genotype frequencies of healthy individuals in "Caucasian" populations (Table 3). Although these comparative studies were studies investigating gastric tissue differentiation, the control groups were completely composed of healthy individuals. This finding shows that *TP53* (Arg72Pro) polymorphism might be associated with obesity. Our findings can also contribute to the definition of the physiopathology of obesity, and other diseases such as cardiovascular diseases that may develop due to obesity. It is important in preventing complications and developing preventive approaches.

**Table 3.** The distribution of *TP53* genotypes in Caucasian control populations and in this study.

| *Study (Reference)* | *Country/ Region* | *Ethnicity* | | *Arg72Arg* | | *Arg72Pro* | | *Pro72Pro* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Total n* | *n* | *%* | *n* | *%* | *n* | *%* |
| Capella et al. (2008) | Europe | Caucasian | 1056 | 588 | 56 | 399 | 37.8 | 69 | 6.2 |
| De Feo et al. (2009) | Italy | Caucasian | 295 | 169 | 57.3 | 102 | 34.5 | 24 | 8.2 |
| Belyavskaya et al. (2006) | Russia | Caucasian | 125 | 60 | 48 | 46 | 36.8 | 19 | 15.2 |
| Alpizar-Alpizar et al. (2005) | Spain | Caucasian | 47 | 26 | 55.3 | 17 | 36.2 | 4 | 8.5 |
| Zhang et al. (2003) | UK | Caucasian | 277 | 125 | 45.1 | 129 | 46.5 | 23 | 8.4 |
| Sul et al. (2006) | USA | Caucasian | 134 | 51 | 38.1 | 61 | 45.5 | 22 | 16.4 |
| Engin et al. (2011) | Turkey | Caucasian | 108 | 52 | 48.1 | 42 | 38.8 | 14 | 13.1 |
| **This study** | **Turkey** | **Caucasian** | **151** | **143** | **94.7** | **8** | **5.2** | **0** | **0** |

## CONCLUSION

In conclusion, regarding the comparative evaluation of the results of the *TP53* polymorphism state obtained in our study with similar studies in the literature, it has been determined that it may be associated with obesity, but further studies are needed to verify this relationship. It was thought that investigating the association of obesity and *TP53* (Arg72Pro) polymorphism in different ethnic groups and larger populations would benefit the emergence of the relationship with metabolic diseases related with obesity. To our knowledge, *TP53* polymorphism was investigated in obese patients for the first time in a Turkish population. In our study, it is hoped that it will be a reference for revealing the genetic backgrounds of obesity, clarifying the molecular mechanism of the disease and developing genetic risk panels for early diagnosis and making this information available in the management of treatment.

## CONFLICT OF INTEREST

All the authors of this article declared no conflict of interest.

## AUTHOR CONTRIBUTION STATEMENT

Designing the concept and drafted the manuscript (MC, HB and SO). Preparing the figures (SO, OD). Ethical approval and sample collection and clinical data (MC, HB, DÖ, and AÜ). Carried out the laboratory applications of this study (MC, OD). AOA and OD reviewed the existing journal policy. Contributing to the writing of the final version of the manuscript (MC, HB, OD, SO, DÖ, AÜ, AOA, Mİ).

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