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TP53 (RS1042522) POLYMORPHISM IN BREAST CANCER

ÖZGÜN ARAŞTIRMA

MEME KANSERİNDE TP53 (RS1042522) POLİMORFİZMİ

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ÖZET

Amaç: *TP53 geni temel olarak DNA tamiri, apoptozis, hücre yaşlanması ve hücre döngüsü kontrolünde görev alan en önemli tümör baskılayıcı genlerden biridir. TP53 rs1042522 (Arg72Pro) polimorfizmi tümör baskılama sırasında P53 protein yapısında değişikliğe neden olan bir polimorfizmdir. Bu verilere dayanarak, bu çalışmanın amacı TP53 rs1042522 polimorfizmi ve meme kanseri riski arasındaki ilişkiyi araştırmaktır.*
Yöntem: *TP53 rs1042522 polimorfizmi için 508 meme kanserli kadın hastadan ve 367 sağlıklı kadından alınan periferik kanlardan DNA izole edilerek PCR-RFLP yöntemi ile genotipleme yapıldı. İstatistiksel analiz, %95 güven aralığında χ2 testi ile yapıldı ve Hardy-Weinberg eşitliği (HWE) test edilen hastalar ve kontrol popülasyonu için doğrulandı.*
Bulgular: *Genotip frekansları sırasıyla hasta ve kontrollerde GG alleli için %48.6, %46.3, GC alleli için %40.7, %44.7ve CC alleli için de %10.6, %9.0 şeklindedir. Vaka ve control genotipleri arasında istatistiksel olarak fark olmadığı bulundu (χ2=1.591, P= 0.451). Allel frekansı G alleli için vakalarda %69.0 ve kontrollerde %69.0, C alleli için vakalarda %31.0 kontrollerde %31.0 şeklinde ortaya çıktı. Sonuçlar istatistiksel olarak anlamsız bulundu (G allel: p=0.424, C allel: p=0.501). TP53 rs1042522 genotip dağılımı kontrol popülasyonu için Hardy-Weinberg eşitliğine göre kararlı bulundu (p>0.05).*
Sonuç: *Çalışmamızda meme kanseri ile p53 geninde yer alan rs1042522 polimorfizminin tek başına değerlendirildiğinde meme kanseri riski ile ilişkisi olmadığı bulunmuştur. P53'ün karsinogenezdeki rolünden ve özellikle de programlı hücre ölümünden sorumlu çeşitli proteinlerle etkileşime girmesinden dolayı rs1042522 polimorfizmini diğer proteinlerdeki değişimlerle birlikte çalışmak daha anlamlı olabilir. Ayrıca, farklı TP53 polimorfizmleri ile hücre döngüsünde görevli siklin, sikline bağımlı kinazlar ve p21 gibi genlerin ortak etkilerine odaklanılabilinir ya da bu polimorfizmin daha geniş bir hasta popülasyonunda tümörlerin klinikopatolojik özellikleri ile birlikte değerlendirilmesi daha anlamlı sonuçlar verebilir.*

Anahtar Sözcükler: P53 geni, rs1042522, polimorfizm, meme kanseri

ABSTRACT

Objectives: *TP53 gene is one of the most important tumour suppressor gene that mainly plays a role in DNA repair, cell apoptosis, cell senescence and cell cycle control. TP53 rs1042522 (Arg72Pro) polymorphism is nonsynonymous polymorphism that alters P53 protein capacity during tumour suppression. Based on the data above, the purpose of this study is to investigate association between TP53 rs1042522 polymorphism and breast cancer risk in hospital-based Turkish women population.*
Methods: *After DNA isolation from peripheral blood of 508 breast cancer patients and 367 healthy women the genotyping for TP53 rs1042522 polymorphism in both groups was done by a PCR-RFLP method. Statistical analysis was done by the χ2 test with 95% confidence intervals and the Hardy-Weinberg equilibrium was confirmed for tested patient and control populations.*
Results: *Genotype frequencies were 48.6%, 46.3% for GG, 40.7%, 44.7% for GC and 10.6%, 9.0% for CC in breast cancer and controls, respectively. There was not statistical difference between genotypes in cases and controls (χ2=1.591, P= 0.451). Allele frequencies for G allele were 69.0% in cases and 69.0% in controls, for C allele 31.0% in cases and 31.0 % in controls. Also, this results were found to be not statistically significant for G and C alleles (G allele: p=0.424, C allele: p=0.501). The distribution of TP53 rs1042522 genotypes was in agreement with HWE for controls (p>0.05).*
Conclusion: *There is no statistically significant association between rs1042522 and the risk of breast cancer in studied hospital- based Turkish women population. For further, rs1042522 polymorphism may be associated with other genetic factors due to the interaction of p53 with various proteins that play role in programmed cell death. Also, the combined effect of TP53 polymorphisms and the genes such as cyclins, cyclin-dependent kinases and p21 which are related with cell cycle control can be studied or clinicopathological characteristics of breast cancer patients might be evaluated in terms of p53 rs1042522 genotypes in larger population size.*

Keywords: TP53 gene, rs1042522, polymorphism, breast cancer

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Introduction

Breast cancer is the most common cancer type in women worldwide with an incidence more than one million and 41.000 as a death rate in 2012[1,2]. In Turkey, female breast cancer incidence rate increased nearly three times in the last decades[3]. Although late age at menopause, late age at first birth, body mass index and environmental factors generate risk for breast cancer, genetic factors are supposed to be main contributors of breast cancer development[3,4]. Genetic contributors such as BRCA1/2, ATM, PTEN which are primarily involved in DNA repair mechanisms are well-documented to play role in breast cancer but above mentioned genes are mostly responsible for the familial breast cancer. Recently done genome wide association studies have emphasized the importance of single nucleotide polymorphisms in some genes which can play role in breast cancer development[5,6]. TP53 gene, located on chromosome 17q13, is one of the most important tumour suppressor gene that encodes p53 transcription factor[7]. P53 has a regulatory function in repair of DNA damage, cell apoptosis, cell senescence and cell cycle control through inhibition of cell division when irretrievable DNA damage occurs[5,7,8]. Beside DNA damage, it has a central role in stress responses such as hypoxia, metabolite stress and oncogene activation to protect genomic stability[9]. Also, the high incidence rate of p53 inactivation in human cancers emphasizes the significance of its tumour suppressor function. Studies have approved that p53 influences the frequency and mechanisms of mutagenesis throughout carcinogenesis[10].

TP53 rs1042522 (Arg72Pro) polymorphism is one of the extensively investigated nonsynonymous polymorphism located in exon 4. In codon 72,

substitution of guanine (G) to cytosine (C) ends up with alteration of arginine (Arg) to proline (Pro) in the protein structure of p53[10]. Changing of Arg to Pro leads to altering the targeting capacity of p53 to proteasome (p53-mediated apoptosis) and also alters the stimulation of p73, another important tumour suppressor protein, transcription[8,10].Moreover Dumont et al. (2003) showed that Arg form stimulates apoptosis better at least five times as compared to Pro form. This improved apoptosis in Arg variant depends on the better localization to mitochondria and it has been found that greater mitochondrial localization of Arg variant is related with enhanced binding and ubiquitination of P53 through E3 ubiquitin ligase MDM2. In addition, this substitution modifies degradation competence of human papilloma virus E6 protein and Arg variant was reported as more prone to degradation by E6 protein. Even though some studies supported that this proneness to degradation is associated with risk of developing cancer, there is no general agreement in E6- dependent cancer risk due to the rs1042522[11]. In addition, some studies in Greek and Turkish population and meta-analysis report association of breast cancer and rs1042522[12-15] while others have contrary results[16-19]. Beside some previous well established studies that show influence of p53 inactivation in many cancer types including breast cancer provide the rationale for investigating functional p53 polymorphisms in p53 genes as a breast cancer risk factor[7].

In the light of present evidence, we examine the association between TP53 rs1042522 polymorphism and breast cancer risk in the case–control study among Turkish sporadic breast cancer women population.

Materials and Methods

Study Population

In this study, 508 breast cancer patients [mean age: 53.34±11.74] and age-matched 347 healthy controls [mean age: 53.07±0.629] were included. The 508 breast cancer patients were obtained from General Surgery Departments in Faculty of Medicine of Marmara and Kocaeli University. Familial breast cancer patients were excluded from the study.

Genotyping

Genomic DNA extraction was carried out from blood lymphocytes with a conventional salting-out method[20]. Genotypes were analysed by using PCR-RFLP method and the primer set used for TP53 rs1042522 (Arg72Pro) amplification was 5'-TCCCCCTTGCCGTCCCAA-3' as forward and 5'-CGTGCAAGTCACAGACTT-3'as reverse based on Storey et al. (1988)[21] with some modifications. The PCR conditions for TP53 rs1042522 (Arg72Pro) variant were as follows; 94 °C for 2 min followed by 35 cycles at 94 °C for 30 sec, 60°C for 45 s, 72 °C for 30 s and final extension step with 72 °C for 10 min. The resulting 279 bp fragment was digested overnight at 37 °C with 2U of BstUI enzyme, producing 160 and 119 bp long fragments. Finally digested fragments were separated by %10 PAGE for 35 min at 20 W. After silver staining and scanning, bands for rs1042522 were observed as; 279 bp for CC genotype, 160 and 119 bp for GG genotype and 279, 160 and 119 bp for GC genotype.

Statistical Analysis

The $\chi 2$ test was carried out for comparison of allele and genotype frequencies between cases and controls and student's t- test was performed for age difference. The odds ratio (OR) and 95% confidence intervals (CI) were predicted to confirm the effects of the different genotypes, and alleles using conditional logistic regression. p-value of less than 0.05 was assumed as significant. SPSS version 21.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The Hardy-Weinberg equilibrium was confirmed for tested patients and control populations.

Results

Genotype and allele frequencies of TP53 rs1042522 in breast cancer patients and overall controls were statistically analyzed. The distribution of TP53 rs1042522 genotypes were in agreement with HWE for controls (p>0.05) (Table 1). Genotype frequencies were 48.6%, 46.3% for GG, 40.7%, 44.7% for GC and 10.6%, %9.0 for CC genotypes in breast cancer and controls, respectively. It was found that there was not statistically significant difference between genotypes in cases and controls ($\chi 2$=1.591, p = 0.451). Allele frequencies for G allele were 69.0% in cases and 69.0% in controls, for C allele 31.0% in cases and 31.0% in controls. These results were also not found to be statistically significant in G and C alleles (G allele: p=0.424, C allele: p=0.501) (Table 1).

Discussion

Breast cancer is widely encountered as multifactorial disorder in women worldwide. The roles of genetic factors in breast cancer development have been documented in many studies[5]. Single nucleotide polymorphisms in tumour suppressor genes, DNA repair genes and oncogenes have been identified as a potential cause for tumour development. TP53 is an important tumour suppressor gene mainly playing role in DNA repair and cell cycle control[22]. In the current study, the association of TP53 (rs1042522) with breast cancer was investigated by using PCR-RFLP method in a group of hospital-based Turkish women population.

It has been found that there is no association between rs1042522 and the risk of breast cancer. TP53 (rs1042522) polymorphism was studied in various populations and some recent meta- analysis reported that this polymorphism was not risk factor for breast cancer[12,13,14,15]. Although some studies[16,17,18,19] found that this polymorphism is a risk factor for the breast cancer, the studies consistent with the meta-analysis, in Iranian, Slovakian and Iranian Azeri populations found out that there is no association between rs1042522 and breast cancer[23]. On the other hand, this polymorphism was found as protective against breast cancer in Mediterranean population[14]. According to stratified analysis in Indian population, homozygous mutants may be associated with

decreased breast cancer risk[15]. In the Turkish population few studies were performed for rs1042522; the results of Kara et al. (2010) have suggested that there is no association between this polymorphism and breast cancer risk in Turkish women populations[22] but other studies have contrary results.

Table 1. Genotype and allele frequencies of TP53Arg72Pro (rs1042522) in overall breast cancer patients and controls.

Genotype	Breast cancer patients	Controls	χ^2	*p*	OR; 95%CI
TP53Arg72Pro (rs1042522)	508 (100.0)	367 (100.0)	1.591	0.451	
GG	247 (48.6)	170 (46.3)	0.452	0.501	1.097 (0.838 - 1.435)
GC	207 (40.7)	164 (44.7)	1.353	0.245	0.851 (0.649 - 1.117)
CC	54 (10.6)	33 (9.0)	0.639	0.424	1.204 (0.763 - 1.898)
Allele frequency					
G	701 (69.0)	504 (69.0)	0.639	0.424	0.831 (0.527-1.310)
C	315 (31.0)	230 (31.0)	0.452	0.501	0.912 (0.697- 1.193)
HWE exact (*p*)	0.300	0.544			

HWE; Hardy-Weinberg Equilibrium

In contrary to Buyru et al. (2003), results of Akkiprik et al. (2009) revealed that Pro(C) allele is significantly correlated with increased breast cancer risk in the Turkish population[16,23]. Our results are in agreement with Kara et al. (2010) and indicate no association with breast cancer. Also, our population size is similar to that of Kara et al. (2010) and larger than two other contradictory studies in the Turkish populations. Therefore, the source of inconsistent results in Turkish population might be smaller population size used by Buyru et al. (2003) and Akkiprik et al. (2009). This inconsistency was observed also in different previous studies. While Arg (G) allele was found as increased breast cancer risk in some studies, others have found association of Pro(C) allele with increased breast cancer risk. Different ethnicity and geographical distribution may also be the reason for observed inconsistency[23]. In our previous study, the allele frequencies obtained for TP53 rs1042522 variant were similar to what we obtained in this current study[24].

The widespread Arg (G) allele of rs1042522 is related with a form of p53 protein that more potently triggers apoptosis than the other form including Pro(C) allele. It was also found that patients with GG genotype possibly response to radiation or chemotherapy more properly than other variants. Additionally, studies on Finnish, Spanish and Japanese groups have supported that patients with CC genotype have less survival than patients carrying other genotypes[9].

For further studies, TP53 (rs1042522) polymorphism may be associated with other genetic factors because p53 interacts with various proteins that play role in programmed cell death[5, 9]. For instance, it has been evaluated that some fibroblast growth factor receptor 2(FGFR2) gene polymorphisms are associated with breast cancer and FGFR2 gene accomplishes its function in a p53-dependent manner. Activation of apoptosis is stimulated by interaction between FGFR2 and TP53 [5]. Other genes synthesizing proteins that control cell cycle such as cyclins, cyclin-dependent kinases and p21 protein as a cyclin-dependent kinase inhibitor 1 can also be correlated with breast cancer risk. Especially, it has been shown that transcriptional activation of p21 and cell cycle arrest are related with p53 protein concentration[7]. Thus, the combined effect of these genes and TP53 polymorphisms can be focused in future. Moreover, since response to telomere erosion in first cell cycle check point depends on p53 which guards against tumorigenesis and protects genomic instability caused by telomere shortening, TP53 polymorphisms can be correlated with telomere length erosion in breast tumors[25]. Furthermore correlating clinical features of breast cancer patients with p53 rs1042522 genotypes may give data for usage of TP53 variants as a predictive biomarker for breast cancer treatment response. Although our population size is large as compared to some related studies, our study may be improved with investigating larger population and correlating genotype data with clinical features of breast cancer patients.

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