

# TP53 rs1042522 polymorphism and early-onset breast cancer

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**Background:** Breast cancer is the leading cause of cancer deaths among women. Early-onset breast cancer is well recognized as it clinically differs from old-age diagnosed breast neoplasms. *TP53 rs1042522* polymorphism relates to the risk of breast neoplasms, but this relationship in Turkish early-onset breast cancer patients has not been investigated yet. We aimed to search the relationship between *TP53 rs1042522* polymorphism and young Turkish breast cancer patients. **Materials and Methods:** Ninety-six female breast cancer patients who were  $\leq 40$  years of age and 96 healthy controls were enrolled in our study. Participants were genotyped by the hybridization probe system. **Results:** We identified that the genotype frequencies of *rs1042522* were significantly different between controls and cases ( $P = 0.027$ ). Participants carrying CG genotype had also reduced breast cancer risk (odds ratio = 0.4196, 95% confidence interval: 0.1941–0.9067,  $P = 0.027$ ). Our results revealed that there is an association between GG and CG + CC genotype groups with progesterone receptor (PgR) status ( $P = 0.0219$ ). **Conclusion:** Our findings indicate that the CG genotype is a protective factor against breast neoplasms. No other clinicopathologic parameters except for PgR status were found to be related to *rs1042522* polymorphism in young Turkish breast cancer patients.

**Key words:** Breast neoplasms, early onset, genotype, *rs1042522*, TP53

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## INTRODUCTION

Breast neoplasms are one of the most frequent diseases that lead to death among women in the world.<sup>[1]</sup> According to previous studies, different gene expression profiles characterize early-onset breast cancers.<sup>[2]</sup> Unlike other breast cancer types, findings have shown that early-onset breast cancers have poor response to treatment<sup>[3,4]</sup> and their features are more aggressive.<sup>[5]</sup> In contrast to older women diagnosed with breast cancer, several specific issues including recurrence for patients who have undergone mastectomy or breast-conserving therapy,<sup>[6,7]</sup> early menopause risk,<sup>[8]</sup> and chemotherapy-related infertility<sup>[9]</sup> are reported. Unlike cases of normal breast cancer, this situation is a clear indication that cases of breast cancer in young

women have various biological characteristics; therefore, a better understanding of the disease pathogenesis is required to facilitate the success of treatment for such forms of cancer and create emergent specific treatment and diagnostic strategies for the affected patients as in normal breast cancer.<sup>[10]</sup>

In improving breast cancer treatment, different types of genetic, epigenetic, and polymorphic alterations are crucial. The TP53 gene is the most altered in human cancer.<sup>[11]</sup> This tumor suppressor gene is a transcription factor that governs cell cycle, apoptosis, DNA repair, and senescence.<sup>[12]</sup> In addition to mutations, TP53 single-nucleotide polymorphisms (SNPs) can influence breast cancer. Only two of the polymorphisms found in the TP53 coding region can modify the amino acid sequence: SNPs 72 and 47.<sup>[13,14]</sup> The codon 72

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polymorphism (*rs1219648*) arises in the nonconserved proline-rich part of exon 4. This polymorphism results in the expression of either arginine (CGC) or proline (CCC), which results in 3 genotypes Arg/Arg, Arg/Pro, and Pro/Pro that have different apoptotic potential.<sup>[15,16]</sup> A few studies have shown the effect of the *TP53 rs1042522* polymorphism in premenopausal breast cancer patients in different populations.<sup>[17-20]</sup> Even though a study conducted on premenopausal Turkish women revealed that there is an association between early-onset breast neoplasms and *FGFR2* gene *rs1219648*,<sup>[21]</sup> there is no investigation on *TP53 rs1042522* polymorphism in Turkish young breast cancer patients.

In our experiment, we explored the relationship between *TP53 rs1042522* polymorphism and risk of early-onset breast cancer in Turkish women. Our research will provide evidence of the function of genetic polymorphism in early-onset breast cancer. The study will also provide information for further studies on genetically targeted therapies.

## MATERIALS AND METHODS

### Patient features and clinicopathological classification

Overall, the study had a sample of 96 breast cancer patients undergoing treatment at Dicle University's Faculty of Medicine. Ninety-six women aged below 40 years without family history and history of any form of cancer formed the control group. The patients were histopathologically diagnosed with breast neoplasms between 2010 and 2015 years, and selection of patients was based on age restriction. The mean age for women in the study group for breast cancer was  $33.59 \pm 5.52$  years ranging between 18 and 40. Selected patients and control populations were similar in terms of features such as ethnicity and age. Patient follow-up forms were used for collecting the required information on patients, including Her-2 status, progesterone/estrogen receptor (ER) status, tumor grade, and age. Age and ethnicity features constituted the similarities that characterized control populations and selected patients. Written informed consent was obtained from participants' supplied blood samples for genetic tests, and this study was approved by the Firat University Ethics Committee with 97132852 number.

### DNA isolation

Consistent with the requirements of the manufacturing firms from paraffin-embedded blocks of tissues, Cobas DNA Sample Preparation Kit FFPET (Roche, USA) was used to isolate DNA from sample of patients. However, QIAamp Blood Kit (Qiagen) was used to isolate DNA from healthy women's peripheral blood. Nanodrop (BioDrop ULITE,

UK) was used for spectrophotometric measurement of DNA quality and concentrations.

### Determination of *rs1042522* polymorphism in *TP53* gene

Hybridization probe system (TIB Molbiol, Germany) was used to genotype DNA of 96 control cohort and 96 breast cancer tissue samples on LightCycler 480 device (Roche Diagnostics, Germany) to determine *TP53* gene (*rs1042522*) SNP. 96-well plates were used to carry a total volume of 20  $\mu$ l volume containing LC 480 plate, 5  $\mu$ l DNA (50 ng), 10.4  $\mu$ l DNase-free water, and 1.6  $\mu$ l (3 mM)  $MgCl_2$  for 15  $\mu$ l reaction mixture, 2.0  $\mu$ l FastStart DNA Master, and 1.0  $\mu$ l reaction mixture. The PCR reaction was performed via LightCycler 480 II under following conditions; Initial denaturation at 95°C for 10 min, followed by 45 cycles at 95°C for 10 sec, at 60°C for 10 sec, at 72°C for 15 sec and followed by one cycle each at 95°C for 30 sec, at 40°C for 2 min and cooling from 75°C to 40°C. LightCycler 480 software (Roche, Rotkreuz, Switzerland) within LightCycler 480 II was used for conducting melting curve analyses. Evaluation of deviations in temperature was used for determining mutant and normal genotypes in controls and patients after normalization of dissociation curves.

### Statistical analysis

Windows computing program's SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for performing statistical tests. The Chi-square tests were used for evaluating genotypic distribution differences among controls and patients, statistically significant  $P < 0.05$ . For calculation of 95% confidence intervals (CI) and odds ratio (OR) as relative risk estimates for genotypes and alleles, unconditional logistic regressions were used.

## RESULTS

*TP53* gene *rs1042522* polymorphism was successfully determined by real-time PCR in all patients and controls. We observed all 3 genotypes in both patients and controls. The allele and genotype frequencies of *TP53 rs1042522* polymorphism are summed up in Table 1. It has been determined that the proportion of the *rs1042522* polymorphism in the young Turkish women patients was as follows: GG – 26%, CG – 30.2%, and CC – 43.8%. Statistically, the frequencies of these genotypes in disease-free young Turkish women are 17.7%, 49%, and 33.3%, respectively.

Our results showed that the CG genotype is more abundant among the control group, whereas allele frequencies had no significant differences between the control group and the patients. The G allele frequencies were 42.2% and 41.1% in the control group and the patients, respectively. In contrast, the frequencies for the corresponding C allele were 57.8% and 58.9% among the

**Table 1: Genotype allele frequencies, odds ratio, 95% confidence interval (in parentheses), and P values of TP53 gene (rs1042522) in early-onset breast cancer cases and controls**

Genotype	Patients (n=96), n (%)	Controls (n=96), n (%)	OR (95% CI)*	P*
GG	25 (26)	17 (17.7)	Reference	-
CG	29 (30.2)	47 (49)	0.4196 (0.1941-0.9067)	0.027
CC	42 (43.8)	32 (33.3)	0.8925 (0.4137-1.9254)	0.772
CG + CC	71 (74)	79 (82.3)	0.6111 (0.3051-1.2240)	0.165
Alleles				
G	79 (41.1)	81 (42.2)	Reference	-
C	113 (58.9)	111 (57.8)	1.0438 (0.6956-1.5662)	0.836

\*P value was obtained by Chi-square test. \*Odds ratio for genotype was calculated as selected genotype versus other genotypes. OR indicates crude odds ratio. Due to missing values, percentages may not be totally 100%. OR=Odds ratio; CI=Confidence interval

control group and the patients, respectively. In addition, our result indicated that there was a statistically significant relationship between heterozygote genotype and breast cancer (OR = 0.4196, 95% CI: 0.1941–0.9067,  $P = 0.027$ ). We have also compared normal homozygote (GG) genotype with the mutant homozygote (CC) and heterozygote plus mutant homozygote (CG + CC) genotype, and the ORs were found to be 0.8925 (95% CI: 0.4137–1.9254,  $P = 0.772$ ) and 0.6111 (95% CI: 0.3051–1.2240,  $P = 0.165$ ), respectively [Table 1].

Then, we analyzed GG, CG, CC, and CG+CC genotypes and compared them against clinicopathologic and demographic factors. The parameters included ER status, progesterone receptor (PgR) status, HER-2 status, tumor grade, tumor size, and tumor localization within the breast cancer patient group. The relationship between genotypic frequencies and these variables are summarized in Table 2. We have also examined the demographic and clinicopathologic parameters of GG and CG + CC genotype groups in the premenopausal cancer patients to figure out whether early-onset breast cancer genetic risk factors are linked to these features. Even though our results revealed that there is an association between genotypic differences with PgR status ( $P = 0.0219$ ), we did not find any significant correlation between other variables [Table 2].

## DISCUSSION

Although the relationship between TP53 rs1042522 SNP and breast cancer has been evaluated in the Turkish population, most of the studies conducted to date are directed to patients with postmenopausal breast cancer. In our previous study, we have identified that polymorphism in the second intron of the FGFR2 gene rs1219648 related to early-onset breast cancer in the Turkish population.<sup>[21]</sup> However, research to establish the effect of TP53 rs1042522 polymorphism in patients among young Turkish women with early-onset breast cancer is yet to be conducted. The purpose of this investigation was to reveal the effect of TP53 rs1042522 polymorphism on early age breast cancer in 96 patients diagnosed with breast cancer at age of 40 years

and under, and 96 women without cancer and without a family history of cancer by evaluating the TP53 rs1042522 polymorphism, which is found to have different apoptotic potential. As a result of our study, genotype distribution of TP53 rs1042522 polymorphism in the patient group was found as GG 25 (26%), CG 29 (30.2%), CC 42 (43.8%), G allele frequency 79 (41.1%), and C allele frequency 113 (58.9%); in the control group, it was found as GG 17 (17.7%), CG 47 (49%), CC 32 (33.3%), G allele frequency 81 (42.2%), and C allele frequency 111 (57.8%). According to our findings, rs1042522 CG genotype ( $P = 0.027$ , OR: 0.4196) may be protective in early-onset breast cancer, and these results were statistically significant.

In a study conducted on 221 breast cancer patients and 205 healthy Iranian women, no association was found between codon 72 polymorphism of TP53 gene and breast cancer. The patient group was separated as under 35 years and after 35 years of age, but there was no difference observed in the distribution of the alleles. The same situation did not differ when the patients were separated as premenopausal and postmenopausal. No association was observed between ER status, PgR status, and tumor histological type in the same study.<sup>[17]</sup> In our study, we investigated the relationship between early onset breast cancer and ER status, PgR status, Her-2 status, histological grade, tumor size, and tumor localization. No statistically significant relationship was found between the other clinicopathological features.

In another study, researchers investigated the association of MDM-2 and TP53 gene polymorphisms with breast cancer in Indian premenopausal and postmenopausal women, and it has been found that heterozygous variant Arg/Pro (GC) ( $P = 0.007$ , OR = 0.42) and combined variant Arg/Pro + Pro/Pro (GC + CC) ( $P = 0.007$ , OR = 0.46) of the p53 gene have statistically important protective effect on cancer in all participants (both on premenopausal and postmenopausal women). The same relationship was obtained only when postmenopausal women were included in the study ( $P = 0.009$ , OR = 0.25 for CG variant;  $P = 0.013$ , OR = 0.27 for the CG + CC variant). Contrary to our study, no

**Table 2: Association between TP53 gene (rs1042522) genotypes and clinicopathologic characteristics among early-onset breast cancer cases**

Variables	GG, n (%)	CG, n (%)	CC, n (%)	CG + CC, n (%)	$\chi^2$	P*
ER status						
ER (+)	12 (26.1)	14 (30.4)	20 (43.5)	34 (73.9)	0.4017	0.5262
ER (-)	12 (32.4)	10 (27)	15 (40.6)	25 (67.6)		
PgR status						
PgR (+)	8 (18.2)	14 (31.8)	22 (50)	36 (81.8)	5.2489	0.0219
PgR (-)	16 (41)	10 (25.7)	13 (33.3)	23 (59)		
HER-2 status						
HER-2 (+)	12 (28.6)	13 (31)	17 (40.4)	30 (71.4)	0.0049	0.9441
HER-2 (-)	12 (29.3)	11 (26.8)	18 (43.9)	29 (70.7)		
Tumor grade						
Grade I	3 (25)	6 (50)	3 (25)	9 (75)	0.6579	0.7196
Grade II	13 (31.7)	11 (26.8)	17 (41.5)	28 (68.3)		
Grade III	7 (23.3)	9 (30)	14 (46.7)	23 (76.7)		
Tumor size (cm)						
≤2	4 (14.8)	9 (33.3)	14 (51.9)	23 (85.2)	2.7482	0.2530
>2-≤5	12 (32.4)	8 (21.6)	17 (46)	25 (67.6)		
>5	7 (30.4)	10 (43.5)	6 (26.1)	16 (69.6)		
Tumor localization						
Right breast	12 (25.5)	15 (32)	20 (42.5)	35 (74.5)	0.3855	0.82469
Left breast	13 (27.1)	14 (29.2)	21 (43.7)	35 (72.9)		
Right + left breast	0 (0)	0 (0)	1 (100)	1 (100)		

Due to missing values, percentages may not be totally 100%. \*P value was obtained by Chi-square test. ER=Estrogen receptor; PgR=Progesterone receptor

relation was found between codon 72 gene polymorphism and breast cancer in premenopausal women.<sup>[18]</sup>

A study was also conducted with Iranian Azeris that include 100 premenopausal individuals with breast cancer and 100 normal controls. While there was no correlation between alleles and breast cancer, there was a relationship between heterozygote genotype Arg/Pro (GC) and premenopausal breast cancer ( $P = 0.043$ , OR = 0.45).<sup>[19]</sup> In the same study, the distribution of *FGFR2* rs1219648 and *TP53* rs1042522 genotype combination in breast cancer patient and healthy groups was investigated, and *FGFR2* major genotype (AA) and *TP53* hetero genotype (GC) were found to have a protective effect in breast cancer (AA and G;  $P = 0.047$ , OR = 0.512)<sup>[19]</sup> as shown in our study. It has also been reported that the frequencies of Pro72 and Arg72 did not substantially vary between Sudanese premenopausal patients. This implies that the codon 72 Arg72 and Pro72 polymorphism has no role in the susceptibility of premenopausal breast cancer in another population.<sup>[20]</sup>

## CONCLUSION

From our results, CG genotype is a protective factor against breast cancer in early-onset Turkish women. Furthermore, the association was found to be related to PgR status. The other clinicopathologic variables were not found to be associated with *TP53* rs1042522 polymorphism among the study population. There is a need to conduct further

studies with a larger population to confirm the results of this study.

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## Conflicts of interest

There are no conflicts of interest.

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