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The effect of survivin gene promoter polymorphism on breast cancer

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Abstract: Breast cancer is the most common malignant tumor in women and accounts for about 25% of all cancer diagnoses. Survivin is a member of the apoptosis inhibitor protein family of antiapoptotic proteins. In our study, we investigated one of those, the survivin gene promoter 31G/C polymorphism. Included in this study were 111 breast cancer patients who were operated on in our hospital and 101 healthy female subjects. Blood samples from the healthy subjects and paraffin-embedded tissue samples from the patients were used for DNA extraction and subsequent genetic analysis. PCR-RFLP was used for genotype analysis. We established the clinicopathologic characteristics of patients. No significant difference was found between survivin 31G/C promoter polymorphism of tumor characteristics and breast cancer. Between the control and breast cancer groups, survivin promoter polymorphism 31G/C differences were not significantly different (P = 0.058). The risk of developing cancer, having the relevant GC or CC genotype, is 1.413 times higher than those having genotype GG (95% confidence interval: 1.040 to 1.918). Carrying the C allele was statistically significant in terms of susceptibility to breast cancer. In conclusion, the use of survivin gene polymorphism as a risk factor in breast cancer is recommended based on the results of this study.

Key words: Breast cancer, apoptosis inhibitor protein, survivin, survivin gene promoter polymorphism, SNP

1. Introduction

Breast cancer is the most common malignant tumor in women and accounts for about 25% of all cancer diagnoses (Liu et al., 2014) and its incidence continues to increase. Although significant progress in the diagnosis and treatment of breast cancer has been achieved over the past several decades, it remains the second highest cause of cancer-related deaths in women (Christensen et al., 2006). The early identification of high-risk women is an important issue because of the availability of medical and surgical treatment options for breast cancer (Visvanathan et al., 2009). A new gene profiling method has recently been introduced as a powerful tool for predicting the clinical outcomes of women with breast cancer.

Dysregulation of the balance between cell proliferation and cell death contributes to carcinogenesis (including the development and progression of breast cancer) by prolonging cell survival, promoting the accumulation of transforming mutations, and enhancing cell resistance to chemotherapy (Bayram et al., 2011). Survivin is a member of the inhibitor of apoptosis protein family, and is thought to inhibit apoptosis and regulate mitosis. It has unique properties, with bifunctional roles as a cell-cycle regulator and an apoptosis inhibitor (Altieri, 2003). Although survivin is strongly expressed in embryonic and fetal organs, it is undetectable in most terminally differentiated normal adult tissues (Ambrosini et al., 1997). However, survivin is overexpressed in various cancers, including lung, breast, colon, stomach, esophageal, pancreatic, bladder, uterine, and ovarian cancers; large-cell non-Hodgkin's lymphoma; leukemia; neuroblastoma; melanoma; and nonmelanoma skin cancer, compared with its expression in normal tissues (Gazouli et al., 2009).

The survivin gene in humans spans 14.7 kb, and is located in the telomeric region of chromosome 17q25 (Ambrosini et al., 1998). Several single-nucleotide polymorphisms (SNPs) have been identified within the promoter region of the survivin gene. A polymorphism at position –31 promoter region, which involves the substitution G > C (rs 9904341), is the most frequently documented SNP. This polymorphism is located in a cell-cycle-dependent element and the cell-cycle homology region repressor

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binding motif in the promoter region. The G > C mutation correlates with the increased expression of survivin at both the transcriptional and translational levels (Yazdani et al., 2012). Several case–control studies have examined the association between the –31G > C polymorphism and the risk of cancer (Srivastava et al., 2012), including nasopharyngeal carcinoma (Yang et al., 2009), esophageal cancer (Upadhyay et al., 2011), gastric cancer (Yang et al., 2009; Borges et al., 2011), hepatocellular carcinoma (Bayram et al., 2011), pancreatic cancer (Theodoropoulos et al., 2010), and urothelial carcinoma (Wang et al., 2009), but the results are controversial.

Because of the critical role of survivin in carcinogenesis and considering the prognostic and therapeutic implications (Ambrosini et al., 1997), we evaluated the correlation between this polymorphism and clinicopathologic characteristics of patients. We also evaluated the predisposition in breast cancer patients having this polymorphism. To the best of our knowledge, no study has yet determined the association between the –31G > C polymorphism in the survivin gene and the risk of breast cancer.

2. Materials and methods

2.1. Study design and patients

After approval was obtained from the Clinical Research Ethics Board of Turgut Özal University School of Medicine, 111 patients with a diagnosis of invasive breast carcinoma who had undergone surgery at the Department of General Surgery, Turgut Özal University School of Medicine between January 2004 and May 2011 were retrospectively recruited and 101 healthy females subjects having no history or diagnosis of cancer or genetic disease and aged ≥30 years were recruited from among individuals who had visited the hospital for a physical examination. In the cancer group, we excluded patients with systemic disease at baseline, patients receiving neoadjuvant chemotherapy, patients with pure in situ carcinoma, and patients missing during the follow-up period.

After informed consent was obtained from all the participants, blood samples were collected from the healthy subjects for genetic analysis. Paraffin-embedded tissue samples from the women with breast cancer were stored at the Department of Pathology in our faculty and were used for genetic analysis. The genetic studies were conducted at the Genetics Laboratory of the Medical Genetics Department.

We retrieved from our breast cancer database the age, age at menarche, menopausal status, estrogen hormone use, breastfeeding status, smoking status, family history of breast cancer, tumor type, tumor size, tumor grade, lymphatic-duct invasion, vascular invasion, perineural invasion, multifocal/multicentric tumor presence, lymphatic metastasis presence, number of metastatic lymph nodes, estrogen/progesterone receptor status, C-erbB2 status, and the recurrence and survival data for each woman with breast cancer.

2.2. DNA isolation from paraffin-embedded tissue blocks

DNA was isolated and purified from paraffin-embedded tissue blocks using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) and was stored at –20 °C for PCR analysis.

2.3. DNA isolation from peripheral blood

DNA was isolated from peripheral blood samples from the healthy women using the phenol–chloroform method and was stored at –20 °C.

2.4. DNA amplification

To detect the –31G > C (rs 9904341) polymorphism in the survivin gene, a 151 bp region was amplified using the following primers: 5′ -AAGAGGGCGTGCGCTCCCGACA - 3′ (forward) and 5′ - GAGATGCGGTGGTCCTTGAGAAA - 3′ (reverse). Primers were designed using the NCBI/Primer-BLAST database. For the polymerase chain reaction (PCR) 2 µL of DNA (150 ng/µL) solution in a total volume of 25 µL, 2.5 µL of 10X Taq (NH_4) SO_2 buffer, 2.5 mM $MgCl_2$, 100 µM each dNTP, and 0.5 µL of 20 pmol each primer, 1 U of Taq DNA polymerase, and 16.3 µL of dH_2O were used. The conditions for PCR were as follows: denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s, annealing at 62.2 °C for 30 s, and elongation at 72 °C for 1 min, which was repeated for 35 cycles; final elongation was at 72 °C for 5 min.

2.5. Agarose gel electrophoresis

To check whether the PCR products were successfully amplified, they were run on 3% agarose gel (Invitrogen) in 1X Tris–acetic acid–EDTA buffer. For electrophoresis, 5 µL of the PCR products were mixed with 1 µL of 6X loading dye and the samples were loaded into the wells. Electrophoresis was performed at 110 V for 15 min, after which the PCR products were visualized with an ultraviolet (UV) transilluminator (Figure 1).

2.6. DNA digestion and detection of the survivin –31G > C (rs9904341) polymorphism (restriction fragment length polymorphism (RFLP) analysis)

The DNA samples were digested using the *Msp*1 restriction enzyme (FastDigest; Thermo Scientific, Waltham, MA, USA). This enzyme digested the 151-bp DNA sequence into 2 fragments (90 and 61 bp) by cutting the C base at the polymorphic site. After digestion, 5 µL of the restricted DNA was mixed with 1 µL of 6X loading dye. The samples were then loaded into individual wells of an agarose gel and separated electrophoretically at 100 V for 30 min, before visualization with a UV transilluminator. To confirm the genotype at position –31, DNA sequencing was performed.

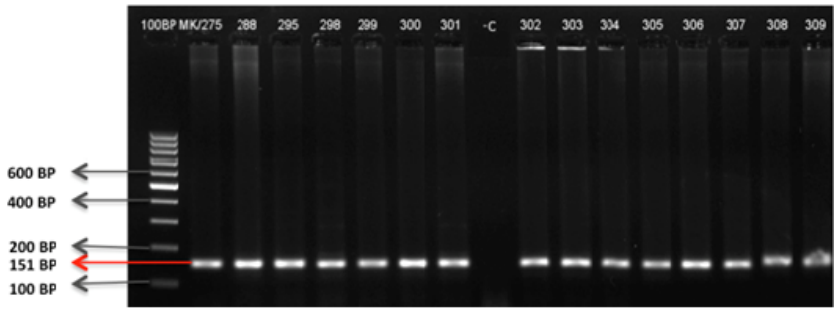

Figure 1. Patient group of PCR amplification products by agarose gel electrophoresis. – C is negative control, 100 bp marker is used for product size, and the numbers in the lanes indicate the patients' PCR products followed by separation on 3.0% agarose gel.

2.7. Sequence analysis

Five representative samples were sequenced by ABI 3130 system. Survivin PCR products of several samples were exposed to ExoSAP IT treatment (GML A.G., Wallerau, Switzerland). Then sequence-specific PCRs were performed on these samples followed by DNA precipitation and they were run on an ABI 3130 sequencer.

3. Statistical analysis

The data were analyzed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Intervals and continuous data are expressed as means ± standard deviations, whereas categorical variables are expressed as numbers of observations and percentages. The significance of differences between the 2 groups was evaluated with Student's *t* test for continuous variables, whereas categorical variables were compared using Pearson's chi-squared test, Fisher's decisive chi-squared test, or the likelihood ratio test.

4. Results

A total of 111 patients with breast cancer and 101 controls were enrolled in this study. There were no significant differences between the 2 groups in terms of their age at menarche, menopausal age, lactation status, smoking history, or family history of breast cancer, which suggests that our matching of the demographic characteristics was satisfactory. The demographic and clinical characteristics of both groups are shown in Table 1.

Table 1. The demographic and clinical characteristics of both groups.

Variables	Patient group (n =111)	Healthy control group (n = 101)	P value
Age	52.9 ± 14.3	40.2 ± 7.3	*<0.001*
Age of menarche	13.3 ± 1.2	13.2 ± 1.5	0.542
Age of menopause	47.9 ± 5.6	44.1 ± 9.0	0.120
Postmenopausal patients	64 (57.7%)	13 (12.9%)	*<0.001*
Hormonal therapy	26 (23.4%)	54 (53.5%)	*<0.001*
History of lactation	102 (91.9%)	89 (88.1%)	0.358
Smoking habits			0.286
No	93 (83.8%)	77 (76.2%)	
Yes	17 (15.3%)	21 (20.8%)	
Presence of previous smoking habits	1 (0.9%)	3 (3.0%)	
Family history			0.166
Absent	88 (79.3%)	87 (86.1%)	
1st degree relatives	12 (10.8%)	4 (4.0%)	
2nd degree relatives	11 (9.9%)	10 (9.9%)	

We detected all 3 genotypes in our groups (Figures 2a and b). There was a slight difference in the prevalence of the survivin promoter polymorphism (–31G > C) between the 2 groups, although the P value (P = 0.058) slightly exceeded the threshold for statistical significance (i.e. P < 0.05). Notably, the combined prevalence of the GC and CC genotypes (GC + CC), reflecting the prevalence of the C allele, was significantly greater in the breast cancer group than in the control group (P = 0.023), with rates of 59% and 38%, respectively. These results imply that the C allele at position –31 in the promoter region of the survivin gene increases an individual's susceptibility to breast cancer. The distributions of the subjects in the breast cancer and control groups according to the survivin promoter polymorphism are shown in Table 2.

The risk of developing cancer was 1.413 times higher in patients with the GC or CC genotype (GC + CC) than in patients with the GG genotype, and this difference was statistically significant (95% CI: 1.040–1.918; Table 3).

In the breast cancer group, the GG genotype was present in 52 patients, whereas the GC + CC genotype was present in 59 patients. There were no significant differences

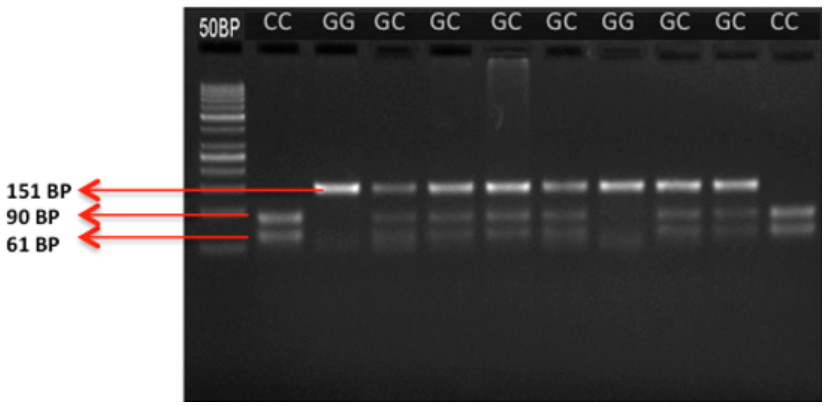

Figure 2a. Genotyping of survivin –31G >C polymorphism by PCR-RFLP analysis followed by separation on 3% agarose gel. Lane 1 is 50 bp marker; lanes 2 and 11 are CC genotype; lanes 3 and 8 are GG genotype; lanes 4–7, 9, and 10 are GC genotype.

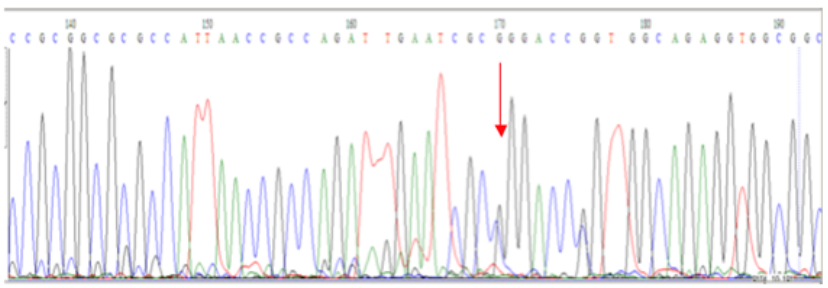

Figure 2b. Electropherogram image of heterozygous polymorphisms of surviving (GC) genotype .

Table 2. The distributions of the subjects in the breast cancer and control groups according to the survivin promoter polymorphism.

	Patient group (n = 111)	Healthy control group (n = 101)	P value
Survivin 31GC			0.058
GG	52 (46.8%)	63 (62.4%)	
GC	46 (41.4%)	32 (31.7%)	
CC	13 (11.8%)	6 (5.9%)	
Survivin			0.023
GG	52 (46.8%)	63 (62.4%)	
GC+CC	59 (53.2%)	38 (37.6%)	

Table 3. Presence of GC+CC polymorphism and odds ratio.

Survivin promoter polymorphism	Healthy control group (n = 101)	Patient group (n = 111)	Odds ratio (95% confidence interval)
GC+CC	38 (37.6%)	59 (53.2%)	1.413 (1.040–1.918)

between these 2 groups in terms of age, menopausal age, age at last birthday, hormone therapy, duration of hormone therapy, history of lactation, smoking history, family history of breast cancer, or the patient's relationship to family members with a history of breast cancer (Table 4).

In terms of the histopathological characteristics of the breast cancer specimens, we found no significant differences between the GG and GC + CC genotypes in terms of multifocality/multicentricity, tumor diameter (mm), tumor stage (TNM), histological grade, lymphovascular invasion, perineural invasion, presence of lymphatic metastasis, number of metastatic lymph nodes, presence of an extensive intraductal component, or extracapsular perinodal invasion. There were no significant differences between these 2 groups in terms of the incidence of invasive ductal carcinoma or invasive lobular carcinoma. There were also no differences in the rates of estrogen receptor, progesterone receptor, or C-erbB2 positivity (Table 5). Furthermore, there were no differences between the 2 groups in the local recurrence and distant recurrence rates, the median time to relapse in patients with recurrence.

5. Discussion

Survivin is an antiapoptotic protein belonging to the inhibitor of apoptosis protein family. It is a bifunctional protein that regulates cell division and suppresses apoptosis. Survivin is highly expressed in various human malignancies, but its expression is very low or below the level of detection in normal adult tissues (Ambrosini et al., 1998; Yazdani et al., 2012). Survivin is usually expressed in the G_2/M phase of the cell cycle, when it is thought to disrupt the apoptosis signaling pathways and to promote the survival of abnormal cells, offering a significant advantage to tumor cells overexpressing survivin (Upadhyay et al., 2011). The elevated expression of survivin is thought to be a negative prognostic factor for tumors and its overexpression is reported to be associated with shortened survival (Altieri, 2003). Many

Table 4. The demographic and clinical characteristics of GG and GC + CC genotype groups in breast cancer patients.

Variables	GG (n = 52)	GC+CC (n = 59)	P value
Age	51.4 ± 12.9	54.3 ± 15.4	0.287
Age of menopause	48.3 ± 7.4	47.6 ± 4.0	0.787
Postmenopausal patients	28 (53.8%)	36 (61.0%)	0.445
Presence of hormonal therapy	9 (17.3%)	17 (28.8%)	0.214
Duration of hormonal therapy (months)	12.5±9.0	38.9±48.9	0.075
History of lactation	47 (90.4%)	55 (93.2%)	0.732
Smoking habits			0.455
No	45 (86.5%)	48 (81.4%)	
Yes	7 (13.5%)	10 (16.9%)	
Presence of previous smoking habits	-	1 (1.7%)	
Family history	12 (23.1%)	11 (18.6%)	0.565
Absent	40 (76.9%)	48 (81.4%)	
1st degree relatives	5 (9.6%)	7 (11.9%)	
2nd or more degree relatives	7 (13.5%)	4 (6.8%)	

Table 5. The pathological characteristics of GG and GC + CC genotype groups in breast cancer patients.

Variables	GG (n = 52)	GC+CC (n = 59)	P value
Multifocal–multicentric	29 (55.8%)	25 (42.4%)	0.159
Unifocal	23 (44.2%)	34 (57.6%)	
Invasive ductal CA	44 (84.6%)	53 (89.8%)	0.409
Invasive lobular CA	8 (15.4%)	6 (10.2%)	
Tumor diameter (mm)	26.6 ± 15.9	27.0 ± 17.0	0.894
T			
≤2 cm	22 (42.3%)	25 (42.4%)	0.508
>2–<5 (2.1–4.9 cm)	27 (51.9%)	27 (45.8%)	
≥5 cm	3 (5.8%)	7 (11.9%)	
Grade			
I and II	35 (67.3%)	37 (62.7%)	0.613
III	17 (32.7%)	22 (37.3%)	
Lymphatic canal invasion	30 (57.7%)	34 (57.6%)	0.994
Vascular invasion	6 (11.5%)	8 (13.6%)	0.749
Perinoral invasion	14 (26.9%)	20 (33.9%)	0.426
Lymphatic metastasis (yes/no)	32 (61.5%)	34 (57.6%)	0.675
Number of lymph nodes	3.7 ± 6.4	5.4 ± 9.7	0.310
N			
(N0) absent	20 (38.5%)	25 (42.4%)	
(N1) 1–3 nodes positive	19 (36.5%)	11 (18.6%)	0.158
(N2) 4–9 nodes positive	7 (13.5%)	14 (23.7%)	
(N3) 10 or more	6 (11.5%)	9 (15.3%)	
Extensive intraductal component	19 (36.5%)	22 (37.3%)	0.935
Perinodal invasion (yes/no)	14 (26.9%)	16 (27.1%)	0.982
ER* positivity	40 (76.9%)	44 (74.6%)	0.774
PR* positivity	41 (78.8%)	44 (74.6%)	0.596
C-erb B2 positivity	15 (28.8%)	16 (27.1%)	0.880

* ER= estrogen receptor, PR= progesterone receptor

studies have also shown that tumors expressing survivin are resistant to the apoptosis that is induced by anticancer drugs (Tran et al., 2002; Rödel et al., 2003). Targeting of survivin using adenoviral antisense vectors was reported to enhance the sensitivity of tumor cells to chemotherapy and radiotherapy (Yamamoto et al., 2003). Clarification of the survivin signaling pathway will provide new predictive and prognostic information for cancer diagnosis and could lead to the development of new therapeutic alternatives for a variety of cancers (Altieri, 2001; Yamamoto and Tanigawa, 2001).

High survivin expression has been detected in several cancer types in humans, including colorectal cancer, hepatocellular cancer, lung cancer, pancreatic cancer, and osteosarcoma (Wang et al., 2009; Yang et al., 2009; Theodoropoulos et al., 2010; Upadhyay et al., 2011;

Borges et al., 2011; Srivastava et al., 2012). Survivin is also expressed in breast cancer (Yamashita et al., 2007).

It has been suggested that the expression of survivin is transcriptionally controlled by cell-cycle-dependent elements located in the proximal region of the survivin promoter and in the cell-cycle homology region (Altieri, 2001). Several nucleotide polymorphisms have been detected in these regions of the survivin DNA sequence. One SNP is –31G > C, which is located in the cell-cycle-dependent element/cell-cycle homology region repressor binding site, 31 bp upstream from the first nucleotide of the ATG start codon. An earlier study suggested that this polymorphism is associated with the overexpression of survivin at the transcriptional and translational levels (Xu et al., 2004). Based on these earlier findings, we focused on this SNP in the present study and investigated the association between it and breast cancer.

Although numerous reports have described the association between the survivin –31G > C polymorphism and a variety of different cancers, to our knowledge, this polymorphism has not been examined in breast cancer. Therefore, we also compared our results with those for other types of cancer associated with the survivin – 31G > C polymorphism. Qin et al. (2012) reported that this polymorphism significantly enhanced the development and progression of renal cell carcinoma in Chinese individuals. Upadhyay et al. (2011) reported that survivin promoter region polymorphism (–31G > C) is associated with susceptibility to esophageal cancer in a northern Indian population. However, Borbely et al. (2007) reported that this polymorphism did not increase the risk of developing cervical cancer, and Borges et al. (2011) found no differences in the –31G > C genotypes or allele frequencies of patients with gastric cancer and the control group. In our study, we found a slight difference in the prevalence of this polymorphism in the breast cancer and control groups, although the P value (P = 0.058) slightly exceeded the threshold for statistical significance.

Several studies have examined the potential association of the –31 C allele in the survivin promoter region with susceptibility to various cancers or with prognostic cancer markers. For example, Yazdani et al. (2012) reported that the survivin –31G > C polymorphism was associated with an increased risk of papillary thyroid cancer and that the frequency of the GC + CC genotype was significantly higher in papillary thyroid cancer patients than in the control group. Therefore, they suggested that the C allele is a predisposing factor for papillary thyroid cancer. In their study, the frequency of the C allele was higher in patients with poor prognostic factors, especially lymph-node involvement, vascular involvement, and multifocality. Zahedi et al. (2012) reported that the frequency of the C allele was significantly greater in endometrial cancer patients. In our study, the risk of developing breast cancer was 1.413 times greater in GC + CC patients than in GG patients (95% CI: 1.040–1.918). The prevalence of the GC + CC genotype was also significantly higher in the breast cancer group than in the control group (P = 0.023), detected in 59% and 38% of patients, respectively. According to these results, the presence of the C allele in the promoter region (–31G > C) of the survivin gene has a statistically significant effect on an individual's susceptibility to breast cancer. In this respect, our results are consistent with those reported by Yazdani et al. (2012) and Zahedi et al. (2012). However, unlike Yazdani et al. (2012), we found no significant differences in the prevalence of the GG and GC + CC genotypes in terms of multifocal/multicentric breast cancer (P = 0.159), lymph-duct invasion, vascular invasion, perineural invasion, lymphatic metastasis, or the number of metastatic lymph nodes (P > 0.05). Gazouli et al. (2009) reported that the C allele and the CC genotype frequencies were significantly higher in colorectal cancer patients than in healthy subjects (P < 0.0001). In a study of patients with lung cancer and healthy controls with at least one G allele, Jang et al. (2008) reported that the risk of lung cancer was significantly lower in those with at least one G allele than in those with the CC genotype. These authors reported that the G allele correlated with significantly lower promoter activity than the C allele. Yang et al. (2009) reported that the C allele increased the risk of developing esophageal squamous cell carcinoma, and Wang et al. (2009) reported that the risk of developing urothelial carcinoma was significantly greater in individuals with the GC + CC genotype than in individuals with the GG genotype. Consistent with the studies by Yang et al. (2009) and Wang et al. (2009), we found that the risk of developing breast cancer was significantly greater (by 1.413 times; 95% CI: 1.040–1.918) in individuals with the GC or CC genotype than in individuals with the GG genotype.

Bayram et al. (2011) studied the association between the –31G > C polymorphism and hepatocellular carcinoma in a Turkish population, but found no difference in the genotype distributions of patients with hepatocellular carcinoma and cancer-free individuals. However, genetic polymorphisms may differ among ethnic groups. Similar to the results reported by Bayram et al. (2011), we found that the distribution of the –31G > C polymorphism did not differ significantly between the breast cancer and control groups (P = 0.058) in a Turkish population.

In conclusion, in terms of survivin gene promoter polymorphisms when compared to 31GG, (31G/C + 31CC) with polymorphisms, carrying the C allele (31GC or 31CC) in the survivin gene promoter region was statistically significant in terms of susceptibility to breast cancer. Because genetic polymorphisms may differ between ethnic groups, further studies are required to examine the associations between polymorphisms in genes such as

survivin and breast cancer in other ethnic populations. The present study should provide a foundation for future studies in this important field. To confirm our findings, other studies of larger populations are needed. Our results indicated that the CC homozygote genotype in the patient group is twice as common as the CC homozygote genotype in healthy controls in Turkish population, but the number of all CC genotypes is 19 out of 212 cases. If the number of cases as well as CC genotypes is increased with further studies, results may display a tendency to breast cancer in the CC genotype, which may be used for diagnostic approaches. If our findings are supported by the results of future studies, we think that the assessment of polymorphisms in the survivin gene promoter region may also add a new dimension to therapeutic approaches to breast cancer. For example, we think that knowledge of the patient's genotype will help oncologists make better decisions regarding the need for prophylactic mastectomy in women with a high risk of breast cancer.

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