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ORIGINAL ARTICLE

Association of WNT Antagonist Gene Polymorphisms with Breast Cancer in a Turkish Population

WNT Antagonist Gen Polimorfizmlerinin Türk Popülasyonunda Meme Kanseri ile İlişkisi

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Abstract

Introduction: Changes in protein encoding genes involved in various signalling pathways are effective in the development of breast cancer. The (WNT)/β-catenin pathway is known to have a role in many cancer types, including breast cancer. In this study we aimed to investigate the relationship between the risk of breast cancer development and WNT antagonist gene polymorphisms in a selected Turkish population.

Methods: In total, 100 patients who were diagnosed with breast cancer and 100 age-matched and sex-matched healthy individuals were evaluated in this study. We genotyped 4 single nucleotide polymorphisms including DKK3 non-synonymous (Exon7 Arg335Gly), DKK3 (Intron4 G/C), DKK4 synonymous (Exon4 V169V), sFRP4 non-synonymous (Exon6 R340K) by performing polymerase chain reactions (PCR) and restriction fragment length polymorphism (RFLP).

Results: In statistical analysis using Chi-square (χ^2) test, we observed that there was no significant difference between case and control groups for distribution of DKK3 nonsynonymous exon 7 Arg335Gly, DKK3 intron 4 G/C polymorphisms and sFRP4 non-synonymous (Exon6 R340K) ($p>0.05$). On the other hand, distribution of DKK4 synonymous exon 4 V169V polymorphism between case and control groups was significantly different ($p<0.05$). However, a statistically significant correlation between breast cancer risk and CC genotype of DKK4 synonymous exon 4 V169V polymorphism (adjusted for BMI and sFRP4) has been defined [p=0.001, OR: 16.38 CI: 95% (6.37–42.12)].

Discussion and Conclusion: These results suggest that the CC genotype of DKK4 synonymous exon 4 V169V polymorphism is associated with the development of breast cancers in Turkish population.

Keywords: Breast cancer; Polymorphism; Turkish population; WNT antagonists

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Breast cancer is one of the most common malignant tumors in women worldwide. Furthermore, it is among the top causes of cancer-related deaths.[1] Median survival of metastatic breast cancer appears to have improved due to new effective agents. Besides, clinical and biological factors affect the long-term outcomes.[2] Thus, new factors have to be investigated.

The Wnt/β-catenin signaling pathway is highly conserved throughout evolution. It is known that this pathway is important in tumor formation, invasion, and metastasis.[3,4] Wnt signaling pathways are divided into canonical and noncanonical pathways. The canonical Wnt signaling is activated by the Wnt receptor complex, which has two components (Frizzled and LRP5/LRP6). Non-canonical Wnt signaling is mediated by the Frizzled family Wnt receptor. Extracellular antagonists of the Wnt signaling pathway can be divided into two main groups. Both classes of molecules inhibit ligand-receptor interactions. However, this inhibition occurs through distinct mechanisms. The first group consists of sFRP (Secreted Frizzled-related protein) family, WIF-1 (Wnt inhibitor factor-1) and Cerberus. They alter the ability of members of this group to bind directly to Wnt protein bonds and Wnt binding complexes. The second group is DKK (Dickkopf) family members, which bind to LRP5 and LRP6 and inhibit Wnt signals. Therefore, DKK family proteins inhibit only the Canonical pathway, while sFRP family proteins inhibit both.[5–7]

The first type of cancer to which WNT signaling has been associated is breast cancer.[4] However, recent studies have been reported that Wnt silencing of Wnt pathway genes and polimorphisms have been reported in many cancers.[7,8] In our study, we investigated the relation between wnt antagonist gene polymorphisms and clinicopathologic data.

Materials And Methods

Study Group

Totally 200 Turkish women were examined in the current study. One hundred patients with breast cancer, who were diagnosed with invasive ductal cancer and treated in Oncology unit of Cumhuriyet University in year 2011–2013, were confirmed as patient group. The histological classification of these patients diagnosed with breast cancer and also the determination of their clinico-pathological stages were created according to the UICC Tumor-Node-Metastasis Classification (TNM), seventh edition, 2010. The control group enclosed 100 healthy, voluntary individuals who were chosen from among living in same city and have similar life characteristics; frequency of age matched with patient group, without known any chronic disease and familial cancer history. Approval was received from the Cumhuriyet University Ethics Committee for this study (number of the committee decision: 2011/027). A written informed consent form has been filled out and signed by both of groups. Our study was conducted in conformity with Helsinki Declaration. Artificial intelligence (AI) did not used in the production of this work.

Samples Collection and Genotype Analysis

Peripheral blood samples of 4 ml were obtained from all individuals and collected in tubes containing EDTA. DNA was isolated by using a "salting out" method". DNA samples were kept at -20°C up to the next step. Genotype analysis was performed nested-PCR and RFLP methods. An Applied Biosystems Gene AmpR PCR system 9700 (USA) thermal cycler was used for PCR amplifications. For each nested-PCR step, PCR reactions were performed in a reaction volume of 25 mL containing 10 pmol of each primer sets, 1 unit of Taq DNA polymerase (Fermentas), 10 mmol/L Tris-HCl (pH 8.3 at 25°C), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 5 nmol each of four deoxynucleotide triphosphates (Fermentas), and 50 ng of genomic DNA. PCR amplifications were performed at the following conditions: one cycle initial denaturation step at 94°C for 5 min, followed by 40 cycles at 94°C for 30 sec, for first step of nested-PCR at 52°C-for second step of nested-PCR at 58°C for 1 min, at 72°C for 1 min, one cycle final extension step at 72°C for 5 min. For genotype analysis (RFLP) of four SNPs, PCR products of 8 µl were digested with restriction endonuclease enzyme of 5U specific to SNPs (Table 1) and 1X reaction buffer in a total reaction volume of 10 µl and incubated at 37°C for overnight (O/N). PCR and RFLP products were run on 2% agarose gel, respectively. Sizes of these products were shown at Table 1. Additionally, the agarose gel image of the RFLP results of 4 polymorphisms is given in Figure I.

Statistical Analyses

SPSS version 22.0 was used for all statistically analyses in current study. Statistically significant departure from Hardy-Weinberg equilibrium was assessed using Chi-square test (χ^2). Independent-Samples T-test test was used to compare mean age. Distributions of smoking habits, alcohol consumption, Body mass index (BMI; were categorized as <25 and >=25kg/m^2), familial cancer history, age at menarche and menopause status between patient-control groups were evaluated by using Binary Logistic Regression analysis. Frequencies of genotypes and alleles among groups, and the correlation between

Table 1. Conditions for the identification of WNT antagonist gene polymorphisms

SNPs	Forward and reverse primer sequences (5'→3')	PCR products (bp)	Restriction enzymes	Alleles and product size (bp)	
DKK3 non - synonymous (Exon7 Arg335Gly	F: GAGGTCCCCGATGAGTATGA R: TAGGAAGAAGCCTGGTCAGC F: GGTCCCCGATGAGTATGAAG R: AGCACACACCTGGGGAAATA	242 210	DdeI (HpyF3I)	G A	210 115+95
DKK3 (Intron4 G/C)	F: TTCCTTAGGTCCCTAGGTCCA R: AGGGCAAAGGAGACTCTTCA F: ACAGGGCATGGCAGTTAGAG R: CTCTTCACCCAACAGGCATT	377 245	SatI (Fnu4HI)	G C	245 171+74
DKK4 synonymous (Exon4 V169V)	F: GCCATGGCATTACTGCTTTT R: ATTGCTGGTCAATTGGCTTC F: CTGCGTGCTGTGTCTGTTTT R: AACGCTGGAAGATTTCTGGA	384 292	XagI (EcoNI)	C T	224+68 292
sFRP4 non - synonymous (Exon6 R340K)	F: AAGAGAGGCTGCAGGAACAG R: TCTGTACCAAAGGGCAAACC F: AGAGCGGAGAACAGTTCAGG R: TGGCCTTACATAGGCTGTCC	397 246	EarI (Eam1104I)	G A	134+112 246

SNP: Single nucleotide polymorphism; DKK3: Dickkopf 3; DKK4: Dickkopf 4; sFRP4: Secreted frizzled-related protein 4; A: Adenine; C: Cytosine; G: Guanine; T: Thymine; bp: Base pair.

clino-pathological characteristics and genotypes were analysed using χ^2 test. The odds ratios (OR) are calculated and corresponding 95% CI's are constructed by using Wald statistic. Forward stepwise logistic regression analysis was applied by considering alcohol consuption, smoking habit, BMI, familial cancer history, menarche, menopause status and all genotype variables. P values <0.05 were considered to be statistically significant.

Results

Characteristic features of study groups have been included in Table 2. The mean age of individuals with breast cancer was similar to controls. It was determined that there was no significant difference between the groups in terms of smoking habits and alcohol consumption. The menarche age was found similar between patient and controls. The ratio of BMI and familial history of cancer in patients was found to have increased rather than that of the controls [p<0.001; OR: 5.44, 95% CI (2.45–12.10) and p<0.001; OR: 1.299, 95% CI (1.17–1.44) respectively]. The majority of patients (69%) had post-menopause status, whereas post-menopause frequency of individuals in the control group was %23 [p<0.001; OR: 7.452, 95% CI (3.97–13.99)]. Frequencies of genotypes and alleles, and OR values in both groups for each polymorphism were shown in Table 3. Distributions of genotypes for each polymorphism except for DKK4 synonymous exon 4 V169V were found to be compatible with HWE for both groups [For DKK3 non-synonymous (Exon7 Arg335Gly); χ^2=0.21, p=0.64; OR: 1.15, 95% CI (0.62–2.12). For DKK3 (Intron4 G/C); χ^2=0.33, p=0.86; OR: 1.06, 95% CI (0.52–2.17). For DKK4 synonymous (Exon4 V169V); χ^2=42.66, p=0.001; OR: 0.20, 95% CI (0.12–0.33). For sFRP4 non-synonymous (Exon6 R340K); χ^2=0.046, p=0.83; OR: 1.04, 95% CI (0.68–1.59)]. For DKK3 nonsynonymous exon 7 Arg335Gly, DKK3 intron 4 G/C and sFRP4 nonsynonymous exon 6 R340K polymorphisms the genotype distribution among the study groups was determined to be insignificant.

According to the genotype distribution result in terms of DKK4 synonymous exon 4 V169V polymorphism between the groups, the TT genotype was determined to be a protective genotype for breast cancer [p=0.001; OR: 0.36; 95% CI, (0.19–0.68)]. Forward stepwise logistic regression analysis was applied by considering alcohol consuption, smoking habit, BMI, familial cancer history, menarche, menopause status and all genotype variables. (As a result of the analysis, only the influencing variables are given in Table 4). We found that individuals with CC genotype have 16 times more risk for breast cancer development than individuals with CT and TT genotypes for this polymorphism [p=0.001; OR: 16.38; 95% CI, (6.37–42.12)]. Moreover, other risk factors include BMI and sFRP4 nonsynonymous exon

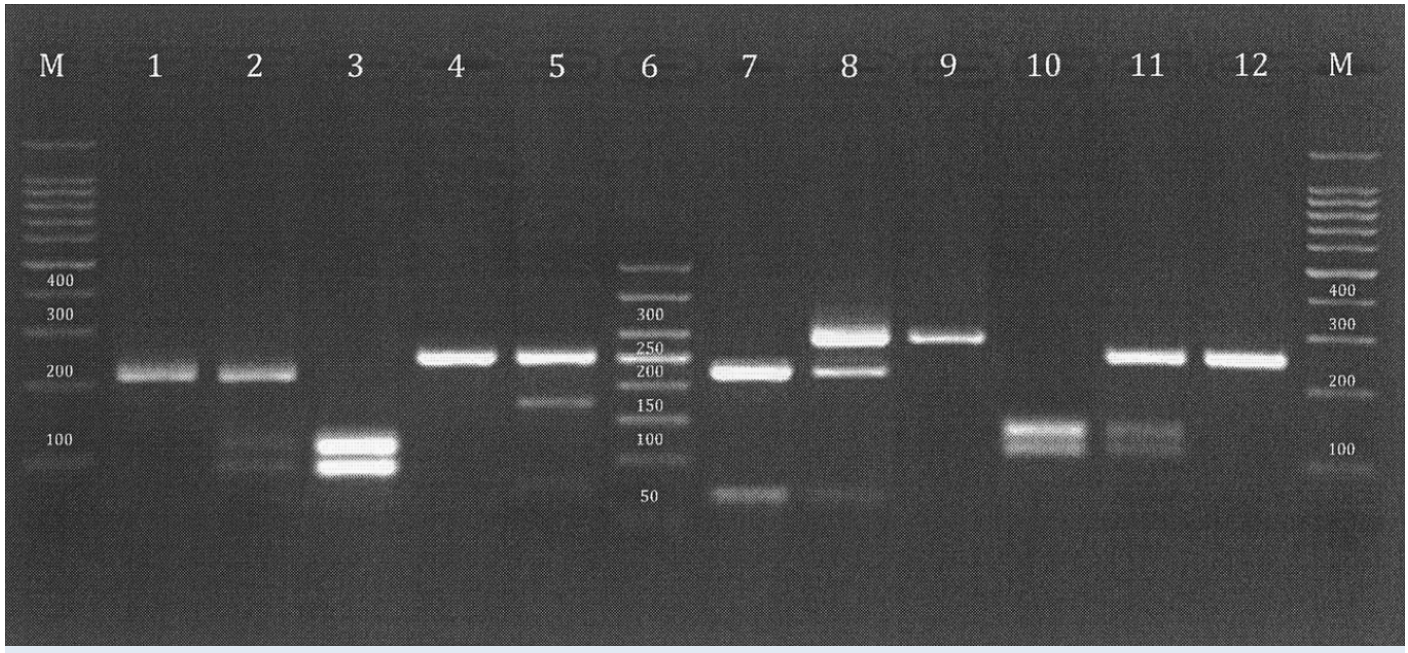

Figure 1. Illustration of products of PCR and RFLP on 2 % agarose gel.

M: 100 bp marker (Biomatics); for DKK3 non-synonymous (Exon7 Arg335Gly) polymorphism: 1-GG wild type (210 bp), 2-GA heterozygous type (210+115+95 bp), 3-AA polymorphic type (115+95 bp); for DKK3 (Intron4 G/C) polymorphism: 4-GG wild type (245 bp), 5-GC heterozygous (245+171+74 bp); 6- 50bp marker (Biomatics); for DKK4 synonymous (Exon4 V169V) polymorphism: 7-CC wild type (224+68 bp), 8-CT heterozygous type (292+224+68 bp), 9- TT polymorphic type (292 bp); for sFRP4 non-synonymous (Exon6 R340K) polymorphism: 10-GG wild type (134+112), 11-GA heterozygous type (246+134+112bp), 12-AA polymorphic type (246 bp); M: 100 bp marker (Biomatics).

6 R340K genotype differences [p=0.001; OR: 6.67, 95% CI (2.38–18.71) and p=0.001; OR: 0.24, 95% CI (0.10–0.57) respectively] (Table 4).

Haplotype structure of DKK3 gene was analysed (Table 5). A linkage was determined for the two alternative forms of this gene in both study groups (χ^2=39.520, p=0.000 for patients; χ^2=68.112, p=0.000 for controls). Since it was prevalent in both groups, the GG served as referent haplotype. As a result of the analysis of 2 polymorphisms of this gene, no statistically significant difference was found for haplotype between the groups (p>0.05).

Additionally, we investigated whether the four SNPs had an effect on clinicopathological parameters. There were no significant effects of SNPs on grade, pT, pN, and pM, except for DKK3 nonsynonymous exon 7 Arg335Gly polymorphisim effect on pM (Table 6).

Discussion

Cancer is a complex disease affecting cells and tissues that can be defined as a signal transduction pathway disorder. The etiology is unknown in the majority of breast cancer cases. Nevertheless, gender, age, presence of a family history of cancer, early menstruation age, menopause at an older age, estrogens, alcohol consumption, tobacco use, genetic mutations are thought to have important effects on the development of breast cancer. However, the genetic abnormalities that occur in many signal transduction pathways have a great contribution to mammary gland carcinogenesis. Among these, the Wnt pathway is known to be of critical importance in the epithelial to mesenchymal transition during development and in breast cancer.[9]

Age has an important place among breast cancer risk factors, and as age increases, the risk of individuals developing this cancer also increases.[10] However, there are also studies in Brazil[11] and China[12] indicating an increased likelihood of breast cancer in adolescent and young adult women. In addition, a study conducted in America reported that breast cancer has increased in women under the age of 40.[13] Consistent with our results (mean age of the patient group; 47.60±8.92, between 31 and 71 years), in a study conducted throughout Colombia, the age range that poses a risk was found to be 45 to 64 years.[14]

Smoking, which is among the environmental factors, contains possible carcinogen substances.[15] It has been stated that breast cancer development increases by 35% in people who actively smoke[16] and that the risk increases in women who started smoking early in life and continued smoking for at least 20 years.[17] According to our data, no significant difference was found between the patient and

Table 2. The characteristic features of patients and controls

Features	Patients (n=100)	Controls (n=100)	p	OR (95% CI)
Age (year±SD)	47.60 ± 8.92	46.05 ± 9.84	0. 245	
Smoking habit Smoker/Non-smoker	11/89	8/92	0. 469	1.42 (0.55–3.70)
Alchool consuption (yes/no)	0/100	2/98	0. 497	1.02 (0.99–1.05)
BMI (<25kg/m^2 /≥25kg/m^2)	9/91	35/65	<**0.001**	**5.44 (2.45–12.10)**
Familial cancer history (Yes/No)	23/77	0/100	<**0.001**	**1.299 (1.17–1.45)**
Age at menarche (<13 /≥13 years)	23/77	26/74	0. 622	1.176 (0.62–2.24)
Menopause status			<**0.001**	**7.452 (3.97–13.99)**
Pre-menopause	31	77		
Post-menopause	69	23		
Tumour size (Tx-T4)				
Tx	3 (3%)			
T1	28 (28%)			
T2	54 (54%)			
T3	14 (14%)			
T4	1 (1%)			
Tumour grade (G1-G3)				
G1	28 (28%)			
G2	51 (51%)			
G3	21 (21%)			
Lymph node + (%)	69 (69%)			
Metastasis + (%)	20 (20%)			

OR: Odd ratios; CI: Confidence interval; SD: Standard derivation; BMI: Body mass index; P<0.05: Statistically significant; P<0.001: Statistically highly significant.

control groups in terms of smoking (p=0.469). When we evaluated alcohol use, which is another risk factor,[18–20] we found that it did not pose a risk for our patient group (p=0.497). As with all types of cancer, the presence of individuals in their families who have had breast cancer is an important risk for this cancer. And familial cancer history increases the risk of breast cancer by 2–3 times.[21,22] Our findings suggest that people with a family history of cancer are at risk (p<0.001). On the other hand, consistent with other studies,[23,24] when BMI was compared, there was a significant difference between the groups (p<0.001). Our results showed that early menarche did not pose a risk for breast cancer, while there was a significant difference between the groups when menopausal status was assessed (p<0.001). The increased number of cycles due to late menopause causes increased DNA damage in the proliferating duct tissue, which may increase the risk of mutations that can directly lead to breast cancer. Inconsistent with the literature, in our study, it was found that the number of individuals in the patient group who entered menopause was higher than in the control group. In people who receive chemotherapy and radiotherapy due to cancer treatment, the ovaries are severely affected and may lead to early menopause. The type of cancer, the extent to which the ovaries are affected, the course of treatment, and the type and dose of medications used for treatment can trigger menopause.[25] The risk of early menopause is higher in women treated for breast cancer.[26] As a result, the fact that the number of menopausal individuals in the patients is higher is attributed to the treatment they received.

By this time, no studies have examined breast cancer risk for the four SNPs mentioned in this study, but their associations have been investigated in different types of cancer.[7,27] Our case–control study investigating the four polymorphisms also revealed its importance in breast cancer, especially with DKK4 synonymous (Exon4 V169V) SNP. The T allele was found to be protective and

Table 3. Distributions of genotypes and alleles for each polymorphism

Polymorphism	Genotypes and alleles	Patients n (%)	Controls n (%)	p	OR (95%CI)
DKK3 non-synonymous (Exon7 Arg335Gly)	GG	76 (76%)	79 (79%)		Reference
	GA	23 (23%)	20 (20%)	0.605	1.19 (0.6–2.35)
	AA	1 (1%)	1 (1%)	0.740	1.03 (0.06–16.9)
	G	175 (87.5%)	178 (89%)		Reference
	A	25 (12.5%)	22 (11%)	0.641	1.15 (0.62–2.12)
DKK3 (Intron4 G/C)	GG	83 (83%)	84 (84%)		Reference
	GC	17 (17%)	16 (16%)	0.849	1.07 (0.5–2.27)
	CC	0 (0%)	0 (0%)		
	G	183 (91.5%)	184 (92%)		Reference
	C	17 (8.5%)	16 (8%)	0.856	1.06 (0.52–2.17)
DKK4 synonymous (Exon4 V169V)	CC	75 (75%)	40 (40%)		Reference
	CT	25 (25%)	37 (37%)	**0.001***	**0.36 (0.19–0.68)**
	TT	0 (0%)	23 (23%)		
	C	175 (88.1%)	117 (58.5%)		Reference
	T	25 (11.9%)	83 (41.5%)	**0.001***	**0.20 (0.12–0.33)**
sFRP4 non-synonymous (Exon6 R340K)	GG	47 (47%)	44 (44%)		Reference
	GA	40 (40%)	48 (48%)	0.407	0.78 (0.43–1.40)
	AA	13 (13%)	8 (8%)	0.396	1.52 (0.57–4.02)

OR: Odd ratios; CI: Confidence interval; DKK3: Dickkopf 3; DKK4: Dickkopf 4; sFRP4: Secreted frizzled-related protein 4; A: Adenine; C: Cytosine; G: Guanine; T: Thymine; *: P<0.05: Statistically significant.

Table 4. Variables affecting breast cancer

Variables	B (regression coefficient)	SE (standard error)	p	Exp (B) (OR)	(95% CI)
BMI	1.89	0.52	0.001	6.67	2.38–18.71
	2.38–18.71				
					6.37–42.12
DKK4	2.79	0.48	0.001	16.38	
	6.37–42.12				0.10–0.57
sFRP4	-1.38	0.42	0.001	0.24	
	0.10–0.57				

OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; DKK4: Dickkopf 4; sFRP4: Secreted frizzled-related protein; P<0.05: Statistically significant.

reduce breast cancer risk (ORT: 0.20; 95% CI: 0.12–0.33) for this SNP. We did not obtain significant results for the other three SNPs that we thought could pose a risk. In a study conducted by Hirata et al.,[7] it was found that DKK3 nonsynonymous exon 7 Arg335Gly polymorphism was associated with renal cancer and that the GG+AA genotype was higher in the patient group than in the control group. In contrast, similar to the findings of studies conducted with lung cancer patients,[27,28] we find that genotype distributions of the DKK3 rs3206824 gene polymorphism (nonsynonymous exon 7 Arg335Gly) in two study groups was insignificant (p>0.05). In addition, the effect of another polymorphism (DKK3 intron 4 G/C) in the DKK3 gene was also investigated, but it was determined that this polymorphism was not associated with breast cancer. Unlike our findings, Hirata et al.[7] indicated that DKK3 rs7396187 (intron 4 G/C) genotypes frequency in patients with renal cell carcinoma was found significant

Table 5. Haplotype analysis for DKK3 rs3206824 and rs7396187 polymorphisms

DKK3 rs3206824 (exon7 G/A) - rs7396187 (intron G/C) haplotype

		Patients n (%)	Controls n (%)	p	OR (95% CI)
G	G	85	60		Reference
G	C	2	0	0.51	0.97 (0.94–1.009)
A	G	6	12	**0.04***	**2.83 (1.007–7.97)***
A	C	7	28	**0.000***	**5.66 (2.32–13.82)***

*: P<0.05: Statistically significant; OR: Odds ratio; CI: Confidence interval; DKK3: Dickkopf 3; A: Adenine; C: Cytosine; G: Guanine.

different compared to controls. In another study, it was determined that this polymorphism did not pose a risk for lung cancer.[27] We also found that for breast cancer, DKK3 gene haplotypes do not expose risk.

Another member of DKK family is DKK4. It was determined that DKK4 synonymous (Exon4 V169V) SNP, whose relationship we investigated with breast cancer, did not pose a risk for some types of cancer.[27,29] Contrasting these findings, Hirata et al.[7] stated in their study TT genotype was higher in the patient group than in the control group. Our results for this polymorphism showed that allele and genotype distributions were significantly different between the groups (Table 3). In contrast to the results found by Hirata et al.,[7] our results showed that the TT genotype and the T allele were protective against breast cancer (Table 3). In our study, Forward Stepwise logistic regression analysis was also performed by considering demographic characteristics and genotype variables. Regression analysis showed that the variables affecting breast cancer were body mass index, DKK4 and sFRP4 genotype differences (Table 4). When evaluated in terms of DKK4 polymorphism, it was determined that the risk of CC genotype in patient individuals was 16.38 times higher (p=0.001) (OR: 16.38, %95 CI: 6.37–42.12).

Table 6. Genotype distributions of five SNPs according to clinicopathological parameters of BC

SNP	Tumour stage n (%)					Tumour grade n (%)			Lymph node n (%)		Metastasis n (%)	
Gen.	pTx	pT1	pT2	pT3	pT4	G1	G2	G3	Absent	Present	Absent	Present
DKK3 non- synonymous (Exon7 Arg335Gly-c.1003 A/G)												
GG	3 (100)	18 (64.3)	41 (75.9)	13 (92.9)	1 (100)	23 (82.1)	41 (80.4)	12 (57.1)	21 (67.7)	55 (79.7)	58 (72.5)	18 (90)
GA	0 (0)	9 (32.1)	13 (24.1)	1 (7.1)	0 (0)	5 (17.9)	9 (17.6)	9 (42.9)	10 (32.3)	13 (18.8)	22 (27.5)	1 (5)
AA	0 (0)	1 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (1.5)	0 (0)	1 (5)
P value	0.494					0.149			0.283		**0.017***	
DKK3 (Intron4 G/C)												
GG	3 (100)	22 (78.6)	45 (83.3)	12 (85.7)	1 (100)	23 (82.1)	41 (82)	12 (57.1)	25 (80.6)	58 (84.1)	65 (81.3)	18 (90)
GC	0 (0)	6 (21.4)	9 (16.7)	2 (14.3)	0 (0)	5 (17.9)	9 (18)	9 (42.9)	6 (19.4)	11 (15.9)	15 (18.7)	2 (10)
CC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P value	0.864					0.475			0.775		0.356	
DKK4 synonymous (Exon4 V169V-57C/T)												
CC	3 (100)	21(75)	39 (72.2)	11 (79)	1 (100)	18 (64.3)	38 (74.5)	19 (90.5)	21 (67.7)	54 (78.3)	17 (85)	58 (72.5)
CT	0 (0)	7 (25)	15 (27.8)	3 (21)	0 (0)	10 (35.7)	13 (25.5)	2 (9.5)	10 (32.3)	15 (21.7)	3 (15)	22 (27.5)
TT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
P value	0.800					0.111			0.320		0.248	
sFRP4 non- synonymous (Exon6 R340K-c.1019 G/A)												
GG	2 (66.7)	11 (39.3)	28 (51.9)	6 (42.9)	0 (0)	10 (35.7)	29 (56.9)	8 (38.1)	11 (35.5)	36 (52.2)	12 (60)	35 (43.8)
GA	0 (0)	1 (46.4)	21 (38.9)	5 (35.7)	1 (100)	15 (53.6)	16 (31.4)	9 (42.9)	14 (45.2)	26 (37.7)	6 (30)	34 (42.5)
AA	1 (33.3)	4 (14.3)	5 (9.3)	3 (21.4)	0 (0)	3 (10.7)	6 (11.8)	4 (19)	6 (19.3)	7 (10.1)	2 (10)	11 (13.8)
P value	0.610					0.266			0.227		0.428	

SNP: Single nucleotide polymorphism; BC: Breast cancer; Gen: Genotype; *: P<0.05: statistically significant.

In studies conducted with both lung cancer[27] and renal cancer patients,[7] the effect of the sFRP4 nonsynonymous exon 6 R340K polymorphism was revealed, and it was determined that the AA genotype creates a predisposition for both types of cancer. Another study revealed that the GA genotype was significantly increased in prostate cancer patients.[30] In our study, genotype distribution was found indifferent between the groups (p>0.05). However, according to logistic regression analysis, the risk was found to be 0.24 times higher in individuals with the AA genotype (p=0.001) (OR: 0.24, %95 CI: 0.10–0.57). Consequently, it was thought that sFRP4 nonsynonymous exon 6 R340K SNP is a candidate risk factor for breast cancer.

Our study also investigated whether these four polymorphisms had an effect on clinicopathological factors. There are studies suggesting that single nucleotide polymorphism may be associated with metastasis, although not with prognosis.[31,32] Apart from this, genetic possibilities that may have an impact on breast cancer development and prognosis were determined in another study.[33] As a result of our evaluation, it was determined that the DKK3 nonsynonymous exon 7 Arg335Gly polymorphism had an effect only on the formation of distant metastases (p=0.017). It is thought that the reason why no significant relationship was found between polymorphisms and clinicopathological factors is due to the fact that single nucleotide polymorphisms, which act as a risk factor, are not always a prognostic factor. Because risk-creating single nucleotide polymorphisms are part of the early stages of carcinogenesis in almost normal cells. Single nucleotide polymorphisms that determine prognosis are necessary for the maintenance of fully transformed cells.[7]

The mechanisms through which these polymorphisms impact breast cancer still remain unclear. Non-synonymous SNPs cause amino acid changes. As a result, protein function may be affected. For this reason, it is thought that non-synonymous single nucleotide polymorphisms may be associated with cancer susceptibility.[34] Additionally, synonymous single nucleotide polymorphisms can change mRNA folding and minimize mRNA stability. Therefore, there may be differences in translation through changes in RNA structure.[35,36] In the light of the data obtained, differences that may occur in the gene structure of Wnt antagonists will also affect the interactions of the protein. It can be said that these antagonists, which modulate the Wnt pathway, also regulate target gene transcription. As a result, cancer development may occur as a result of abnormalities in Wnt signal transmission.

Conclusion

As a result, this study is the first preliminary study investigating the possible relationship between breast cancer and these four antagonist gene polymorphisms. In particular, expanding the patient group and studying DKK4 synonymous exon 4 V169V and sFRP4 nonsynonymous exon 6 R340K polymorphisms in different populations may support our results. It is thought that expanding the study group could make the study meaningful in terms of the two polymorphisms of the DKK3 gene. In addition to these results, understanding Wnt antagonists will also lead to the development of new treatment methods.

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