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RESEARCH ARTICLE

Effect of *HOTAIR* rs920778 polymorphism on breast cancer susceptibility and clinicopathologic features in a Turkish population

Süleyman Bayram · Ahmet Taner Sümbül · Celal Yücel Batmacı · Ahmet Genç

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Abstract Overexpression of Hox transcript antisense intergenic RNA (*HOTAIR*), a long non-coding RNA (lncRNA), is associated with cancer cell proliferation, invasion, progression, and metastasis as well as poor survival in a variety of human cancers including breast cancer (BC). A common functional single nucleotide polymorphism (SNP) rs920778 (T→C) in the intronic enhancer of the *HOTAIR* has been reported to influence *HOTAIR* expression and cancer predisposition, but the association of *HOTAIR* rs920778 polymorphism with BC susceptibility and clinicopathological features has yet to be investigated. We genotyped *HOTAIR* rs920778 polymorphism in 245 Turkish women including 123 BC patients and 122 age-matched healthy controls by a real-time polymerase chain reaction (PCR) with the TaqMan assay. We found that the CC genotype of *HOTAIR* rs920778 polymorphism significantly increased the risk of BC in both codominant (odds ratio (OR)=2.12, 95 % confidence interval (CI) 1.00–4.51, P=0.05) and recessive (OR=2.40, 95 % CI 1.22–4.73, P=0.01) inheritance genetic models. Our research also indicated an association between the CC genotype of *HOTAIR* rs920778 polymorphism and clinicopathologic features of tumor, including advanced tumor–node–metastasis (TNM) stage, larger tumor size, distant metastasis, and poor histological grade (P<0.05). Because our findings suggest for the first time that the CC genotype of *HOTAIR* rs920778 polymorphism might play important roles in genetic susceptibility to BC development and aggressiveness in a Turkish population, further independent studies are required to validate our findings in a larger series, as well as in patients of different populations.

Keywords Breast cancer · *HOTAIR* · lncRNA · *HOTAIR* rs920778 polymorphism · Genetic susceptibility · Clinicopathologic features

Süleyman Bayram and Ahmet Taner Sümbül contributed equally to this work.

S. Bayram (✉)
Department of Nursing, Adıyaman School of Health, Adıyaman University, 02040 Adıyaman, Turkey
e-mail: slymnbyrm81@gmail.com

A. T. Sümbül
Department of Medical Oncology, Medical Faculty, Mustafa Kemal University, 31000 Hatay, Turkey

C. Y. Batmacı
Department of Internal Medicine, Medical Faculty, Mustafa Kemal University, 31000 Hatay, Turkey

A. Genç
Vocational School of Health Services, Adıyaman University, 02040 Adıyaman, Turkey

Introduction

Breast cancer (BC) is the second most common type of human malignancies worldwide and the first leading cause of cancer-related deaths among women with an estimated 1,677,000 new cases and 522,000 deaths in 2012 [1, 2]. In Turkey, BC is the most common cancer and is ranked the first leading cause of cancer-related death in Turkish women [2]. It is now well established that various epidemiological risk factors contribute to breast carcinogenesis, including age, early menarche, late menopause, late first full-term pregnancy, no breast feeding, nulliparity, oral contraceptive use, hormone replacement therapy, obesity, no physical activity, cigarette smoking, excessive alcohol intake, other unhealthy lifestyles, exposure to low-dose ionizing radiation, family history of BC in two or more first-degree relatives, previous benign breast disease,

and high-fat-based diets [3, 4]. Besides these epidemiological risk factors, evidence has also shown that genetic susceptibility factors were also found to be associated with BC risk and prognosis [5, 6]. Single nucleotide polymorphisms (SNPs) represent the largest class of genetic susceptibility factors on breast carcinogenesis, and this class of genetic susceptibility factors attracts much attention because of its effect on a wide range of cellular functions [4–7]. Therefore, identification of additional potential SNPs may result in the increased prediction of BC susceptibility and provide the earlier application of proper therapeutic strategies to decrease mortality rate of BC [8].

A new group of regulatory non-coding RNAs that is longer than 200 nucleotides (nt) in length (frequently up to 100 kbp) is named long non-coding RNA (lncRNA) [9–11]. lncRNAs are crucial players in a wide range of biological processes including chromatin remodeling, genome packaging, genome rearrangement, dosage compensation, gene imprinting, and regulation of gene expression [12–16]. A great number of lncRNAs arrange gene expression via their interaction with chromatin modification complexes [16]. One of the best known lncRNA-regulated chromatin modification complexes is polycomb-repressive complex 2 (PRC2) that consists of histone H3-lysine 27 (H3K27)-specific histone methyl transferase EZH2 (enhancer of zeste homolog 2) and several other polycomb groups of proteins such as Suz12 and EED [16–18]. Within the PRC2-interacting lncRNAs, *Hox transcript antisense intergenic RNA* (*HOTAIR*) is expressed from the antisense strand of the homebox C (HOXC) that recruits PRC2 to its specific target genes [16–18]. Gupta et al. [18] reported that high expression of *HOTAIR* in breast cancer cell lines could promote colony growth and invasion by modifying the expression of many genes related to tumor aggressiveness via the PRC2 chromatin modification complex. They also showed that overexpression of *HOTAIR* in patients with BC was a significant predictor of subsequent metastasis and poor prognosis [18]. Moreover, Wu et al. [19] reported that overexpression of *HOTAIR* is correlated with cancer cell proliferation, invasion, progression metastasis, and poor prognosis in a variety of human carcinomas. All these convincing proofs indicate the oncogenic role of *HOTAIR* in the course of several human carcinogeneses including breast cancer.

The *HOTAIR* gene is located on the long arm of chromosome 12 (12q13.13). There are a few SNPs which are reported in the *HOTAIR* gene (http://www.ncbi.nlm.nih.gov/snp/). Even with the potential significance of the *HOTAIR* gene in human carcinogenesis, only two studies have been conducted in human carcinomas about SNPs and their functional significance in the *HOTAIR* gene [20, 21]. For example, functional analysis of *HOTAIR* rs920778 polymorphism has been performed so far only [20]. *HOTAIR* rs920778 polymorphism occurs as a result of substituting cytosine to thymine (C→T) which is located on intron 2 of the *HOTAIR* gene [20]. A study conducted recently found that a new intronic enhancer is located on intron 2 of the *HOTAIR* gene which lies *HOTAIR* rs920778 polymorphism in this region [20]. *HOTAIR* rs920778 polymorphism located within the new intronic enhancer region of the *HOTAIR* gene has a genotype-specific effect on *HOTAIR* expression, which results in a higher *HOTAIR* expression among T allele carriers [20]. Additionally, Zhang et al. [20] found that the TT genotype of *HOTAIR* rs920778 polymorphism had enhanced esophageal squamous cell carcinoma (ESCC) susceptibility risk in Chinese populations. In another work related to the *HOTAIR* polymorphism, it was shown that *HOTAIR* rs12826786 polymorphism has a genotype-specific effect on HOTAIR expression and is associated with the risk of gastric adenocarcinoma as well as poor clinicopathologic features in a population of North China [21].

According to our latest knowledge, no research has been executed to evaluate the *HOTAIR* rs920778 polymorphism and risk of BC as well as clinicopathologic features of BC. Given the very important role of *HOTAIR* in breast carcinogenesis, the present study was aimed to investigate the role of *HOTAIR* rs920778 polymorphism in BC susceptibility and clinicopathologic features of BC in a Turkish population. To test the hypothesis that the *HOTAIR* rs920778 polymorphism is related with susceptibility of BC and clinicopathologic features of BC, we carried out genotyping analysis by real-time polymerase chain reaction (PCR) with TaqMan assay in a hospital-based case–control study design comprising 123 BC patients and 122 age-matched healthy controls from Turkey.

Materials and methods

Ethics statement

The study was approved by the Human Ethics Committee of the Faculty of Medicine, Mustafa Kemal University (Hatay, Turkey). All the participators ensured their written informed consent to be included in the study concerning the use of their blood specimens for research studies. The study continued in agreement with the statement on the Declaration of Helsinki confirmed by the World Medical Association meeting in Edinburgh.

Study population

This hospital-based case–control study comprised a total of 245 women subjects including 123 sporadic BC cases and 122 healthy controls. Informed consent about the study was taken from all the participants. All participants were over 18 years old and genetically unrelated Turkish and were from the surrounding areas of southern Turkey. Healthy controls

were frequency matched to BC cases on age and recruited from volunteers who came to the hospital for their routine checkups. Selection criteria for controls included no evidence of any personal history of cancer or other malignant conditions. All BC cases were newly diagnosed, clinically and histologically confirmed with primary BC and were gathered from the Department of Medical Oncology between October 2013 and November 2014. Classification of BC was carried out according to the seventh edition of the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) staging system [22].

All clinical and pathological data of patients are taken as follows. Clinicopathological variables of BC patients including age, age at onset, histologic type of cancer, TNM stage, tumor size, lymph node status, nodal status, distant metastasis, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER-2/neu) status, lymphovascular invasion, and perineural invasion were collected from the patients' medical records with the help of the oncologist.

DNA extraction

Whole blood samples were collected into a test tube containing EDTA from BC patients and healthy women controls. None of the BC patients received chemotherapy or radiotherapy prior to whole blood collection. Genomic DNA was isolated from the whole blood specimen of all participants using the AxyPrep Blood Genomic DNA Miniprep KitAP-MN-BL-GDNA-250 (Wujiang, Jiangsu, China) according to the manufacturer's directions. The quantity and quality of DNA was identified by the Qubit® Fluorometer (Invitrogen, Carlsbad, CA, USA).

Genotyping

SNP rs920778 in the *HOTAIR* gene is genotyped using a TaqMan allelic discrimination assay (TaqMan® SNP Genotyping AssayID numbers for rs920778: C___9162435_20) according to the protocols described by the manufacturers (Applied Biosystems, Foster City, CA, USA). For each sample, 10 ng of DNA was used per reaction with 5 μL of 2× TaqMan® Universal Master Mix II, 200 nM primers, and 250 nM TaqMan probes (Applied Biosystems, Foster City, CA, USA). TaqMan PCR was conducted with the LightCycler 96 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The following cycling conditions were used: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. All genotypes were determined by LightCycler Genotyping software with endpoint genotyping analysis (Roche Diagnostics GmbH, Mannheim, Germany). To provide quality control, 15 % of the study populations were randomly selected without knowledge of the subjects' case–control status and subjected to repeat analysis by different persons; reproducibility was 100 %. The success rate of genotyping was 100 %, and 123 BC patients and 122 healthy controls were finally included for subsequent statistical analyses.

Statistical analysis

An effective sample size to detect significant differences in the distributions of genotypes/alleles between BC patients and healthy controls was calculated by Quanto (version 1.1) software (http://hydra.usc.edu/gxe), and the power was set at 80 % [23]. Statistical Package for Social Sciences (SPSS) software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for data handling, dataset management, and statistical analyses. Descriptive statistics of BC patients and healthy controls were presented as the mean and standard deviations (SD) for continuous variables, while frequencies and percentages were used for categorical variables. Genotype and allele frequencies of *HOTAIR* rs920778 polymorphism were computed and checked for deviation from the Hardy–Weinberg equilibrium (HWE) in both BC patients and healthy controls by using HWE calculator software (http://www.oege.org/software/hwe-mr-calc.shtml) [24]. The online tool SNPStats (http://bioinfo.iconcologia.net/SNPstats) was used to assess the strength of the association between *HOTAIR* rs920778 polymorphism and breast cancer risk in all genetic models (codominant, dominant, recessive, overdominant, and log-additive) of inheritance [25]. BC risk estimates were expressed as the odds ratio (OR) and its corresponding 95 % confidence interval (CI). To evaluate the relationship between *HOTAIR* rs920778 polymorphism and clinicopathological features including age at onset, histologic type of cancer, TNM stage, tumor size, lymph node status, nodal status, distant metastasis, histological grade, ER status, PR status, HER-2/neu status, lymphovascular invasion, and perineural invasion were assessed through Pearson's chi-square (χ^2) test or Fisher's exact test. All tests were two sided, and a P value ≤0.05 was considered to be statistically significant.

Results

A total of 245 age-matched Turkish women subjects (123 BC patients and 122 healthy controls) were genotyped to find out the possible association between *HOTAIR* rs920778 polymorphism and BC risk in this study. Clinicopathological characteristics of 123 BC patients are shown in Table 1. The mean age was 51± 11.28 and 52.24±11.26 years for BC patients and healthy controls, respectively. As expected, the mean age for two groups paired quite well (P>0.05). The

Table 1 Clinicopathological features of breast cancer patients

Variable	*N*=123 (%)
Age at diagnosis, mean±SD (range 25–77)	51.58±11.28
Age at diagnosis	
≤35	8 (6.50 %)
>35	115 (95.50 %)
Histologic type of cancer	
Infiltrating ductal carcinoma	100 (81.30 %)
Infiltrating lobular carcinoma	13 (10.57 %)
Mixed	10 (8.13 %)
TNM classification	
I	8 (6.50 %)
II	49 (39.84 %)
III	36 (29.27 %)
IV	30 (24.39 %)
Tumor size	
T1	27 (21.95 %)
T2	55 (44.71 %)
T3	29 (23.58 %)
T4	12 (9.76 %)
Lymph node status	
Negative (−)	26 (21.14 %)
Positive (+)	97 (78.86 %)
Nodal status	
N0	26 (21.14 %)
N1	40 (32.52 %)
N2	25 (20.33 %)
N3	32 (26.02 %)
Distant metastasis	
M0 (present)	93 (75.61 %)
M1 (absent)	30 (24.39 %)
Histological grade	
I (good)	13 (10.57 %)
II (intermediate)	63 (51.22 %)
III (poor)	47 (38.21 %)
Estrogen receptor status	
ER negative (−)	31 (25.20 %)
ER positive (+)	92 (74.80 %)
Progesterone receptor status	
PR negative (−)	48 (39.02 %)
PR positive (+)	75 (60.98 %)
HER-2/neu status	
Negative (−)	96 (78.05 %)
Positive (+)	27 (21.95 %)
Lymphovascular invasion	
Negative (−)	57 (46.34 %)
Positive (+)	66 (53.66 %)
Perineural invasion	
Negative (−)	81 (65.85 %)
Positive (+)	42 (34.15 %)

genotype frequencies of *HOTAIR* rs920778 polymorphism did not depart from the Hardy–Weinberg expectation both in BC patients and in the healthy control groups ($P>0.05$).

Distribution of the *HOTAIR* rs920778 polymorphism genotypes and alleles in BC patients and in healthy control subjects is presented in Table 2. The frequencies of the T allele and TT genotype of *HOTAIR rs920778* polymorphism were predominant in BC patients and in healthy control subjects. The minor allele (C allele) frequencies (MAF) in BC patients and in healthy control subjects were 0.463 and 0.393, respectively (Table 2). The C allele of *HOTAIR* rs920778 polymorphism was not associated with BC risk when compared with the T allele of *HOTAIR* rs920778 polymorphism (OR=1.33, 95 % CI 0.93–1.91, $P=0.12$). As shown in Table 2, a statistically significant association was observed in both codominant and recessive genetic inheritance models. In the codominant genetic inheritance model, the CC genotype of *HOTAIR* rs920778 polymorphism has a higher frequency in BC patients when compared to healthy control subjects (25.2 and 12.3 %) and is associated with an increased risk of BC (TT vs. CC: OR=2.12, 95 % CI 1.00–4.51, $P=0.05$). Moreover, the CC genotype of *HOTAIR* rs920778 polymorphism increased BC risk to 2.40-fold in the recessive genetic inheritance model (TT+TC vs. CC: OR=2.40, 95 % CI 1.22–4.73, $P=0.01$). However, no significant association between *HOTAIR* rs920778 polymorphism and the risk of BC was observed in the dominant (TT vs. TC+CC: OR=1.05, 95 % CI 0.62–1.79, $P=0.86$), overdominant (TT+CC vs. TC: OR=0.62, 95 % CI 00.38–1.03, $P=0.07$), and log-additive (OR=1.33, 95 % CI 0.93–1.90, $P=0.07$) genetic inheritance models.

Moreover, we demonstrated the association of *HOTAIR* rs920778 polymorphism genotypes with the demographical and clinicopathological features in Table 3, including the patient's age at diagnosis, TNM stage, tumor size, lymph node status, nodal status, distant metastasis, histological grade, ER status, PR receptor status, HER-2/neu status, lymphovascular invasion, and perineural invasion. According to the recessive genetic inheritance model, we found that the CC genotype of *HOTAIR* rs920778 polymorphism was associated with advanced TNM (III and IV) classification ($P=0.025$), larger tumor size (T3 and T4) ($P=0.033$), presence of distant metastasis ($P=0.022$), and poor (III) histological grade ($P=0.028$) in BC patients, but was not related with the patient's age at diagnosis, histologic type of cancer, lymph node status, nodal status, ER status, PR status, HER-2/neu status, and lymphovascular invasion. Interestingly, we determined a statistically significant inverse relationship between the CC genotype of *HOTAIR* rs920778 polymorphism and positive perineural invasion ($P=0.024$).

Table 2 Allele/genotype frequency and model inheritance for *HOTAIR* rs920778 polymorphism among breast cancer patients and control subjects as well as the association with risk of breast cancer

	Breast cancer, n=123 (%)	Controls, n=122 (%)	OR (95 % CI)	P value[a]	AIC	BIC
Allele						
T	132 (53.7 %)	148 (60.7 %)	1.00 (reference)			
C	114 (46.3 %)	96 (39.3 %)	1.33 (0.934–1.91)	0.12		
Codominant						
TT	40 (32.5 %)	41 (33.6 %)	1.00 (reference)		338.3	348.8
TC	52 (42.3 %)	66 (54.1 %)	0.81 (0.46–1.42)	0.46		
CC	31 (25.2 %)	15 (12.3 %)	2.12 (1.00–4.51)	0.05		
Dominant						
TT	40 (32.5 %)	41 (33.6 %)	1.00 (reference)		343.6	350.6
TC+CC	83 (67.5 %)	81 (66.4 %)	1.05 (0.62–1.79)	0.86		
Recessive						
TT+TC	92 (74.8 %)	107 (87.7 %)	1.00 (reference)		336.8	343.8
CC	31 (25.2 %)	15 (12.3 %)	2.40 (1.22–4.73)	0.01		
Overdominant						
TT+CC	71 (57.7 %)	56 (45.9 %)	1.00 (reference)		340.2	347.2
TC	52 (42.3 %)	66 (54.1 %)	0.62 (0.38–1.03)	0.07		
Log-additive	–	–	1.33 (0.93–1.90)	0.12	341.2	348.2

AIC Akaike's information criterion, *BIC* Bayesian information criterion

[a] Data were calculated by logistic regression analysis

Discussion

The discovery of genetic factors liable for susceptibility to BC is needed to understand tumorigenesis and explore effective prevention and therapeutic approaches [7, 8]. In the current molecular epidemiological study, we try to find the association between *HOTAIR* rs920778 polymorphism on the risk of BC susceptibility and clinicopathologic features of BC through a hospital-based case–control study in a Turkish population. To the best of our knowledge, this is the first study to examine the role of *HOTAIR* rs920778 polymorphism in BC tumorigenesis. The major finding of this study is that the CC genotype of *HOTAIR* rs920778 polymorphism is associated with significantly increased risks of BC susceptibility in both the codominant (TT vs. CC: OR=2.12, 95 % CI 1.00–4.51, P=0.05) and recessive (TT+TC vs. CC: OR=2.40, 95 % CI 1.22–4.73, P=0.01) inheritance genetic models (Table 2). In contrast to our findings, Zhang et al. [20] found that the TT genotype of *HOTAIR* rs920778 polymorphism increased the risk of esophageal squamous cell carcinoma (ESCC) in Chinese Jinan, Shijiazhuang, and Huaian populations when compared with the CC genotype of *HOTAIR* rs920778 polymorphism. Rational explanation for the significant difference in the results of studies may be attributable to differences in the genetic background of studied population, discrepancies in sample sizes, diversities of cancer types, and varieties of genotyping techniques as well as random errors. For example, ethnic differences have been reported regarding the allele frequency of several polymorphisms (International HapMap Project). According to the HapMap data (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=920778), the allele frequency of *HOTAIR* rs920778 polymorphism shows significant differences between different ethnic populations (Table 4). In this study, we found that the C allele frequency of *HOTAIR* rs920778 polymorphism was 0.393 in our healthy Turkish women subjects, similar to the determined allele frequencies in Caucasian populations (HapMap-CEU and HapMap-TSI) (Table 4). In the study conducted by Zhang et al. [20], the frequency of the C allele was determined to be 0.790 in Chinese Jinan, Shijiazhuang, and Huaian populations. However, the C allele frequency of *HOTAIR* rs920778 polymorphism in Chinese Jinan, Shijiazhuang, and Huaian populations is not similar to HapMap data of Chinese populations (HapMap-CH, HapMap-CHD, and HapMap-HCB) (Table 4). In addition, in a recently conducted study form Chinese population, it has been shown that genotype and allele frequencies of another *HOTAIR* polymorphism (rs4759314) were different between healthy controls and HapMap data [21]. In spite of this, the subjects of healthy controls and HapMap data were an ethnically homogeneous Chinese Han population [21]. Differences from HapMap data may have resulted from the method used for genotyping. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping technique is used in both of these studies [20, 21].

Table 3 Genotype distribution of *HOTAIR* rs920778 polymorphism with respect to clinicopathological features of breast cancer patients

Variables		*HOTAIR* rs920778 polymorphism		Total	χ^2	*P*
		TT+TC	CC			
Age at diagnosis	≤35	5 (62.50 %)	3 (37.50 %)	8	0.68	0.41
	>35	87 (75.70 %)	28 (24.30 %)	115		
Histologic type of cancer	Ductal	74 (75.60 %)	26 (24.40 %)	100	2.27	0.32
	Lobular	12 (92.30 %)	1 (7.70 %)	13		
	Mixed	7 (70.00 %)	3 (30.00 %)	10		
TNM classification	Early (I and II)	48 (84.20 %)	9 (15.80 %)	57	4.99	*0.025*
	Advanced (III and IV)	44 (66.70 %)	22 (33.30 %)	66		
Tumor size	T1+T2	68 (82.90 %)	14 (17.10 %)	82	4.53	*0.033*
	T3+T4	27 (65.90 %)	14 (34.10 %)	41		
Lymph node status	Negative (−)	21 (80.80 %)	5 (19.20 %)	26	0.48	0.49
	Positive (+)	72 (74.20 %)	25 (25.80 %)	97		
Nodal status	N0+N1	50 (75.80 %)	16 (24.20 %)	66	0.07	0.79
	N2+N3	42 (73.70 %)	15 (26.30 %)	57		
Distant metastasis	M0 (Absent)	75 (80.60 %)	18 (19.40 %)	93	5.24	*0.022*
	M1 (Present)	18 (60.00 %)	12 (40.00 %)	30		
Histological grade	I+II (good+intermediate)	62 (56.80 %)	14 (19.20 %)	76	4.85	0.028
	III (poor)	30 (63.80 %)	17 (36.20 %)	47		
Estrogen receptor status	ER negative (−)	23 (74.20 %)	8 (25.80 %)	31	0.008	0.93
	ER positive (+)	69 (75.00 %)	23 (25.00 %)	92		
Progesterone receptor status	PgR negative (−)	32 (66.70 %)	16 (33.30 %)	48	2.76	0.10
	PgR positive (+)	60 (80.00 %)	15 (20.00 %)	75		
HER-2/neu status	Negative (−)	73 (76.00 %)	23 (24.00 %)	96	0.36	0.55
	Positive (+)	19 (70.40 %)	8 (29.60 %)	27		
Lymphovascular invasion	Negative (−)	41 (71.90 %)	16 (28.10 %)	57	0.08	0.77
	Positive (+)	49 (74.20 %)	17 (28.80 %)	56		
Perineural invasion	Negative (−)	54 (66.70 %)	27 (33.30 %)	81	5.11	*0.024*
	Positive (+)	36 (85.70 %)	6 (14.30 %)	42		

The clinicopathological features are considered as the most important prognostic factor. The distribution of clinicopathological features and genotypes of *HOTAIR* rs920778 polymorphism in BC patients were estimated to

Table 4 Allele frequencies of *HOTAIR* rs920778 polymorphism according to the HapMap data

Population ID	Individual group	C allele frequency of *HOTAIR* rs920778 polymorphism	T allele frequency of *HOTAIR* rs920778 polymorphism
HapMap-CHB	Asian (Han Chinese)	0.171	0.829
HapMap-JPT	Asian (Japanese)	0.198	0.802
HapMap-CHD	Chinese in metropolitan Denver, CO	0.253	0.747
HapMap-TSI	Toscans in Italy	0.290	0.710
HapMap-HCB	Asian (Han Chinese)	0.314	0.686
HapMap-CEU	European	0.336	0.664
Present study	Turkish	0.393	0.607
HapMap-GIH	Gujarati Indians in Houston, TX	0.420	0.580
HapMap-MEX	Mexican ancestry in Los Angeles, CA	0.420	0.580
HapMap-MKK	Maasai in Kinyawa, Kenya	0.597	0.403
HapMap-ASW	African ancestry in Southwest USA	0.663	0.337
HapMap-YRI	Sub-Saharan African (Nigeria)	0.690	0.310
HapMap-LWK	Luhya in Webuye, Kenya	0.706	0.294
Zhang et al. [20]	Chinese (Jinan, Shijiazhuang, and Huaian)	0.790	0.210

clarify the role of *HOTAIR* rs920778 polymorphism in the clinicopathologic state of BC. Clinicopathological feature assessments included age at onset, histologic type of cancer, TNM stage, tumor size, lymph node status, nodal status, distant metastasis, histological grade, ER status, PR status, HER-2/neu status, and lymphovascular invasion as well as perineural invasion. According to the recessive inherited genetic model, the CC genotype of *HOTAIR* rs920778 polymorphism was significantly associated with various clinicopathological features involved in BC progression like advanced TNM stage (III and IV), larger tumor size (T3 and T4), distant metastasis (M1), and poor histological grade (III). Our findings suggest that the CC genotype of *HOTAIR* rs920778 is responsible for the invasiveness and metastatic potential of BC. It is reasonable to assume that BC patients with the CC genotype of *HOTAIR* rs920778 polymorphism are more likely to have poor prognosis.

The results of the study are biologically reasonable and consistent in several ways to the laboratory and clinical findings of previous studies. A growing body of literature documents has shown that *HOTAIR* is pervasively overexpressed in both primary and metastasized tumors of a variety human cancer types including breast cancer [18], hepatocellular carcinoma [26], pancreatic cancer [27], lung cancer [28], colorectal cancer [29], ESCC [30], and others. Moreover, high expression of *HOTAIR* is correlated with tumor cell proliferation, invasion, metastasis, and poor prognosis [19]. Recently carried out studies have shown that *HOTAIR* rs920778 and rs12826786 polymorphisms in the *HOTAIR* gene affect the *HOTAIR* expression according to the genotypes of these polymorphisms [20, 21]. In contrast to our expectations, higher *HOTAIR* expression has been reported in the T allele of *HOTAIR* rs920778 polymorphism in normal esophageal tissues [20]. But, differences in *HOTAIR* expression according to *HOTAIR* rs920778 polymorphism in the ESCC tissues were not observed [20]. However, the overlapping situations are observed in the *HOTAIR* rs12826786 polymorphism. Guo et al. [21] have determined that *HOTAIR* rs12826786 polymorphism has a genotype-specific effect on *HOTAIR* expression both in gastric adenocarcinoma tissues and normal gastric tissues.

Several potential limitations of the current case–control study are as follows. (i) Since it was a hospital-based case–control study and BC cases and healthy women subjects were from a single center, inherent choice bias might exist. Thus, it is important to confirm the findings of our case–control study in a population-based prospective study in the future. (ii) The statistical power of the present study may be restricted by the sample size, especially for statistical analyses of subgroups which are stratified by clinicopathological features. Therefore, large sample size prospective studies are needed to confirm the impact of the *HOTAIR* rs920778 polymorphism on BC predisposition, clinicopathological features, and prognosis. (iii) This case–control study is limited by the Turkish population because of differences in allele frequency observed for *HOTAIR* rs920778 polymorphism in different populations (Table 4). Further studies on different populations are required to confirm our results and to achieve more credible findings on evaluating the association between *HOTAIR* rs920778 polymorphism and BC susceptibility risk. (iv) Zhang et al. [20] reported that the T allele of *HOTAIR* rs920778 polymorphism leads to increased expression of *HOTAIR* in normal esophagus tissues but not in esophageal squamous cell carcinoma. Due to the controversial situation of the expression of *HOTAIR* according to the allele of *HOTAIR* rs920778 polymorphism in normal and cancerous tissues as well as lack of data on *HOTAIR* expression according to the allele of *HOTAIR* rs920778 polymorphism in our study, future research needs to be done in order to clarify, according to the allele of *HOTAIR* rs920778 polymorphism, the effects of expression of HOTAIR in both normal and cancerous tissues of the breast.

In summary, our results reveal for the first time that *HOTAIR* rs920778 polymorphism was associated with BC susceptibility and poor clinicopathological features of BC in a Turkish population. *HOTAIR* rs920778 polymorphism may be a helpful genetic marker to predict BC predisposition and prognosis. Therefore, further investigations are required to understand the exact mechanisms of *HOTAIR* rs920778 polymorphism in BC cells. Because this is the first study regarding *HOTAIR* rs920778 polymorphism and the risk of BC susceptibility in the literature, further independent studies are needed to validate our results in larger sample sizes, as well as in patients of different populations.

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Conflicts of interest None

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