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The Relationship between Matrix Metalloproteinase Gene Polymorphisms and Tumor Type, Tumor Size, and Metastasis in Women with Breast Cancer in Central Iran

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Abstract

Background: Matrix metalloproteinases-2 and -9 play important roles in the development of breast cancer by hydrolyzing the extracellular matrix. Since −1306C/T and −1562C/T polymorphisms are located at the promoter regions of the matrix metalloproteinase-2 and -9 genes, respectively, C to T substitution may affect promoter activity and impact the rate of extracellular matrix degradation and cancerous cell proliferation. Therefore, we aimed to determine the genotype and allele frequencies of these polymorphisms in Iranian healthy women and women with breast cancer. We have also examined the correlation of genotypes with clinicopathological parameters such as tumor type, tumor size, and metastasis to lymph nodes.

Methods: This case-control study enrolled 200 women with breast cancer and 200 age-matched healthy women. DNA was extracted, and we determined the genotype and allele frequencies of −1306C/T matrix metalloproteinase-2 and −1562C/T matrix metalloproteinase-9 polymorphisms by the polymerase chain reaction-restriction fragment length polymorphism method. Additionally, tumor size (<20 mm/>20 mm), tumor type (ductal/non-ductal), and metastasis (yes/no) were determined.

Results: Genotype and allele frequencies of the −1306C/T matrix metalloproteinase-2 polymorphism showed no significant association with the occurrence of breast cancer. Genotype and allele distribution differed in the −1562C/T matrix metalloproteinase-9 polymorphism and indicated a 4.83-fold increase in the risk of breast cancer for T allele carriers. There was no likelihood of any interaction found between the two polymorphisms and susceptibility to breast cancer. In addition, the −1562C/T matrix metalloproteinase-9 T allele showed an association with metastasis to lymph nodes but we observed no association between the −1306C/T matrix metalloproteinase-2 polymorphism and clinicopathological features.

Conclusion: The –1562C/T matrix metalloproteinase-9 polymorphism is involved in the pathogenesis of breast cancer in Iranian women. The T allele may increase the risk of disease.

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Introduction

Breast cancer, characterized by malignant cells in the epithelial tissue of mammary glands, is one of the major causes of cancer deaths in women worldwide, particularly in Asia, Africa, and South America.[1] Breast cancer is the most frequent cancer among women with approximately 1.7 million new cancer cases that were diagnosed in 2012. It accounts for 11.9% of all cancers.[2] Studies in Iran have shown that breast cancer is the second cause of female deaths in Iran with a mortality rate that increased from 1.97 per 100,000 in 2006 to 2.45 per 100,000 in 2010.[3]

A wide variety of factors is involved in breast cancer development, some of which include early menopause, the lack of breastfeeding, postmenopausal obesity, smoking, hormone therapy, radiation exposure, and excessive alcohol consumption.[4] Breast cancer development is characterized by the proliferation and systemic spread of transformed cells to distant sites. Fundamentally, the breast is composed of a non-cellular component known as the extracellular matrix (ECM) that plays important roles in prevention of expanding growth and migration of cancer cells.[5]

Degradation of the ECM by matrix metalloproteinases (MMPs) is a key step for invasive and metastatic breast cancer. Broadly speaking, degradation of the ECM and components of the basement membrane by MMPs facilitates the detachment of tumor cells, their crossing over tissue boundaries, and invasion into tissue compartments.[6] Matrix metalloproteinases are a family of endopeptidases implicated in cancer development and progression.[7] Approximately 23 members of the human MMP gene family have thus far been identified, among which MMP-2 and MMP-9 were shown to be involved in various cancers, particularly breast cancer.[8,9] Matrix metalloproteinase-2 (gelatinase A; type IV collagenase) represents a unique ability to hydrolyze collagen types IV, V, VII, and X - main structural components of the basement membrane. In addition, MMP-9 is one of the most important members of this family (known as gelatinase-B or type IV collagenases), which can degrade gelatin and type IV collagen, the most important components of the basement membrane.[8]

A variety of studies demonstrated the relationship between genetic polymorphisms, such as single nucleotide polymorphisms (SNPs) and breast cancer risks.[6,10] More recently, the C/T transition SNP located at −1306 in the MMP-2 promoter, has been reported to disrupt the Sp1-type promoter site (CCACC box), which resulted in decreased promoter and transcriptional activities.[11] The SNP at position −1562 in the MMP-9 promoter region is also a binding site for a transcription repressor protein. Therefore, the C/T substitution at this polymorphic site prevents the DNA-protein interaction that leads to changes in the expression of this gene.[5]

Based on proposed roles of MMP-2 and MMP-9 in breast cancer progression and metastasis, the aim of the present study is to investigate the genotype frequencies of −1306C/T MMP-2 and −1562C/T MMP-9 polymorphisms in healthy women and patients with breast cancer. Additionally, we have further investigated the relationship between genotype frequencies and clinicopathological features such as tumor type, tumor size, and metastasis in patients with breast cancer.

Materials and Methods

Sample collection

This case-control study enrolled 400 subjects, 200 women with breast cancer and 200 healthy control women aged 35-59 years. We randomly selected participants from those who admitted to Sina Hospital (Tehran, Iran) by the caliper matching method. Control subjects comprised individuals who visited the hospital for regular health checkups and were later evaluated by physicians to confirm their healthiness. In patients, breast cancer was initially approved by a physician and diagnosed by pathologists after examination of tumor samples. The clinical and pathological records that included patients' ages, tumor type (ductal or non-ductal), tumor size (<20 mm or >20 mm), and presence or absence of armpit lymph

Table 1. Cinicopathological features of breast cancer patients.

Tumor size		Tumor type		Metastasis to lymph nodes	
<20 mm	>20 mm	Ductal	Non-ductal	Yes	No
174 (87%)	26 (13%)*	189 (94.5%)	11 (5.5%)	38 (19%)	162 (81%)

*Data are presented as number of subjects and percent (brackets).

node metastasis were assessed according to the tumor-node-metastasis (TNM) classification based on the Union for International Cancer Control (UICC) Guidelines.[12] We excluded subjects older than 60 years, those with a previous history of cardiovascular, thyroid, liver, or kidney diseases, other types of cancers, diabetes, or chronic diseases as well as individuals with a history of tobacco smoking from the study. Participants provided written informed consent for participation and the Research Ethics Committee of Hamadan University of Medical Sciences (Iran) approved this project. Tumor samples were collected by surgical resection and paraffin-embedded slides were freshly prepared.

DNA extraction

Blood samples were collected from control subjects into EDTA vacutainer tubes and genomic DNA was extracted by the salting out method. For breast cancer patients, genomic DNA was prepared from fresh frozen paraffin embedded (FFPE) breast tumor samples using proteinase K digestion, and extracted by a YTA Genomic DNA Extraction Mini Kit (Yekta Tajhiz Azma, Tehran, Iran). The concentration of genomic DNA was measured at 260 nm, and samples were stored at -20˚C until further analysis.

Matrix metalloproteinase (MMP)-2 and MMP-9 genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) analysis was performed for MMP-2 and MMP-9 genotyping. Forward F: 5'-CTTCCTAGGCTGGTCCTTACT-GA-3' and reverse R: 5'-CTGAGACCTG AAGAGCTAAAGAGGT-3' primers were used to amplify a 193 bp PCR product that contained a –1306C/T polymorphism on the MMP-2 promoter site.[13] In order to amplify a 436 bp product that contained a -1506C/T polymorphism site on the MMP-9 promoter, we used a pair of forward (5'-GCCTGGCACATAGTAGGCCC-3') and reverse (5'-CTTCCTAGCCAGCCGGCATC-3') primers.[3] PCR reactions were performed in a final volume of 25 µl using the PCR Super Master Mix (Yekta Tajhiz Azma, Tehran, Iran), according to the manufacturer's instructions in a peqSTAR DNA thermal cycler (VWR Peqlab, Vienna, Austria). We amplified the MMP-2 gene as follows: an initial denaturation step at 94˚C for 5 min followed by 35 cycles (94˚C for 45 s), annealing at 58˚C for 45 s, and extension at 72˚C for 45 s followed by a final step at 72˚C for 10 min to allow for complete extension of all PCR fragments. Apart from the 1 min annealing at 65˚C, similar PCR conditions were also applied to amplify the MMP-9 gene. PCR products for both MMP-2 and MMP-9 were electrophoresed on a 1.5% agarose gel and stained with SYBR safe (Invitrogen, Paisley, UK) to confirm the sizes of the products.

For RFLP analysis, the 193 bp (MMP-2) and 436 bp (MMP-9) PCR products were digested by XspI and SphI restriction enzymes (Fisher Scientific Ltd., Paisley, UK), respectively and separated on appropriate agarose gels. The XspI enzyme detected the C*TAG sequences on the MMP-2 promoter site and resulted in two separate bands (188 bp and 5 bp) for the homozygous TT genotype, whereas the homozygous CC genotype that lacked the XspI restriction site yielded a single 193 bp fragment. In contrast, the heterozygous CT genotype resulted in 3 fragments of 193, 188, and 5 bp bands. Similarly, the SphI enzyme detected the GCATG*C sequences on the MMP-9 promoter site and generated two separate fragments (205 bp and 258 bp) for the homozygous TT genotype, a single fragment of 436 bp for the homozygous CC genotype, and three fragments of 463, 205, and 258 bp for the heterozygous CT genotype.

Table 2. Genotype distribution and allele frequencies of –1306C/T matrix metalloproteinase (MMP)-2 and –1562C/T MMP-9 gene polymorphisms in control healthy women and patients with breast cancer.

Genotype	Total n (%)	Control n (%)	Patients n (%)	*P*-value (χ2, df)	Odds ratio (OR), 95% CI (Lower-upper; *P*-value)
–1306 C/T MMP-2					
CC	298 (74.5)	143 (71.5)	155 (77.5)		Reference group
CT	97 (24.25)	57 (28.5)	40 (24.3)	0.011 (8.322, 2)	1.545, (0.971-2.456; 0.065)
TT	5 (1.25)	0 (0.0)	5 (2.5)		***
CT+TT	102 (25.5)	57 (28.5)	45 (22.5)	0.207 (1.592, 1)	1.373, (0.874-2.158; 0.169)
MMP-2 alleles					
C	693 (86.6)	343 (85.75)	350 (87.5)		Reference group
T	107 (13.4)	57 (14.25)	50 (12.5)	0.533 (0.388, 1)	0.860, (0.572, 1.293; 0.467)
–1562 C/T MMP-9					
CC	347 (86.75)	189 (94.5)	158 (79.0)		Reference group
CT	47 (11.75)	11 (5.5)	36 (18.0)	0.000 (22.532, 2)	3.915 (1.930, 7.943; 0.000)
TT	6 (1.5)	0 (0.0)	6 (3.0)		***
CT+TT	53 (13.25)	11 (5.5)	42 (21.0)	0.000 (20.902, 1)	4.567 (2.276, 9.167; 0.000)
MMP-9 alleles					
C	741 (92.62)	389 (97.25)	352 (88.0)		Reference group
T	59 (7.38)	11 (2.75)	48 (12.0)	0.000 (25.051, 1)	4.822 (2.466, 9.432;0.000)

Data are presented as Mean ± SD. CI: Confidence interval, df: Degree of freedom, χ2: Chi-square. ***:Statistical limitation due to the number of cases.

Statistical analysis

All statistical analyses were carried out using the Statistical Package for Social Sciences version 16 (SPSS, Inc., Chicago, IL, USA). Values were presented as mean±SD and statistical significance was defined as *P*-values less than 0.05. The MMP-2 and MMP-9 genotypic distributions were tested for accordance with the Hardy-Weinberg equilibrium. The chi square (χ2) and Fisher's exact tests were used as appropriate to assess the differences between cases and controls in frequencies of genotypes. Statistically significant differences in means between genotypes were assessed by the t-test. In addition, the association between the MMP-2 or MMP-9 polymorphisms and the risk of breast cancer were estimated by odds ratios (ORs) and their 95% confidence intervals (CI), which were calculated by unconditional logistic regression models. Additionally, STATA software version 11.2 (StataCorp LP., TX, USA) was used to determine the likelihood interaction of −1306C/T (MMP-2) and −1506C/T (MMP-9) polymorphisms.

Results

In this case-control study, we investigated the genotype and allele frequencies of −1306C/T MMP-2 and −1506C/T MMP-9 gene polymorphisms in 200 women with breast cancer and 200 control women. Pathological parameters that included tumor type, tumor size and lymph node metastasis were determined in patients (Table 1). The results showed that 87% of patients had tumor sizes less than 20 mm. Ductal tumor was the predominant tumor type in 94.5% of the patients and no metastasis to lymph nodes was observed in 81% of patients.

Genotype frequencies of the –1306C/T matrix metalloproteinase (MMP)-2 polymorphism

Table 2 described the genotype frequencies of the −1306C/T MMP-2 gene polymorphism. The genotypic distributions were in Hardy-Weinberg equilibrium. The CC genotype was predominant (74.5%) in the entire study population of control subjects and patients whereas the homozygous TT genotype was the least frequent and present in 1.25% of participants. Frequency analysis of the CC, CT, and TT genotypes in women with breast cancer and control subjects showed a significant difference in distribution of genotype frequencies between patients and healthy individuals (*P*=0.011). However, when we examined the risk estimate of different genotypes (CC, CT, and TT) of the −1306C/T polymorphism for breast cancer development the results showed no significant association with the CT and TT genotypes to breast cancer when compared to the CC reference

Table 3. Interaction of different genotypes of the –1306C/T matrix metalloproteinase (MMP)-2 polymorphism with –1562C/T MMP-9 genotypes in control women and breast cancer patients.

–1306C/T MMP-2 genotypes	–1562 MMP-9 genotypes (Control)			*P*-value	–1562 MMP-9 genotypes (Patients)			*P*-value
	CC	CT	TT		CC	CT	TT	
CC	164	9	0	0.647	121	33	4	0.801
CT	25	2	0		28	7	1	
TT	0	0	0		6	0	0	

genotype (*P*=0.065). A total of 85.75% of the control subjects had the C allele, whereas 14.25% had evidence of the T allele for MMP-2 compared with 87.50% for the C allele and 12.5% for the T allele in breast cancer patients (Table 2). Although T allele displayed the least frequency in this study, there was no significant association found between allele frequencies in patients with breast cancer and controls for the −1306C/T polymorphism. Additionally, statistical analysis showed that the presence of the T allele did not have protective effect or was not a risk factor for breast cancer (Table 2).

Genotype frequencies of the –1562C/T matrix metalloproteinase (MMP)-9 polymorphism

We examined the genotype distributions and risk estimates for the −1562C/T MMP-9 gene polymorphism among the patient and control groups. Our results showed that the frequencies of genotypes in women with breast cancer were 79% (CC), 18% (CT), and 3% (TT) whereas the corresponding genotype frequencies in controls were 94.5% (CC), 5.50% (CT), and 0.0% (TT). We observed a significant difference in genotype frequencies between the patient and control groups which indicated an association of the CT and TT genotypes with a higher risk of breast cancer (Table 2). The risk estimates of different genotypes (CC, CT, and TT) of the −1562C/T polymorphism were also examined for their possible potential risks for breast cancer development. The results showed that the CT and CT+TT genotypes, compared to the CC reference genotype, represented significantly higher risks for breast cancer, with a 3.9-fold (CT) and 4.5-fold (CT+TT) incline in the risk of breast cancer (Table 2). The allele frequencies for the −1562C/T MMP-9 gene polymorphism showed that the frequency of the T allele in women with breast cancer was 4.83-fold higher than that in the control group. Therefore, the T allele might be associated with a 4.83-fold increased risk of developing breast cancer.

Likelihood of interaction between the –1306C/T matrix metalloproteinase (MMP)-2 and –1562C/T MMP-9 gene polymorphisms

In table 3, an analysis of possible interactions between the two polymorphisms has indicated that different genotypes of the −1306C/T MMP-2 polymorphism had no effect on the occurrence of a given genotype in the −1562C/T MMP-9 polymorphism or vice versa in both patients (*P*=0.801) or controls (*P*=0.647).

Genotypes and pathological features

The relationship between genotype frequencies of the −1306C/T MMP-2 and −1562C/T MMP-9 polymorphisms and pathological features that included tumor type (ductal and non-ductal), tumor size (>20 mm and <20 mm), and lymph node involvement were examined in patients with breast cancer. There was no significant association observed between different genotypes of the −1306C/T MMP-2 polymorphism and tumor size, tumor type, and metastasis to lymph nodes (Table 4). On the other hand, statistical analysis of the relationship between pathological features and genotype frequencies of the −1562C/T MMP-9 polymorphisms in women with breast cancer showed a significant association between metastasis and the −1562C/T MMP-9 polymorphism (*P*=0.029). In contrast to the CC genotypes, patients with CT or TT genotypes had

Table 4. Frequencies of pathological features based on genotype distributions of the –1306C/T matrix metalloproteinase (MMP)-2 and –1562C/T MMP-9 gene polymorphisms in breast cancer patients.

Pathological features	–1306C/T MMP-2 Genotypes			*P*-value	–1562C/T MMP-9 Genotypes			*P*-value
	CC	CT	TT		CC	CT	TT	
Tumor type								
Ductal	146	38	5	1.000	150	33	6	0.597
Non-ductal	9	2	0		8	3	0	
Tumor size								
<20 mm	133	36	5	0.806	135	33	6	0.523
>20 mm	22	4	0		23	3	0	
Metastasis								
Yes	31	7	0	0.735	36	2	0	0.029
No	124	33	5		122	34	6	

significantly lower metastasis to lymph nodes (Table 4) and T allele carriers showed nearly a 5-time lower risk of metastasis. Interestingly, when frequencies of metastasis were determined based on the different combinations of the −1306C/T MMP-2 and −1562C/T MMP-9 genotypes, the results showed that 30 patients with metastasis (out of 38) were CC/CC genotype carriers whereas 91 patients (out of 162) with no metastasis had the CC/CC genotype (data not shown). In other words, CC/CC genotype combinations of −1306C/T MMP-2 and −1562 C/T MMP-9 polymorphisms were observed in 79% of patients with metastasis while this genotype combination was found only in 56% of patients without metastasis.

Discussion

Breast cancer is one of the leading causes of cancer deaths among women in Western countries and Asia, particularly in Iran.[3] In addition to the environmental risk factors for breast cancer,[14] genetic variation may also impact the development and progression of breast cancer. It is well known that MMPs have a significant impact on cancer progression due to their ability to degrade the ECM and basement membrane, two physical barriers that play important roles in preventing and expanding growth and migration of cancer cells.[14] Genetic polymorphisms of MMP-2 and MMP-9 play key roles in their enzyme activity and transcriptional regulation. The present study has aimed to determine a possible association of these polymorphisms with the risk of breast cancer development.

Sp1, a ubiquitously expressed transcription factor, binds to GC/GT-rich elements on the promoter sites and regulates transcription of a variety of genes. The CCACC box has been shown to be essential for Sp1 binding and promoter function in several genes such as MMPs. Binding of Sp1 to MMP promoter sites invariably alters MMP gene expression, changes their enzyme activity, and hence reshapes the ECM structure.

In the present study, we investigated the genotype frequencies of the −1306C/T polymorphism within the promoter site of the MMP-2 in women with breast cancer and healthy controls. The results showed that although frequencies of the −1306C/T MMP-2 genotypes had a different distribution among patients and controls, none of CC, CT, or TT genotypes was found to have protective elements or risk factors for breast cancer. Additionally, there was no statistically significant difference in the C or T allele distributions between healthy controls and patients with breast cancer. Therefore, it could be concluded that susceptibility to breast cancer did not correlate to a specific genotype or allele of the −1306C/T MMP-2 polymorphism in Iranian women. Several studies investigated the effects of the MMP-2 −1306C/T polymorphism on breast cancer incidence with varied findings. While no association of the MMP-2 −1306C/T polymorphism and breast cancer has been observed in Brazilian and Swedish populations,[15,16] studies reported significantly

reduced risk of breast cancer in Chinese and Saudi Arabian ethnicity[11,17] or a smaller size of tumors and lower promoter activity in Australian populations for TT homozygous subjects.[10] A recent meta-analysis of studies that covered over 30000 subjects also led to inconsistent results that showed significantly higher frequency of CC genotypes of −1306C/T MMP-2 in breast cancer cases[18] or the lack of association between breast cancer and −1306C/T MMP-2 polymorphism,[19] particularly among Europeans and Asians.[20] Considering the literature data and the lack of allelic-association between −1306C/T polymorphism and breast cancer as observed in this study in Iranian women, it has been postulated that susceptibility to breast cancer might occur in ethnicity-based manner and differ among various populations.

Unlike the −1306C/T MMP-2 polymorphism, the genotype distribution and allele frequencies of the −1562C/T MMP-9 polymorphism significantly differed between control women and patients with breast cancer in the present study. The CT+TT genotypes showed a 4.56-fold increased risk for breast cancer compared to the homozygous CC genotype. The T allele carriers were found to be 4.83 times more susceptible to breast cancer than individuals with the C allele. Therefore, we have observed a strong association between the −1562C/T MMP-9 polymorphism and breast cancer incidence in Iranian women. There is evidence that a C/T polymorphism located at nucleotide position −1562 in the MMP-9 gene promoter has an effect on MMP-9 expression,[6] which results in higher transcriptional activity of the T-allele promoter through the loss of binding site for a repressor protein.[21] Accordingly, our observation about the higher risk of breast cancer in T allele carriers has supported previous reports in Indian,[5] Swedish,[16] and British[22] populations, which can be explained by the loss of repressor binding site as a result of polymorphism. In contrast, there is considerable evidence that the genotype distribution of the −1562C/T MMP-9 polymorphism is not associated with breast cancer in Polish[21] and Brazilians.[15] It is believed that −1562C/T MMP-9 genotype frequencies may vary according to ethnic and race characteristics. Several meta-analysis studies have reported that this polymorphism might only be associated with an increased risk of metastasis[23] and good prognosis.[10] A recent study has reported a significant association with the TT genotype in the recessive model with breast cancer when only a fixed-effect model and not the other genetic models were applied.[18,24] In our opinion, in population-based studies, ethnicity plays a key role in correlation of the −1562C/T MMP-9 genotype distribution and breast cancer. The correlation between the T allele and breast cancer observed in the current study (OR=4.82; $P<0.001$) was similar to that reported by Sadeghi et al. (OR=3.27; $P=0.004$) in the central part of Iran (Isfahan), which indicated a higher susceptibility of T allele carriers to breast cancer.[3]

We investigated the likelihood of an association of −1306T/C MMP-2 and −1562C/T MMP-9 gene polymorphisms in women with breast cancer. There was no linkage between different genotypes or alleles of the −1306T/C MMP-2 polymorphism and the occurrence of a specific −1562C/T MMP-9 genotype in patients. None of the genotype combinations of MMP-2/MMP-9 polymorphisms increased the risk of breast cancer or protected women from the disease. This observation was supported by the result of a previous study which indicated no evidence of a linkage between the −1306T/C MMP-2 and −1562C/T MMP-9 polymorphisms.[10] While the likelihood of an association of two polymorphisms has not been reported, an occurrence of particular genotype combinations with some clinicopathological features of breast cancer was observed. Lei et al. reported that patients homozygous both for TT (−1306C/T MMP-2) and CC (−1562C/T MMP-9) more frequently had progesterone receptor (PR) negative tumors than carriers of the other genotype combinations.[16] Clinicopathological features of breast cancer that included tumor size, tumor type, and metastasis to lymph nodes were previously investigated with conflicting results.

Saeed et al. reported a −1306C/T MMP-2

polymorphic status with the clinicopathological characteristics in patients above 48 years old.[17] However, there was no significant association reported between this polymorphism and tumor stage and metastasis[11] or with regional lymph node metastasis, histological grade, and tumor size.[16] Grieu et al. showed an association between the −1562C/T MMP-9 T allele with non-ductal type histology and better prognosis of breast cancer,[10] Chiranjeevi et al. reported no difference in genotype distribution of this polymorphism based on histopathological classification.[5] A recent meta-analysis that addressed 10516 different cancer cases confirmed the lack of association between pathological symptoms manifested by patients and the −1306C/T MMP-2 polymorphism. The Asian population with a −1562C/T MMP-9 polymorphism showed higher metastatic risk compared to the European population.[23] Consistently, our study revealed that metastasis in Iranian women as an Asian population had a significant correlation with the −1562C/T MMP-9 polymorphism but not the −1306C/T MMP-2 polymorphism. In line with our observation, the absence of a relationship between the genotypes of −1306C/T MMP-2 polymorphism and lymph node metastasis was also previously reported in China, Australia, and Brazil.[10,11,15] It has been postulated that −1306C/T MMP-2 polymorphism is not a key contributor to the pathological manifestation of breast cancers. Interestingly, the association of the −1562C/T MMP-9 polymorphism with metastasis, as observed in the present study, confirmed a previous report which showed an elevated OR for outcome of the −1562C/T MMP-9 T allele with progression and invasion of breast cancer in Iranian women from central Iran.[3] Unlike metastasis, we did not find any association between −1306C/T MMP-2 or −1562 C/T MMP-9 polymorphisms to tumor size and type in breast cancer patients.

Breast cancer is an epithelial tumor which its growth, invasion and metastasis is facilitated by MMP through the proteolytic degradation of the ECM and basement membrane. The MMP-2 and MMP-9 promoters contain binding sites for specific transcription factors and repressor proteins. Clearly, any variant that impact on binding property of these promoters (e.g., −1306C/T MMP-2 and −1562C/T MMP-9 polymorphisms) has the potential to affect MMP-2 and MMP-9 gene transcription and proteolytic activities. In conclusion, this study indicated that the −1306C/T MMP-2 polymorphism had no association with breast cancer occurrence or clinicopathological manifestations. However, there was a clear correlation between the −1562C/T MMP-9 polymorphism and breast cancer.

The study had some shortcomings. Although we did not correct our results for multiple comparisons, only a few associations were noted. Based on evidence, age is the major risk factor for most types of cancers including breast cancer, but we could not conduct genotype-stratified analyses and age adjustment due to the lack of an adequate number of subjects in some phenotypes. Second, good prognosis of breast cancer, histopathological classification of patients and their survival, is somehow estrogen receptor (ER) and PR dependent. However, due to financial limitations, we did not investigate the ER and PR status. Finally, since ethnicity plays a key role in population-based studies, any heterogeneity in ethnicity may confound the results. Therefore, to overcome this issue, it is necessary to have a larger sample size.

Conflict of interest

No conflict of interest is declared.

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