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Genetic Variation in *MMP*-1 Promoter Region and Breast Cancer Susceptibility

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Abstract

Breast cancer forms from uncontrolled growth of abnormal breast cells, and its metastasis is one of the leading causes of death among women. MMP-1 is a member of matrix metalloproteinase genes family that is involved in many steps of cancer development and invasion. Genetic variation in the *MMP*-1 promoter (insertion or deletion of a Guanine) could modify the level of MMP-1 expression and taught to be a facile factor for tumor development and metastasis. The purpose of the present study is evaluation whether genetic variation in *MMP*-1 promoter could modify the susceptibility to development and metastasis of breast cancer. Therefore, it could be considered as genetic marker for identification highly prone women for breast cancer initiation and/or invasion. For investigation of this hypothesis, we genotyped 200 women with breast cancer and 100 control subjects without any history of genetic disease using PCR-RFLP. The median follow up of patients was 20 months. Metastasis based analysis revealed that 2G/2G genotype had stronger correlation with metastasis group than M- group; both at the time of diagnosis (OR=1.86, %95CI=1.0-3.64) and also at the end of follow-up duration (OR=2.71, %95CI=1.43-5.11). Our data suggest that 2G/2G homozygote polymorphism is involved in metastatic spread, but not in initiation of breast cancer.

Keywords: Breast cancer; Matrix metalloproteinase-1 (MMP-1); Metastasis; 2G/1G polymorphism

Introduction

Breast cancer is the most common cancer among women in the world. This cancer usually affects tissues are involved in milk production. Malignant breast tumor forms from uncontrolled growth of abnormal breast cells. They can invade and destroy surrounding tissues and spread to other parts of the body. In fact, the most difficult problem in the treatment of breast cancer is metastatic spread [14,15].

Only 5 to 10 percent of all breast cancers are hereditary [15]. These cancers can be caused by mutation in particular genes, such as *BRCA*1 or *BRCA*2 [10]. It has been shown that first degree relatives of breast cancer patients have 2 fold elevated risk over the general population, most of which can not be explained

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by *BRCA*1/2. Although some of high and moderate penetrance genes are probably undiscovered, inherited predisposition to breast cancer in human is more likely to be the result of low-penetrance alleles at several or many genetic loci [17,18]. However, some mutation in particular genes associated with breast cancer are more common among certain geographic or ethnic groups [14].

Matrix metalloproteinase is a large family of zinc endopeptidase that degrades all components of extra cellular matrix (ECM) and basement membrane [7,11]. The extra cellular matrix (ECM) and basement membrane act as two physical barriers that play important roles in preventing expanding growth and migration of cancer cells [21,25,29]. There is much evidence that matrix metalloproteinases (MMPs) play essential roles in migration, tumor growth and development [6,12,13,22]. The first mammalian MMP, interstitial collagenase-1 (MMP-1) is the principle secreted proteinase that contributes to tumorogenes and invasion by excessive destruction of collagenous ECM and releasing of growth factors and angiogenesis factors [7,19]. MMP-1 belongs to matrix metalloproteinase family and has the ability of initiating degradation of native fibrillar collagens of type I, II, III, VII, and VIII [4]. Since collagens represent the major structural proteins of all tissues and the chief obstacle to tumor cell development and migration, it has long been postulated that this collagenolytic enzyme plays pivotal role in facilitating development and dissemination of cancer [1,4,28]. In addition, MMP-1 could release the cell proliferation factors and angiogenic factors. Therefore, it could participate in tumor growth and angiogenesis [5,19]. Recent report by Boire *et al.* revealed that MMP-1 secreted by host fibroblasts promotes breast cancer cell invasion by activating of PAR-1 [3]. This work is last confirmation in which way increased evidences of high specific roles played by MMP-1 during tumor invasion. Therefore, it seems that *MMP*-1 over-expression being necessary to make appropriate environment for tumor cell growth and migration.

Rutter *et al.* reported that the insertion of an extra guanine base (G) at -1607 of *MMP*-1 promoter creates a core binding site for ETS family of transcription factors (5′-GGA-3′; 20). At least one copy of 2G allele is present in about 75% of human population, where it has the potential to enhance *MMP*-1 transcription in both normal fibroblast and some tumor cells [26,27]. This polymorphism has been correlated to the risk of several tumors such as lung cancer [31] and colorectal cancer [2]. *MMP*-1 genotype polymorphism also had been associated with the invasion of cutaneous malignant melanoma [30] and colorectal cancer [8]. Since 1G/2G polymorphism has a significant influence on the *MMP*-1 gene expression we aimed to determine whether this polymorphism may be associated with varying risk of breast cancer initiation and invasion. In present study, two crowded cities of Iran (Tehran and Isfahan) were selected and *MMP*-1 promoter allelic variant distribution was calculated and compared. We investigated the possible effect of the *MMP*-1 polymorphism on the initiation and invasion of the breast cancer in Iranian women.

Materials and Methods

This study included 200 female patients with confirmed diagnosis of breast cancer that recruited from Imam Khomeini and Omid hospitals, placed in Tehran and Isfahan, respectively, between 2002-2006, and 100 control subjects that were selected from tumor- free volunteer women who visited the same hospital in the same period of time for medical examination. These are the two biggest and equipped hospitals of Iran that the majority of breast patients from Tehran and Isfahan and farther/near surrounding regions refer to these hospitals for treatment. Since patients from various cities refer to Tehran and Isfahan for treatment and cure, we could conclude that *MMP*-1 genotype distribution may be identical in Iranian population.

Patients were between 25 and 86 years of age (mean age 57.9) and controls matched to cases on age, (± 5 years). At recruitment, informed consent was obtained from each subject. In order to investigate the *MMP*-1 polymorphism genotype on initiation and metastasis steps of breast cancer, patients placed in two categories: with detectable metastasis position category (M+) and without detectable metastasis position category (M−). The patients were followed every 3 months by clinical examination during the first two postoperative years and then every 6 months. The median follow up was 20 months (range, 9-36 months), during the follow up period any changes in the disease situation of patients was considered. None of the patients lost of follow up. The effect of *MMP*-1 polymorphism was further evaluated with stratification by age and hormonal receptors (estrogen and progesterone).

DNA Extraction

Genomic DNA was successfully isolated from blood samples for each subject, using Miller *et al.* method with slight modification [16]. DNA concentration was investigated by measurement of OD260 and electrophoresis on %1 agarose gel.

MMP-1 Genotyping

MMP-1 genotype determination was performed with polymerase chain reaction-restriction length polymerphism (PCR-RFLP) method. Primers sequences and PCR reactions are shown in Table 1. The PCR cycling conditions were 4 min at 94°C followed by 30 s at 94°C, 30 s at 58°C, 30 s at 72°C, repeated for 30 step cycles, and the final step at 72°C for 10 min An T to G substitution mutation was introduced at the second nucleotide close to the 3′ end of the reveres primer to create a recognition sequence for the restriction endonuclease *Alu* I (5′-AGCT-3′) in the case of the 1G alleles (but not 2Gs) at the polymorphic site (31, Table 2 and Fig. 1).

Statistical Analyses

All statistical analyses were carried out with SPSS11 soft ware (SPSS, Chicago, IL). *MMP*-1 genotype polymorphism cooperation in different study groups was done by χ^2 test or student's t test when appropriate. OR and %95 confidence interval (CI) were calculated by unconditional logistic regression model as index of *MMP*-1 polymorphism association with breast cancer. A probability of %5 was significant in all of the statistical analyses.

Results

All of the 200 breast cancer patients and 100 healthy subjects were genotyped successfully. Of the patients, 100 cases were collected from Tehran and 100 cases were recruited from Isfahan. Control subjects were also selected as equal ratio from these cities. 1G/1G, 1G/2G and 2G/2G frequencies were 19%, 50% and 29% in patients and 26%, 52% and 22% in controls of Tehran population, respectively. These frequencies were 20%, 52% and 30% in patients group and 26%, 50% and 24% in healthy group of Isfahan population, respectively. There were no statistical significant differences in *MMP*-1 genotype distribution between patients and controls population of these cities (χ^2=0.0018, P=0.99 in patients, χ^2=0.06, P=0.97 in controls, Table 3). Therefore in the present study, these populations were considered as unique population in all of the statistical analyses.

In patients, *MMP*-1 genotype distribution (2G/2G and 2G/1G) were significantly different compared with healthy subjects (χ^2=5.99 P=0.04, Table 3). 2G homozygote genotype was more frequent among patients than controls (OR=1.40, %95CI=0.80-2.44).

Table 1. Forward and reverse primers including PCR reaction concentrations

F	5′-TGACTTTTAAAACATAGTCTAATGTTCCA-3′
R	5′-TCTTGGATTGATTGAGGATAAGTCATAgC-3′

PCR reaction	Volume	Concentration
Template DNA	2 µl	250 ng
Upstream primer	0.5 µl	50 pM
Downstream primer	0.5 µl	50 pM
$MgCl_2$	2 µl	12 mM
dNTP mix	2 µl	10 mM
10xPCR buffer	5 µl	200 mM Tris-HCl, 100 Mm $(NH_4)_2SO_4$, 100 mM KCl, 1% TritonX-100, 1 mg/ml BSA
Taq DNA pol.	0.5 µl	5 u/µl
dH_2O	Up to 50 µl	

Table 2. The size of fragments resulting from PCR-RFLP

Digestion	Digested fragments	Undigested fragments
PCR product	Allelic dependent	269 bp
Allele G1	269 bp	241 bp and 28 bp
Allele G2	269 bp	269 bp

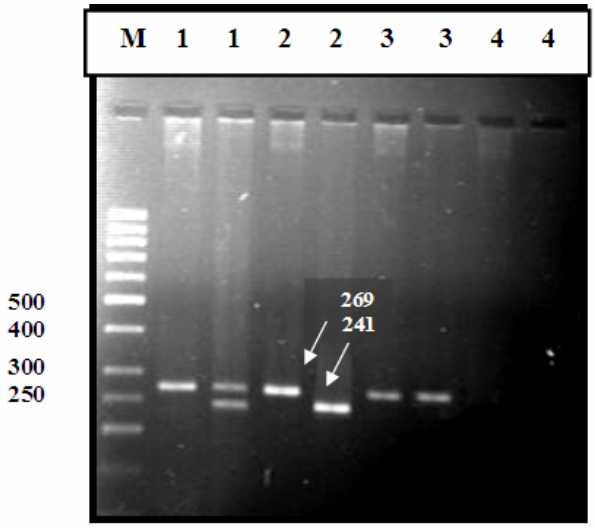

Figure 1. *MMP*-1 genotyping using PCR-RFLP method. **M**: 50 bp DNA marker; **1**: 1G/2G genotype; **2**: 1G/1G genotype; **3**: 2G/2G genotype; **4**: Negative control. Note: in all samples, the first line is related to undigested and second line is related to digested with enzyme (*Alu*I).

Table 3. *MMP*-1 promoter polymorphism distribution at control and patients groups in Tehran and Isfahan population

	Control n (%)	Patient n (%)
Tehran		
1G/1G	13 (26%)	19 (19%)
1G/2G	26 (52%)	50 (50%)
2G/2G	11 (22%)	29 (29%)
Isfahan		
1G/1G	13 (26%)	20 (20%)
1G/2G	25 (50%)	52 (52%)
2G/2G	12 (24%)	30 (30%)
Total[a]		
1G/1G	26 (26%)	39 (19.5%)
1G/2G	51 (51%)	102 (51%)
2G/2G	23 (23%)	59 (29.5%)

[a] At the total population (Tehran and Isfahan), 2G/2G genotype frequency is more frequent among patients versus controls (OR=1.40, 95%CI=0.80-2.44).
There are no statistical significant differences in *MMP*-1 genotype distribution between patients and controls population of Tehran and Isfahan (χ^2=0.0018, P=0.99 in patients, χ^2=0.06, P=0.97 in controls).
In total population, *MMP*-1 genotype distribution is significantly different in breast patients compared with healthy subjects (χ^2=5.99 P=0.04).

Table 4. Association analysis of *MMP*-1 polymorphism with presence or absence of detectable metastasis

Groups	*MMP*-1 genotypes		*P*[a] value
	1G/1G + 1G/2G	2G/2G	
Control	77	23	
Non-metastasis	95	31	0.78
Metastasis	46	28	0.03

[a] P Value is calculated from χ^2 test.
[b] 2G/2G genotype presents a stronger correlation with M+ group versus control group (OR=2.03, 95%CI=1.05-3.94).

Table 5. Association analysis of *MMP*-1 promoter polymorphism with metastasis risk of breast cancer

Groups	A			B		
	M+	*M−*	*OR (95% CI)*	*M+*	*M−*	*OR (95% CI)*
1G/1G + 1G/2G	46	95	Reference	51	90	Reference
2G/2G	28	31	1.86(1.0-3.46)	34	25	2.71(1.43-5.11)

A: At the time of diagnosis.
B: At the end of diagnosis.

As shown in Table 4, further examination with regard to presence of metastasis spread demonstrated that 2G homozygote was more frequent in M+ group than controls (OR=4.51, %95 CI=0.03). However, no statistical differences were observed in M- group versus control group (χ^2=0.078, P=0.78).

As shown in Table 5, it was found, individual carrying 2G/2G genotype with OR of 1.8 (%95CI=1.0-3.46) compared with those who were 1G/1G or 1G/2G, expose on median increased risk for metastasis of breast cancer. In addition, a stronger association was observed between 2G homozygote polymorphism and the M+ group at the end of follow up (OR=2.71, %95CI=1.43-5.11).

In the next step, we demonstrated that 1G/1G genotype and even genotypes with at least one 2G allele, have no important effect on the age of onset of breast cancer initiation (56.5 Vs 57, P=0.08). However 2G homozygote individuals are spatially at risk for early onset of metastasis spread of breast cancer (56.9 Vs 58.8, P=0.02).

However, hormonal receptor analysis shows no interaction between *MMP*-1 genotype polymorphism and statues of hormonal receptors (estrogen and progesterone, data not shown).

Discussion

Statistical analyses showed no significant differences in *MMP*-1 polymorphism distribution in Tehran and Isfahan populations. Our data demonstrated that 2G/2G genotype was higher in breast patients than control subjects. Furthermore, metastasis based analysis revealed that 2G/2G genotype had important association with M+ group compared with M- group both at the time of diagnosis and at the end of follow-up period. During the follow-up period, 24 patients had been affected to metastasis that 46 percent of them had 2G/2G genotype. On the other hand, 2G/2G genotype association with M+ group had been raised from 1.8 to 2.71 at the end of the follow-up. However, no association between 2G/2G genotype and M- group was observed by comparison M- versus control group. These findings suggest there is an important association between the *MMP*-1 polymorphism and risk of metastasis but not at the initiation of breast cancer. In fact, patient women with 2G homozygote genotype compared with patients who carrying other type of genotypes expose on 1.8 fold higher risk for metastasic spread of breast cancer (OR=1.8, 95%CI=1.0-3.46).

Our results seem to be in disagreement with previous observation in Italy by Biondy [2] and Ghilardy [9] who found no correlation between *MMP*-1 promoter

Table 6. Compression of *MMP*-1 promoter polymorphism association with breast cancer in Italian and Iranian population

Population	Study		N	1G/1G	1G/2G	2G/2G	OR (95% CI)
Italy	Biondy *et al.*	Controls	164	42	86	36	
		Patients	43	8	26	9	0.94 (0.41-2.14)
Italy	Ghilardy *et al.*	Controls	110	27	50	33	
		Patients	86	16	48	22	0.80 (0.42-1.51)
Iran	Present study	Controls	100	26	51	23	
		Patients	200	39	102	59	1.86 (1.0-3.46)

polymorphism and breast cancer (Table 6). Several possibilities exist to explain these differences such as difference in study ethnics, genetic and environment background of Iranian and Italian population. In addition, rate of mutation in particular genes that associate with breast cancer and have influence on *MMP*-1 expression such as P53 could be different in geographic or ethnic groups.

P53 has a strong inhibitory impact on the human MMP-1 expression and could disrupt communications between transactivator -72 AP-1 and the basal transcription complex. However, mutated P53 may lose the ability of regulating *MMP*-1 transcription [23,24]. These evidences emphasize the important of genetic effect evaluation on cancer initiation and invasion in various populations. Another interesting finding in present study was the age of onset of initiation and invasion of breast cancer that further confirmed the role of 2G/2G genotype on the metastasis stage. 2G/2G homozygote genotype could decrease the age at onset of metastasis but had no significant influence on the age at onset of breast cancer initiation.

Briefly, this epidemiological study demonstrates a statistical important association between *MMP*-1 genotype polymorphism and the breast cancer metastasis. Taken together, our evidence suggests that 2G/2G genotype maybe a moderate risk factor for breast cancer invasion and metastasis. Since, metastatic spread is the most common cause of the breast cancer related death, detection of cases with high potential for development of metastasis and prevention of its development are important to enhance the survival rate of patients and to treat the cancer. So, *MMP*-1 genotype determination could be a moderate genetic marker to identify highly prone women for breast cancer metastatic spread.

However, it is clear that metastasis processes do not cause by action of a single gene and certainly other genetic and environmental factors, are involved in susceptibility to the breast cancer invasion and metastasis. In addition, acceptance of 2G/2G polymorphism as breast cancer metastasis risk factor can only come through repeated analyses at different studies and by consequent slow progress to consensus judgment.

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