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Association of *MMP-9* promoter polymorphism and breast cancer among Iranian patients

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Summary. *Background and aims:* Breast cancer is one of the most common cancers in women population. This cancer is influenced by environmental and genetic factors. *MMP-9*, a member of *MMPs* family, is located on 20 q11.2- q13.1 and encodes Gelatinase B, a 92 KDa protein which is able to degrade collagen IV, matrix proteoglycan core and elastin. Over expression of *MMP-9* has been shown in some cancers. MMP-9 is involved in the metastasis and cancerous cells migration. *MMP-9* promoter polymorphism (-1562 C>T) is a candidate for cancer susceptibility. It is likely that T allele impedes recruitment of repressor proteins and causes gene over expression in T allele carriers. In the present case-control study we investigate whether *MMP-9* promoter polymorphism (-1562C>T) is associated with breast cancer. *Methods:* A total of 216 Azeri women including 104 patients and 112 healthy women were enrolled in the study. Blood sampling was done after taking a written consent. Genomic DNA was extracted and its purity was determined on 1% agarose gel. Then the given polymorphism was determined by PCR- RFLP method using *SphI*. *Results:* The results showed that T allele and CT genotype are more frequent in the patients as compared to the control group, but there are not significant differences between two groups (P=0.1). *Conclusion:* It is suggested that *MMP-9* polymorphism (-1562C>T) is not associated with the breast cancer occurrence in our population. However, further studies in larger populations including other genetic and environmental factors are required to achieve conclusion.

Key words: breast cancer; gene polymorphism; Iran; RFLP

Introduction

Breast cancer is the most common cancer in the world women population with an estimated 521000 related- deaths in 2012. The rate of breast cancer is increasing in the developing countries (1). This cancer is influenced by environmental and genetic factors (2). BRCA1 and BRCA2, PTEN, TP53, ATM have been shown to have important roles in breast cancer onset, invasion, metastasis and prognosis (3).

Matrix metalloproteinases (MMPs), a group of zinc dependent proteolytic enzymes, was shown to be involved in the degradation of many components of the extracellular matrix during both physiological and pathological processes (4). The regulatory mechanisms of MMP activity occur at several stages, by induction of gene transcription, pro-enzyme activation and inhibition of active MMPs. The process of MMP transcription can be stimulated by cytokines, steroids and growth factors (5). Activation of MMPs can be controlled by proteolytic enzymes, plasmin, while their inhibition is controlled by their specific endogenous inhibitors like α_2-macroglobulin, thrombospondins 1 and 2, membrane-bound RECK, tissue inhibitors of MMPs (TIMPs) (6). MMPs are frequently produced by infiltrating inflammatory cells and stromal cells, including fibroblasts, which can be invigorate by molecules on the surface of cancer cells (7).

MMP-9, a member of gelatinases subfamily, produces gelatinase B. This 92 KD protein have three re-

peats of type II fibronectin domains and is able to bind to gelatin, collagen, laminin of matrix in the physiological conditions and degrade them in a normal way. In the pathologic conditions, this function mediates tumor invasion. There are several studies reporting higher levels of gelatinase B in patients with breast cancer compared with the healthy controls (8, 11, 12)and MMP-9 gene became a candidate for breast cancer onset and progression susceptibility. For example, it has been shown that serum MMP-9 concentrations were higher in cancer than in benign. This study provides evidence supporting the potential role of serum MMP2/9 as biomarkers for breast disease classification (9).

Matrix metalloproteinases (MMPs) have been shown to be implicated in diverse roles in breast cancer development and progression. While many of the different MMPs expressed in breast cancer are produced by stromal cells, MMP-9 is produced mainly by the tumor cells themselves. Tumor cell-produced MMP-9 promotes tumor vascularization (10). Basement membrane degradation is mediated by targeted secretion of various matrix metalloproteinases (MMPs). Specifically, MMP2 and MMP9 (MMP2/9) have the ability to hydrolyze components of the basement membrane and regulate various aspects of tumor growth and metastasis (11).

Because common genetic variants can alter the expression or function of MMPs, we hypothesized that potentially functional single-nucleotide polymorphisms (SNPs) in the MMP9 gene may be associated with the survival of patients with invasive breast cancer. One of these polymorphisms is SNP (rs3918242) or *MMP-9* promoter -1562 C>T polymorphism. The purpose of our study was to investigate the association between *MMP-9* promoter -1562 C>T polymorphism and breast cancer.

Materials and methods

Patients

A total of 216 women were enrolled in the study including 104 patients with breast cancer (median age of 46.9 years) and 112 healthy women (median age of 47.2 years). Breast cancer subjects were operated on in Noore-e-nejat hospital, Tabriz, east Azerbaijan province, Iran. Patients were selected considering breast cancer reports in tumor biopsy or clinical examination. Patients that have been received any type of chemotherapy or radiotherapy was excluded. The controls were selected of women came to the laboratory of Gholestan clinic for health tests considering the absence of cancer in themselves and their first degree relatives and negative report of breast mass in mammography or clinical breast exam and diagnosed psychiatric diseases. All patient and controls were Iranian Azeri Turkish, living in northwest of Iran. Informed consent was obtained from all individual participants included in the study. This form was approved by the ethical committee of health ministry. The project has been accepted by the university ethical committee.

DNA extraction

Genomic DNA was extracted from whole blood specimens using GPP solution kit (Genepagoohan, Iran) according to the manufacturer's protocol. The DNA of samples was analyzed by 1%agarose gel electrophoresis.

Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP)

The genotypes of *MMP-9* (-1562 C/T) were determined by the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. A 690- bp fragment of DNA containing interested polymorphism region was amplified by polymerase chain reaction(PCR) using a Biorad Thermocycler. Primers were provided by takapoo zist (Table 1).

The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 45 s at 94°C, 45 s at 57.5°C and 45 s at 72°C, and with a final step at 72 for 5 min to allow complete extension for PCR products. 5 µl of the template DNA was added to 10 µl of reaction mixture containing 1x CinnaGene PCR mastermix [0.08 units/ µl Taq DNA polymerase in reaction buffer, 3 Mm MgCl2, 0.4 Mm(dATP, dTTP, dCTP, DGTP)], 1 µl of each primer, 3 µl DNase free sterile water.PCR products were analyzed by 2% agarose gel electrophoresis.

Table 1. PCR primers for MMP-9 PCR-RFLP assay.

SNP	Primers	enzyme	Fragments length
MMP-9	5´ ACTTATTACGGTGCTTGACACA 3´	*SphI*	690 bp (C)
(-1562 C/T)	5´ TCACTCCTTTCTTCCTAGCCA 3´		254+ 436 bp (T)

Then, 4 µl of each PCR products were added to 4.8 DNase free sterile water, 0.2 µl restriction enzyme *SphI* (Fermentase), 1 µl Buffer B to be digested by restriction enzyme at 37˚C for 4.5 hours. Digested PCR products were loaded onto 1.5% agarose gel, stained with ethidium bromide and visualized by UV light source. DNA fragment sizes were estimated by comparison with 50-bp DNA ladder (thermo scientific generuller).

Statistical analysis

Statistical analysis was perfumed using χ^2 by Med Calc ver. 12.1.4 Software. P value of less than 0.05 was considered statistically significant.

Results

The frequency of C and T allele in patients with breast cancer was 75.48% and 24.51% and in control group was 81.25% and 18.75%, respectively (Table 2). The CC, CT and TT genotypes of *MMP-9* promoter -1562C>T polymorphism were observed in 53 (50.96%), 51 (49.04%) and 0 (0%) of the breast cancer cases, and 70 (62.5 %), 42 (37.5%) and 0 (0 %) of controls, respectively (Figure 1; Tables 1 and 3). T allele and CT genotype were more frequent in the patients compared with the controls, but this differences were not significant (P=0.1)

Table 2. Allele frequencies of MMP-9 promoter -1562 C>T polymorphism.

Alleles	Patients n (%)	Controls n (%)	P value
C	157 (75.48%)	182 (81.25%)	0.1
T	51 (24.51%)	42 (18.75%)	

χ^2=1.7

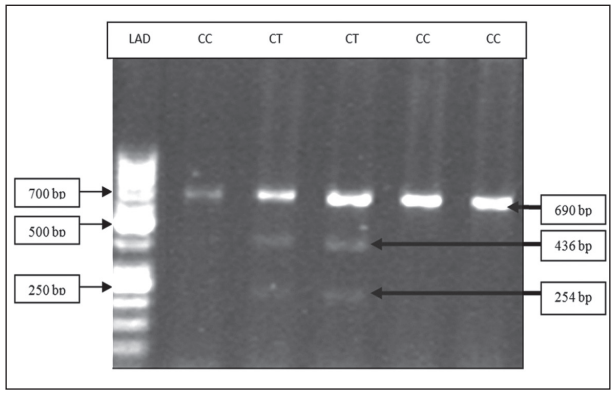

Figure 1. PCR- RFLP analysis of MMP-9 promoter -1562 C>T polymorphism. LAD=Molecular marker. (From left to right: patient CC, patient CT, control CT, control CC, control CC).

Table 3. Genotype frequencies of MMP-9 promoter -1562 C>T polymorphism

Genotypes	Patients n (%)	Controls n (%)	P value
CC	53 (50.96%)	70 (62.5%)	
CT	51 (49.04%)	42 (37.5%)	0.1
TT	0	0	

χ^2=2.4

Discussion

Breast cancer is the most common cancer in women worldwide. Although there are some reliable classic biomarkers and susceptible genes for breast cancer such as CA15-3/CA27.29, carcino-embryonic antigen (CEA), estrogen receptor (ER)/progesterone receptor (PR), HER2/neu, urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) (12), new biomarkers and genes are necessary to detect sooner and more precisely.

Considering MMP-9 roles in carcinogenesis, tumor invasion and angiogenesis (13), and the higher

levels of gelatinase B in cancer patients with T allele, searches to find which mechanism may results in high level of gelatinase B were done. One of these mechanism was enhanced transcription in the presence of T allele in *MMP-9* (-1562 C>T) polymorphism (probably due to not effectively binding of a transcription repressor protein to the T allelic promoter) (14), then *MMP-9* polymorphism became an important candidate for cancer susceptibility.

Several studies has been shown the association of *MMP-9* promoter polymorphism (-1562 C>T) or SNP (rs3918242) with cancers, but there are insufficient information and different results to determine if this SNP is a high risk factor (15, 16). A moderate increased risk of breast cancer in TT homozygotes (OR= 1.88; CI 95% 0.97-3.63) was observed by lei et al in 2007 (17) while przybylowsk reported and absence of correlation between T allele and malignancy [OR= 2.61; CI 95% 1.33;4.87) (18). According to a meta-analysis in 2009 there was no association of MMP 1, 2, 3 or 9 polymorphisms with breast cancer, MMP-1, 3 or 9 with lung cancer or MMP-2, 3 or 9 with colorectal cancer (19). It has been suggested that the polymorphisms in the MMP 9 gene may be genetic modifiers for breast cancer prognosis in this Chinese population (20).

In the present study, for the first time, the association between *MMP-9* promoter -1562C>T polymorphism and breast cancer among Iranian Azeri Turkish patients was investigated. In spite of more frequency of T allele and CT genotype in patients compared with the controls, we did not find significant differences in allele or genotype frequencies among them (P>0.05). Another study in Iran, on Isfahan population, has shown a correlation between the T allele and breast cancer occurrence (21). These inconsistent results might be in consequence of founder effect, other genes variation influence, differences in environmental factors and life style.

Some limitations should be considered in interpreting our results. The ethnicity of participants was Azeri Turkish, so for generalizing the results in Iran it is necessary to study the SNP on other populations. The advantage of the study is comparatively homogeneity of participants in race, ethnicity and habitat, thus this similarity in ancestors, climate and life style can minimize the influence of other genes variation or environmental risk factors on the function of interested gene polymorphism.

Due to relatively small number of participants in this report, expanded studies are needed to confirm this genetic variation relationship with breast cancer progression. We also suggest evaluation of MMP-9 and its inhibitor, active and total ranges in blood and tissue because the activity of protein gelatinase B can be modified by protein-protein interactions.

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References

1. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol 2001; 2: 133-40.
2. Barrow TM, Michels KB. Epigenetic epidemiology of cancer. Biochem Biophys Res Commun 2014; 455: 70-83.
3. Rhiem K, Todt U, Wappenschmidt B, *et al.* Sporadic breast carcinomas with somatic BRCA1 gene deletions share genotype/phenotype features with familial breast carcinomas. Anticancer Res 2010; 30: 3445-9.
4. Amălinei C, Căruntu ID, Bălan RA. Biology of metalloproteinases. Rom J Morphol Embryol 2007; 48: 323-34.
5. Johansson N, Westermarck J, Leppä S, *et al.* Collagenase 3 (matrix metalloproteinase 13] gene expression by HaCaT keratinocytes is enhanced by tumor necrosis factor alpha and transforming growth factor beta. Cell Growth Differ 2007; 8: 243-50.
6. Bäck M, Ketelhuth DF, Agewall S. Matrix metalloproteinases in atherothrombosis. Prog Cardiovasc Dis 2010; 52: 410-28.
7. González LO, Corte MD, Junquera S, *et al.* Expression and prognostic significance of metalloproteases and their inhibitors in luminal A and basal-like phenotypes of breast carcinoma. Hum Pathol 2009; 40: 1224-33.
8. La Rocca G, Pucci-Minafra I, Marrazzo A, *et al.* Zymographic detection and clinical correlations of MMP-2 and MMP-9 in breast cancer sera. Br J Cancer 2004; 90: 1414-21.
9. Somiari SB, Somiari RI, Heckman CM, *et al.* Circulating MMP2 and MMP9 in breast cancer - potential role in classification of patients into low risk, high risk, benign disease and breast cancer categories. Int J Cancer 2006; 119: 1403-11.

10. Mehner C, Hockla A, Miller E, *et al.* Tumor cell-produced matrix metalloproteinase 9 (MMP-9) drives malignant progression and metastasis of basal-like triple negative breast cancer. Oncotarget 2014; 5: 2736-49.
11. Jacob A, Jing J, Lee J, *et al.* Rab40b regulates trafficking of MMP2 and MMP9 during invadopodia formation and invasion of breast cancer cells. J Cell Sci 2013; 126: 4647-58.
12. Harris L, Fritsche H, Mennel R, *et al.* American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007; 25: 5287-312.
13. Littlepage LE, Sternlicht MD, Rougier N, *et al.* Matrix metalloproteinases contribute distinct roles in neuroendocrine prostate carcinogenesis, metastasis, and angiogenesis progression. Cancer Res 2010; 70: 2224-34.
14. Zhang B, Ye S, Herrmann SM, *et al.* Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 1999; 99: 1788-94.
15. Chiranjeevi P, Spurthi KM, Rani NS, et al. Gelatinase B (-1562C/T) polymorphism in tumor progression and invasion of breast cancer. Tumour Biol 2014; 35: 1351-6.
16. Chaudhary AK, Pandya S, Mehrotra R, *et al.* Role of functional polymorphism of matrix metalloproteinase-2 (-1306 C/T and -168 G/T) and MMP-9 (-1562 C/T) promoter in oral submucous fibrosis and head and neck squamous cell carcinoma in an Indian population. Biomarkers 2011; 16: 577-86.
17. Lei H, Hemminki K, Altieri A, *et al.* Promoter polymorphisms in matrix metalloproteinases and their inhibitors: few associations with breast cancer susceptibility and progression. Breast Cancer Res Treat 2007; 103: 61-9.
18. Przybylowska K, Kluczna A, Zadrozny M, *et al.* Polymorphisms of the promoter regions of matrix metalloproteinases genes MMP-1 and MMP-9 in breast cancer. Breast Cancer Res Treat 2006; 95: 65-72.
19. McColgan P, Sharma P. Polymorphisms of matrix metalloproteinases 1, 2, 3 and 9 and susceptibility to lung, breast and colorectal cancer in over 30,000 subjects. Int J Cancer 2009; 125: 1473-8.
20. Fu F, Wang C, Chen LM, *et al.* The influence of functional polymorphisms in matrix metalloproteinase 9 on survival of breast cancer patients in a Chinese population. DNA Cell Biol 2013; 32: 274-82.
21. Sadeghi M, Motovali-bashi M, Hojati Z. *MMP-9* promoter polymorphism associated with tumor progression of breast cancer in Iranian population. IJIB 2009; 6: 33-7.

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