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The Association of *Matrix Metalloproteinase-1* Promoter Polymorphisms with Breast Cancer

CHIEH-LUN HSIAO[1,2,3*], LIANG-CHIH LIU[4*], TZU-CHING SHIH[5*], YI-LIANG LAI[6], SHIH-WEI HSU[3,6], HWEI-CHUNG WANG[1,4], SU-YI PAN[1,5], TE-CHUN SHEN[1,3], CHIA-WEN TSAI[1], WEN-SHIN CHANG[1], CHEN-HSIEN SU[1], TZONG-DER WAY[2], JING-GUNG CHUNG[2] and DA-TIAN BAU[1,2,3,7]

[1]*Terry Fox Cancer Research Laboratory, Translational Medical Research Center,*
[4]*Department of Breast Surgery, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[2]*Ph.D. Program for Biotechnology Industry,* [3]*Graduate Institute of Biomedical Sciences, and*
[5]*Department of Biomedical Imaging and Radiological Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[6]*Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;*
[7]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: The family of matrix metalloproteinases (MMPs) are responsible for the homeostasis of extracellular matrix components and their genetic polymorphisms may be associated with cancer susceptibility. The serum levels of MMP-1 have been reported to be lower in breast cancer patients than healthy subjects. In the current study, we aimed at investigating the contribution of a polymorphism in the promoter region of MMP-1 to breast cancer in Taiwan. Materials and Methods: The MMP-1 rs1799705 polymorphic genotypes were genotyped among 1,232 breast cancer patients and 1,232 healthy controls by the typical polymerase chain reaction-restriction fragment length polymorphism methodology. Results: The percentages of 2G/2G, 1G/2G, and 1G/1G for MMP1 -1607 genotypes were 35.4, 40.6 and 24.0% in the breast cancer group and 34.1, 43.6, and 22.3% in the healthy control group (p trend=0.3025), respectively. The odds ratios (ORs) after adjusting for age, smoking and alcohol drinking status for those carrying 1G/2G and 1G/1G genotypes at MMP1 -1607 were 0.93 (95%CI=0.76-1.11, p=0.2390) and 1.01 (95%CI=0.77-1.23, p=0.7377), respectively, compared to those carrying the wild-type 2G/2G genotype. Supporting this finding, the adjusted OR for those carrying the 1G allele at MMP-1 -1607 was 1.03 (95%CI=0.91-1.18, p=0.8860), compared to those carrying the wild-type 2G allele. Our findings suggest that the polymorphic genotypes at MMP1 promoter -1607 investigated in the current study, may not play a major role in determining cancer susceptibility to breast cancer in Taiwan. Other early diagnostic and predictive markers are urgently needed for personalized and precise breast cancer detection and therapy.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, Translational Medical Research Center, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 Ext. 5805, e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com

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For many years, breast cancer has been the most common malignancy and the leading cause of female cancer mortality all over the world (1). According to the most updated report about burden of disease trends, cancers overall have increased by 34% during 199-2015 while breast cancer related deaths globally have increased to 45%, a much higher level than average overall cancers (1). In Taiwan, breast cancer has the highest incidence and is ranked as the fourth leading cause of mortality among Taiwanese women (2). From the viewpoint of epidemiology, the risk factors of breast cancer in Taiwan included high caloric intake, high-fat diets, early menarche, late menopause, obesity, high levels of stress, and exposure to environmental pollutants (3). Since, the prevalence and mortality rates are both very high in Taiwan and the world, to figure out feasible molecular markers for early detection and prognosis prediction of breast cancer, especially the subtype of triple negative breast cancer (TNBC), are in urgent need.

The matrix metalloproteinases (MMPs), which are also called matrixins, are a group of enzymes involved in tumor progression such as proliferation, invasion and metastasis (4, 5). In the literature, there are a few papers indicating that some MMP polymorphic genotypes, especially those

Table I. *Demographics and lifestyles of the 1,232 breast cancer patients and the 1,232 healthy control women in Taiwan population.*

Characteristic	Controls (n=1,232)			Patients (n=1,232)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at birth of first child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Tumor site							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history							
First degree (Mother, sister, and daughter)				55	4.5%		
Second degree				6	0.5%		
No history				1171	95%		
Habit							
Cigarette smoker	86	7.0%		170	13.8%		<0.0001*[a]
Alcohol drinker	91	7.4%		162	13.1%		<0.0001*[a]

[a]Chi-square or [b]unpaired Student's *t*-test; *Statistically significant.

implicated in the regulation of gene expression, may account for inter-individual differences of susceptibility to several types of cancer (6-14), while some others do not (15-19).

In literature, the commonly investigated *MMP-1* rs1799750 promoter genotypes were reported to be non-significantly associated with breast cancer in the population of Poland (20). In particular, they found significantly higher levels of MMP-1 expression in tumor and normal breast tissue samples for *MMP-1* rs1799750 2G/2G genotype than for 1G/1G and 1G/2G genotypes (20). Furthermore, there was a positive correlation of *MMP-1* rs1799750 2G/2G genotype with metastasis in axillary lymph node (20). However, the contribution of *MMP-1* genotypes to breast cancer still needs to be validated in other populations, especially those in the East. Thus, the current study aimed at examining the genetic frequencies of *MMP-1* rs1799750 promoter genotypes and evaluating the contribution of *MMP-1* genotypes to the susceptibility of breast cancer in Taiwan.

Materials and Methods

Investigated breast cancer and healthy control subjects. Our study was evaluated and approved by the Institutional Review Board of China Medical University Hospital (DMR99-IRB-108). In this study, 1,232 female patients diagnosed with breast cancer were enrolled at the China Medical University Hospital and an equal number of healthy controls were matched with age and gender. Exclusion criteria for the healthy controls included metastatic cancer of another known or unknown origin, previous malignancy, and any hereditary or genetic disease. All the participants were oriented to complete a self-administered questionnaire and gave peripheral blood samples. The content of the questionnaire included questions on medical history and personal habits such as alcohol consumption and cigarette smoking. These factors were well recorded and are selectively summarized in Table I. All the enrolled individuals in this study have provided their informed consent to the tissue bank of China Medical University Hospital.

MMP-1 genotyping methodology. The genomic DNA of the peripheral blood leukocytes of each participant was extracted, aliquoted and stored as previously described (15, 21, 22). The sequences of primer pairs for *MMP-1* rs1799750 polymorphism were designed by our team as previously published (17). Briefly, genotyping polymerase chain reaction (PCR) cycling conditions *via* My Cycler (Biorad, Hercules, CA, USA) for *MMP-1* were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 sec, 57˚C for 30 sec and 72˚C for 30 sec and a final extension at 72˚C for 10 min (19).

Statistical methodology. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotypic frequencies of *MMP-1* polymorphisms in the healthy controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's chi-square test was used to compare the distribution of the *MMP-1* genotypes between case and control groups. The associations between the *MMP-1* polymorphisms and breast cancer risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders when indicated.

Results

Comparison of demographics and lifestyles between breast cancer cases and control groups. The distributions of

Table II. *Distributions of matrix metalloproteinase-1 (MMP1) -1607 genotypic frequencies among 1,232 breast cancer cases and 1,232 healthy controls in Taiwan.*

	Cases (%)	Controls (%)	Adjusted OR (95%CI)[a]	*p*-Value[b]
MMP1 -1607				
2G/2G (wild-type)	436 (35.4)	420 (34.1)	1.00 (reference)	
1G/2G	500 (40.6)	537 (43.6)	0.93 (0.76-1.11)	0.2390
1G/1G	296 (24.0)	275 (22.3)	1.01 (0.77-1.23)	0.7377
p for trend				0.3025
Carrier comparison				
2G/2G+1G/2G	936 (76.0)	957 (77.7)	1.00 (reference)	
1G/1G	296 (24.0)	275 (22.3)	1.05 (0.78-1.51)	0.3160
2G/2G	436 (35.4)	420 (34.1)	1.00 (reference)	
1G/1G+1G/2G	796 (64.6)	812 (65.9)	1.01 (0.88-1.46)	0.4984

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted with confounding factors include age, smoking and alcohol drinking status. [b]Based on Chi-square test without Yates' correction test.

Table III. *Allele frequencies of matrix metalloproteinase-1 (MMP1) -1607 1G/2G among 1,232 breast cancer cases and 1,232 healthy controls in Taiwan.*

Allele	Cases (%) n=2464	Controls (%) n=2464	Adjusted OR (95%CI)[a]	*p*-Value[b]
MMP1 -1607				
Allele 2G	1372 (55.7)	1377 (55.9)	1.00 (reference)	0.8860
Allele 1G	1092 (44.3)	1087 (44.1)	1.03 (0.91-1.18)	

OR: Odds ratio; CI: confidence interval. [a]Data has been adjusted with confounding factors include age, smoking and alcohol drinking status. [b]Based on Chi-square test without Yates' correction test.

frequencies of the basic characteristics and lifestyles of the breast cancer patients and healthy controls are summarized in Table I. Statistically, there was no difference between the two groups as for age, age at menarche, age at birth of first child, or age at menopause ($p>0.05$). Regarding the personal habits, it was found that more breast cancer patients (13.8 and 13.1%) than healthy controls (7.0 and 7.4%) were of smoking and alcohol drinking habits ($p<0.05$) during their life time (Table I).

Association of MMP-1 promoter genotypes and breast cancer risk. The distributions of genetic frequencies for the investigated *MMP-1* -1607 polymorphism among the breast cancer patients and healthy controls are presented and compared in Table II. Compared to the wild-type genotype (2G/2G) sub-group, there was no significant increased or decreased risk for the 1G/2G or 1G/1G genotypes, even after adjustment for the confounding factors including age, smoking and alcohol drinking status (adjusted OR=0.93 and 1.01, 95%CI=0.76-1.11 and 0.77-1.23, $p=0.2390$ and 0.7377; respectively). In addition to the normal model, we also performed the carrier comparison in the recessive (2G/2G+1G/2G *versus* 1G/1G) and dominant (2G/2G *versus* 1G/1G+1G/2G) models. The results showed that either in recessive or dominant models, the 1G-containing genotypes can significantly increase the risk of breast cancer among Taiwanese. In summary, the 1G allele at *MMP-1* -1607 was not a determinant of increased breast cancer risk in Taiwan (Table II).

Association of MMP-1 allelic subtypes and breast cancer risk. The frequencies of the *MMP-1* promoter -1607 alleles among breast cancer patients and healthy control subjects are presented in Table III. Supporting the findings shown in Table II, the variant 1G allele at *MMP-1* -1607 was not significantly associated with breast cancer risk (adjusted OR=1.03, 95%CI=0.91-1.18, $p=0.8860$) (Table III).

Overall, the findings in Tables II and III consistently support each other.

Discussion

The extracellular matrix is dynamically regulated and plays a critical role in carcinogenesis, while MMPs and their inhibitors are responsible for maintaining the homeostasis of the components of the extracellular matrix (23, 24). MMPs were frequently shown to be upregulated in several types of cancer, and their expression was often associated with poor patient prognosis (25-29). The difference in the expression of MMPs in breast cancer cell lines of different tumorigenicity correlates with the biological behavior of these cells: more malignant cells expressed more MMPs than the less malignant ones (30-32).

The promoter polymorphic site of *MMP-1*, -1607, may determine the levels of MMP-1 and influence the personal susceptibility to breast cancer. In the current study, we demonstrated that the genotypes of *MMP-1* -1607 were non-significantly associated with breast cancer in the investigated population in Taiwan (Tables II and III). This finding is similar to those results of Przybylowska, Biondi and Ghilardi, investigating breast cancer subjects in Poland (20) and Italy (33, 34). The contribution of *MMP-1* polymorphic genotypes to breast cancer require further validation in multi-center and multi-population studies.

In 2012, the knockdown of *MMP-1* was found to decrease the growth, invasive and brain metastatic capacities of breast cancer cells (35). Investigating the phenotypic differences in animals according to their genotypes at *MMP-1* -1607, the mice with the 2G allele had higher levels of MMP-1 in their serum than those with 1G (36). Thus, it is reasonable to further examine the effect of the polymorphic site *MMP-1* -1607 and other promoter polymorphic sites in the regulation

of MMP-1 and tumor behaviors, such as brain and/or osteolytic metastasis.

In conclusion, our results suggest that the typical 1G/2G *MMP-1* polymorphic site did not play a direct determinant role in breast cancer among Taiwanese women. Further phenotypic studies such as the determination of MMP-1 levels in the serum of breast cancer patients and genotype-phenotype correlation are warranted before the contribution of *MMP-1* to breast cancer can be fully revealed.

Conflicts of Interest

The Authors declare no conflict of interest in regard to this study.

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