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The Association of Matrix Metalloproteinase-8 Promoter Genotypes in Breast Cancer

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Abstract. *Background/Aim: The family of matrix metalloproteinases (MMPs) controls homeostasis of the extracellular matrix and their genetic polymorphisms may be associated with personal cancer susceptibility. The serum levels of MMP8 was reported to be higher in patients with breast cancer than in healthy individuals. In this study, we aimed to investigate the contribution of a polymorphism in the promoter region of MMP8 (-799C/T) and two nonsynonymous polymorphisms (Val436Ala and Lys460Thr) to breast cancer. Materials and Methods: MMP8 -799C/T, Val436Ala and Lys460Thr polymorphic genotypes were determined for 1,232 patients with breast cancer and 1,232 healthy controls by polymerase chain reaction-restriction fragment length polymorphism methodology. Results: The odds ratios (ORs) after adjusting for age, gender, smoking and alcohol drinking status for those carrying CT and TT genotypes at the MMP8 promoter C-799T were 1.03 (95% CI=0.88-1.23, p=0.7475) and 1.08 (95% CI=0.91-1.53, p=0.3561), respectively, compared to those carrying the wild-type CC genotype. The OR for the combined T-bearing genotypes were of a similar non-significant level (OR=1.05, 95% CI=0.90-1.26, p=0.5176). Supporting this finding, the adjusted OR for those carrying the T allele at MMP8 C-799T was 1.05 (95% CI=0.86-1.21, p=0.3797), compared to those carrying the wild-type C allele. There was also no significant association of MMP8 Lys460Thr with breast cancer. There was no polymorphic genotype at MMP8 Val436Ala found among any of the investigated individuals. Conclusion: MMP8 -799C/T, Val436Ala and Lys460Thr polymorphisms may only play an indirect role in determining personal cancer susceptibility to breast cancer in Taiwan.*

Globally, breast cancer is the most common malignancy and the leading cause of female cancer mortality (1). According to the most updated data regarding disease trends, all types of cancer overall increased by 34% during 1990-2015, while breast cancer deaths globally have increased by 45% (1). In Taiwan, breast cancer has the highest incidence and is the fourth leading cause of mortality among Taiwanese females (2). Epidemiologically speaking, the risk factors for breast cancer include high caloric intake and a high-fat diet, early menarche and late menopause, obesity, high stress, and exposure to environmental pollution (3). Since the prevalence and mortality rates are both very high in Taiwan and worldwide, molecular markers for early detection and prognosis prediction of breast cancer, especially the subtype of triple-negative breast cancer (TNBC), are urgently in need.

The matrix metalloproteinases (MMPs) are a family of enzymes regulating the degradation of extracellular matrix and are involved in carcinogenesis and invasion and metastasis of cancer (4, 5). MMP8 is a collagen-cleaving enzyme which is present in the connective tissue, and the different patterns of regulation of MMP8 may lead to different progression of cancer among individuals with different genetic backgrounds of *MMP8* (6, 7).

In 2007, the T allele of *MMP8* C-799T genotypes was found to be associated with breast cancer risk and lymph node

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Key Words: Breast cancer, genotype, *MMP8*, polymorphism, Taiwan.

Table I. *Demographics and lifestyle habits of the 1,232 patients with breast cancer and the 1,232 healthy control Taiwanese females.*

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				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.

metastasis in Leaven (Belgium) and Shanghai (China) (8). The serum level of MMP8 was also found to be significantly higher in patients with breast cancer than in healthy individuals (9). In addition, electrophoretic mobility shift assays revealed differences in nuclear protein binding to oligonucleotides associated with differences in *MMP8* C-799T genotype (10). Furthermore, promoter constructs containing the CT or TT genotype at *MMP8* C-799T had a 3-fold greater activity in chorion-like trophoblast cells compared to the constructs containing the C alleles (10). However, the genetic background is quite different among Caucasian and Eastern populations, such as Taiwanese. Therefore, the current study aimed to investigate the association of *MMP8* C-799T, Val436Ala and Lys460Thr polymorphisms with the susceptibility of breast cancer in Taiwan.

Materials and Methods

Investigated patients with breast cancer and controls. A total of 1,232 female patients diagnosed with breast cancer were enrolled at the China Medical University Hospital, Taichung, Taiwan. At the same time, an equal number of healthy controls were matched by age and gender. Exclusion criteria for the healthy controls included metastatic cancer from other or unknown origin, previous malignancy, and any hereditary or genetic disease. All the participants completed a self-administered questionnaire and gave their peripheral blood samples. The content of the questionnaire included questions on medical history and personal habits such as alcohol consumption and cigarette smoking. These data were recorded and are selectively summarized in Table I. All the enrolled individuals provided their informed consent to the Tissue Bank of China Medical University Hospital in this study. Our study was evaluated and approved by the Institutional Review Board of China Medical University Hospital (DMR99-IRB-108).

MMP8 genotyping methodology. The genomic DNA from the peripheral blood leukocytes of each participant was extracted, aliquoted and stored as previously described (11, 12). The primers for *MMP8* C-799T, Val436Ala and Lys460Thr polymorphisms were designed by our team as previously published (13, 14). Briefly, genotyping polymerase chain reaction (PCR) cycling conditions *via* My Cycler (Biorad, Hercules, CA, USA) for *MMP8* were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 57˚C for 30 s and 72˚C for 30 s and a final extension at 72˚C for 10 min (15, 16).

Statistical analysis 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 *MMP8* 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 *MMP8* genotypes between case and control groups. The associations between the *MMP8* 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 the breast cancer case and control groups. The distributions of frequencies for the demographics and lifestyles of the breast cancer cases and healthy controls are summarized in Table

Table II. *Distributions of matrix metalloproteinase-8 (MMP8) genotypic frequencies among the 1,232 patients with breast cancer and the 1,232 healthy control Taiwanese females.*

MMP8	Cases (%)	Controls (%)	Adjusted OR (95%CI)[a]	*p*-Value[b]
C-799T				
CC (wild-type)	648 (52.6)	633 (51.4)	1.00 (Reference)	
CT	466 (37.8)	468 (38.0)	1.03 (0.88-1.23)	0.7475
TT	118 (9.6)	131 (10.6)	1.08 (0.91-1.53)	0.3561
CT+TT	582 (47.4)	599 (48.6)	1.05 (0.90-1.26)	0.5176
p_{trend}				0.6510
Lys460Thr				
AA (wild-type)	1199 (97.3)	1201 (97.5)	1.00 (Reference)	
AC	26 (2.1)	23 (1.9)	1.01 (0.68-1.63)	0.6672
CC	7 (0.6)	8 (0.6)	1.08 (0.61-2.83)	0.7994
AC+CC	33 (2.7)	31 (2.5)	1.01 (0.63-2.14)	0.8000
p_{trend}				0.8816
Val436Ala				
TT (wildt-ype)	1232 (100.0)	1232 (100.0)	1.00 (Reference)	
CT	0 (0.0)	0 (0.0)	--	
CC	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. [a]Adjusted for confounding factors including age, gender, cigarette smoking and alcohol drinking status. [b]Based on Chi-square test without Yates' correction.

Table III. *Allelic frequencies for matrix metalloproteinase-8 (MMP8) polymorphisms among the 1,232 patients with breast cancer and the 1,232 healthy control Taiwanese females.*

Polymorphic allele	Cases (%) N=2,464	Controls (%) N=2,464	Adjusted OR (95%CI)[a]	*p*-Value[b]
C-799T				
Allele C	1762 (71.5)	1734 (70.4)	1.00 (reference)	0.3797
Allele T	702 (28.5)	730 (29.6)	1.05 (0.86-1.21)	
Lys460Thr				
Allele A	2424 (98.4)	2425 (98.4)	1.00 (reference)	0.9097
Allele C	40 (1.6)	39 (1.6)	0.98 (0.73-1.48)	
Val436Ala				
Allele T	2464 (100.0)	2464 (100.0)	1.00 (reference)	
Allele C	0 (0.0)	0 (0.0)	--	

OR: Odds ratio; CI: confidence interval. [a]Adjusted for confounding factors including age, gender, cigarette smoking and alcohol drinking status. [b]Based on Chi-square test without Yates' correction.

I. Statistically, there were no differences between the two groups comparing indices such as age, age at menarche, age at birth of first child, or age at menopause ($p>0.05$). Regarding personal behavior, the data showed that more patients with breast cancer (13.8 and 13.1%) than healthy controls (7.0 and 7.4%) had smoking and alcohol drinking habits, respectively ($p<0.05$) (Table I).

Association of MMP8 promoter genotypes and breast cancer risk. The distributions of genetic frequencies for the three investigated *MMP8* polymorphisms among the breast cancer cases and controls are presented and compared in Table II. Firstly, there was noticeably no polymorphic genotype at *MMP8* Val436Ala found among the breast cancer cases nor the controls (Table II, lower panel), that is to say, all the participants were of TT genotype at *MMP8* Val436Ala. Secondly, the ORs after adjusting for possible confounding factors (age, family history of any cancer, smoking and alcohol drinking status) for those carrying CT and TT genotypes at *MMP8* promoter C-799T were 1.03 (95% CI=0.88-1.23, $p=0.7475$) and 1.08 (95% CI=0.91-1.53, $p=0.3561$), respectively, compared to those carrying the wild-type CC genotype (Table II, upper panel). The *p*-values for trend analysis and combined CT+TT genotypes *versus*

wild-type CC were both not significant (p=0.6510 and 0.5176) (Table II, middle panel). To sum up, these data indicate that none of the genotypes of CT or TT at *MMP8* promoter C-799T, AC or CC at *MMP8* Lys460Thr, CT or CC at *MMP8* Val436Ala, may serve as a useful biomarker for determining the risk of breast cancer in Taiwan (Table II).

Association of MMP8 allelic subtypes and breast cancer risk. The adjusted OR for those carrying the T allele at *MMP8* promoter C-399T was 1.05 (95% CI=0.86-1.21, p=0.3797) compared to those carrying the wild-type C allele (Table III, upper panel). The adjusted OR for those carrying the C allele at *MMP8* Lys460Thr was 0.98 (95% CI=0.73-1.48, p=0.9097) compared to those carrying the wild-type A allele (Table III, middle panel). Overall, the findings shown in Tables II and III are consistent supporting each other.

Discussion

MMPs maintain the homeostasis of extracellular matrix components, which are critical mediators for cancer all behavior such as proliferation, survival, invasion, metastasis and angiogenesis (17, 18). In 2008, plasma MMP8 levels were found to be positively associated with lymph node involvement but showed a negative correlation with the risk of distant metastasis, suggesting MMP8 has a protective effect against lymph node metastasis (19). In 2017, the levels of MMP8 in the serum from patients with breast cancer were found to be higher than those from healthy individuals, indicating that MMP8 may play a role in the occurrence and development of breast cancer (9). Thus, it is reasonable to examine the feasibility of detective and predictive markers from the *MMP8* gene for early detection of breast cancer and prediction of prognosis.

In the present study, we examined the genotypes of *MMP8* among a representative Taiwanese breast cancer population, and assessed whether there was an association between the genotypes of *MMP8* C-799T, Lys460Thr and Val436Ala and breast cancer risk. The results show that there was no significant association between the presence of the T allele at *MMP8* C-799T, C allele at *MMP8* Lys460Thr or C allele at *MMP8* C-799T with breast cancer risk in this Taiwanese cohort (Tables II and III). Our findings suggest that these *MMP8* polymorphic genotypes may not play a determinant role in increasing susceptibility to breast cancer. There are very few studies which investigated the association of *MMP8* genotype with breast cancer. The other two studies showed that the T allele at *MMP8* C-799T was associated with better survival rates (20), and was associated with reduced risk of cancer relapse and greater survival, particularly among patients with earlier stage cancer (8). Our study had its limitation in that the serum levels of MMP8 were not available and therefore we were unable to provide further genotype–phenotype analysis to reveal the contribution of MMP8 to breast cancer in the current study. All three studies had investigated different populations with more than 1000 patients and counterpart controls, and made significant contributions in validating the genomic role of *MMP8* in breast cancer. The consistency or inconsistency may be validated and discussed more after further multi-country and multi-center investigations.

In conclusion, our results suggest that the three polymorphisms of *MMP8*, namely C-799T, Lys460Thr and Val436Ala, may only play an indirect role in breast cancer in the Taiwanese. Further phenotypic studies such as the determination of MMP8 levels in serums of patients with breast cancer are warranted before the contribution of MMP8 to breast cancer can be fully ascertained.

Conflicts of Interest

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

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