Matrix Metalloproteinase-1 Genetic Polymorphism in Breast Cancer in Taiwanese

CHEN-HSIEN SU[1], HSIEN-YUAN LANE[1], CHIEH-LUN HSIAO[1,2], LIANG-CHIH LIU[2], HONG-XUE JI[1,2], HSIN-TING LI[2], SHIOU-TING YEN[2], CHUNG-HAO SU[2], TE-CHUN HSIA[2], WEN-SHIN CHANG[1,2*], CHIA-WEN TSAI[2*] and DA-TIAN BAU[1,2,3*]

[1]*Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[2]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Aim: Metalloproteinases (MMPs) are a family of enzymes involved in many physiological processes, such as skeletal development, wound healing, and scar formation, as well as carcinogenesis. However, the contribution of MMP1 genotype to breast cancer has not been elucidated. This study aimed to evaluate the contribution of commonly studied MMP1 promoter 1607 genotype to breast cancer risk. Materials and Methods: In this hospital-based case–control study, contribution of MMP1 genotype to breast cancer risk was evaluated among 1,232 patients with breast cancer and 1,232 gender-matched healthy controls. Results: The distribution of 2G/2G, 1G/2G and 1G/1G for MMP1 promoter 1607 genotype was 36.0%, 41.3% and 22.7% in the breast cancer group and 34.2%, 44.5% and 21.3% in the non-cancer group, respectively (p for trend=0.2820). We also analyzed the allelic frequency distributions and found that the variant 1G allele of MMP1 promoter 1607 conferred similar breast cancer susceptibility as the wild-type 2G allele (odds ratio=0.99, 95% confidence interval=0.89-1.11, p=0.8858). There was no interaction between MMP1 promoter 1607 genotype and cigarette smoking or alcohol drinking habits. Conclusion: The genotype of MMP1 promoter 1607 may not be a major determining factor for breast cancer risk. The contribution of MMP1 promoter 1607 genotype to prognosis and subtypes of breast cancer needs further investigation.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422052121 Ext. 7534, e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com

Key Words: Breast cancer, genotype, *MMP1*, polymorphism, Taiwan.

Statistically, breast cancer is one of the most commonly diagnosed types of cancer in females worldwide, and the incidence of early-onset breast cancer has rapidly increased in Taiwan and other Asian areas compared to Western countries. Although several predictive biomarkers for breast cancer in Taiwanese have been reported in recent years (1-6), the genomic etiology of breast cancer is of great interest but largely unknown.

Matrix metalloproteinases (MMPs), a family of enzymes that can selectively digest individual components of the extracellular matrix in many tissues (7), regulate the cell migration, differentiation, and regeneration in processes of both normal physiological and pathological states (8-11). MMPs are also closely related to regulation of invasion and metastatic capacities of cancer cells (12). Many studies have shown that single nucleotide polymorphisms of MMP genes may be associated with breast cancer risk, but the relationships remaining controversial. Collagenase 1 (also called MMP1) is most abundant among the MMPs and under the control of activator protein-1 (AP1) which binds to the promoter region of mitogen-activated kinase through polyomavirus-enhancing activity-3 (13, 14). MMP1 expression was significantly higher in atypical ductal hyperplasia than in benign breast disease and in invasive breast cancer compared to *in situ* breast cancer (15, 16). The most well-known polymorphic site in the *MMP1* promoter region is at the upstream position of 1607 bp (rs1799750), and was reported to control the transcriptional activity of *MMP1* (17). No significant difference in genotype distribution between breast cancer and non-cancer groups was observed in a US population (15), in mixed ethnicity with Caucasian patients comprising 65% (18), Polish population (19), nor in meta-analysis (20, 21). To date, as far as we are aware, there is neither any study of *MMP1* genotype in Taiwanese nor a single representative *MMP1* genotype study with more

Table I. *Demographics and lifestyle factors of the investigated patients with breast cancer and the control women.*

Characteristic	Controls (n=1232)			Patients (n=1232)			*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]
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%		
Behavioral habits							
Cigarette smoking	86	7.0%		170	13.8%		<0.0001[a]
Alcohol drinking	91	7.4%		162	13.1%		<0.0001[a]

Statistical results based on [a]Chi-square or [b]unpaired Student's *t*-test.

than one thousand cancer patients anywhere else. In the current study, we aimed to reveal the contribution of *MMP1* genotype at the promoter 1607 site to the risk of breast cancer among 1,232 patients with breast cancer and 1,232 non-cancer healthy people in Taiwan.

Materials and Methods

Investigated population. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR96-IRB-240) and written-informed consent was obtained from all participants. One thousand two hundred and thirty-two patients diagnosed with breast cancer were recruited at the outpatient clinics of general surgery at the China Medical University Hospital in Taiwan. The clinical characteristics of patients, including histological details, were all defined by expert surgeons, Dr. Wang, Dr. Liu and their teammates. The slides were reviewed and scored by at least two independent pathologists. All patients voluntarily participated and provided peripheral blood samples. A total of 1,232 age-matched healthy volunteers were selected after initial random sampling from the Health Examination Cohort of the hospital as the controls of this study. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. The ages of the patients and the controls and other detailed information are summarized in Table I.

Genotyping conditions. The genomic DNA from the peripheral blood leucocytes of each patient and control was prepared and stored at −80˚C until processed as per our previous articles (22-24). The primers for *MMP1* genotype at the promoter 1607 site were 5'-TGACTTTTAAAACATAGTCTATGT-3' (forward) and 5'-GAT TGATTTGAGATAAGTCATAGC-3' (reverse), respectively. The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 58˚C for 30 s, and 72˚C for 30 s; and a final extension at 72˚C for 10 min. After amplification, the PCR products were subject to the digestion by *Alu* I restriction endonuclease for 2 h at 37˚C and separation using 3% agarose gel electrophoresis. The genotypes were identified as homozygous 2G/2G with 269-bp product, heterozygous 1G/2G with 269-, 241- and 28-bp products, and homozygous 1G/1G with 241- and 28-bp products. All the genotypic processing was repeated by two researchers independently, and blindly, and the results were 100% concordant.

Statistical analyses. Student's *t*-test was used for the comparison of ages between the case and the control groups. Pearson's Chi-square test was used to compare the distribution of the *MMP1* promoter 1607 genotypes among the subgroups. The associations between the *MMP1* promoter 1607 genotypes and breast cancer risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis. Any difference at $p<0.05$ was considered statistically significant, and all statistical tests were two-sided.

Results

The frequency distributions of selected characters including age at diagnosis, age at menarche, age at first birth of child, age at menopause, cancer site and personal behavioral habits for the 1232 patients with breast cancer and 1232 matched non-cancer controls are summarized and compared in Table I. Since we applied frequency matching to recruit the non-cancer healthy controls, there was no difference in the distributions of age between the control and case groups

Table II. *Distribution of matrix metalloproteinase-1 (MMP1) promoter 1607 genotypes among controls and patients with breast cancer.*

	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
Genotype						
2G/2G	421	34.2%	443	36.0%	1.00 (Reference)	
1G/2G	548	44.5%	509	41.3%	0.88 (0.74-1.06)	0.1739
1G/1G	263	21.3%	280	22.7%	1.01 (0.82-1.25)	0.9105
P_{trend}						0.2820
Carrier comparison						
2G/2G +1G/2G	969	78.7%	952	77.0%	1.00 (Reference)	
1G/1G	263	21.3%	280	22.7%	1.08 (0.90-1.31)	0.4087
2G/2G	421	34.2%	443	36.0%	1.00 (Reference)	
1G/1G+1G/2G	811	65.8%	789	63.2%	0.92 (0.78-1.09)	0.3530

OR: Odds ratio; CI: confidence interval. [a]Based on Chi-square test without Yate's correction.

Table III. *Distribution of allelic frequencies for matrix metalloproteinase-1 (MMP1) promoter 1607 among controls and patients with breast cancer.*

	Controls, n	%	Patients, n	%	OR (95% CI)	*p*-Value[a]
Allele						
2G	1390	56.4%	1395	56.6%	1.00 (Reference)	
1G	1074	43.6%	1069	43.4%	0.99 (0.89-1.11)	0.8858

[a]Based on Chi-square test without Yate's correction.

(Table I). From the viewpoint of epidemiology, the analysis of behavioral habits showed that cigarette smoking and alcohol consumption were more frequent in the breast cancer population studied ($p<0.0001$) (Table I).

The distributions of the *MMP1* promoter 1607 genotype among the non-cancer controls and the patients with breast cancer are presented and statistically analyzed in Table II. The genotypes of *MMP1* promoter 1607 were not differently distributed between breast cancer and non-cancer control groups (p for trend=0.2820) (Table II). In detail, the MMP1 promoter 1607 heterozygous 1G/2G and homozygous 1G/1G were not associated with increased or decreased breast cancer risk (p=0.1739 and 0.9105, respectively; Table II). In the recessive and dominant models, there was still no association between the genotype of *MMP1* promoter 1607 and breast cancer risk (p=0.4087 and 0.3530, respectively; Table II).

To confirm the genotypic findings in Table II, the analyses of allelic frequency distribution for the *MMP1* promoter 1607 among the investigated groups was also performed and the results are presented in Table III. Consistent with the findings that neither heterozygous 1G/2G nor homozygous 1G/1G genotype of *MMP1* promoter 1607 was associated with breast cancer risk, the variant allele 1G was found at 43.4% in the breast cancer group, similar to that of 43.6% in the non-cancer control group (OR=0.99, 95% CI=0.89-1.11, p=0.8858). To sum up, there was no significant difference in the genotypic or allelic frequencies of *MMP1* promoter 1607 between the control and breast cancer patient groups (Tables II and III).

Discussion

In the current case–control association study, the contribution of *MMP1* promoter 1607 genotypes to breast cancer risk was firstly evaluated in a Taiwanese population. The results showed that neither the genotypic nor the allelic frequencies of *MMP1* promoter 1607 were differentially distributed among the breast cancer patients and non-cancer healthy controls (Tables II and III). The sample sizes of previous studies investigating the association of *MMP1* genotypes with breast cancer risk were all less than the current breast cancer patient collection (15, 18, 19). The findings are consistent that genotypes of *MMP1* promoter 1607 may not be a high-penetrant determinant factor for breast cancer susceptibility. In Table I, we found that cigarette smoking and alcohol consumption were more frequent in the breast cancer population investigated (Table I). However, no

obvious interaction between *MMP1* 1607 genotype and cigarette smoking or alcohol consumption on the susceptibility to breast cancer was observed after our performing the stratification analysis (data not shown).

The MMP1 protein is involved in the degradation of native fibrillar collagen, hence it is also called collagenase-1. MMP1 is thought to promote invasion and metastasis through the degradation of the extracellular matrix as the main component of connective tissue (25-27). In 2004, Przybylowska and colleagues explored the association between *MMP1* promoter 1607 1G/2G genotypes and the risk of breast cancer, and the results showed that the frequency of the 2G allele was higher in patients with lymph node metastasis than in the group without metastasis ($p<0.001$) (19). In 2007, Hughes and colleagues found that the genotypes of *MMP1* and *MMP3*, but not those of *MMP3, MMP7, MMP12* or *MMP13*, were associated with lymph node-positive breast cancer (18). In addition, *MMP1* 2G/2G genotype was associated with reduced survival (hazard ratio=3.1, 95% CI=1.1-8.7) (18). A possible mechanism has been proposed by Tower and his colleagues, providing evidence showing that MMP1 2G/2G genotype contributed to higher expression of MMP1 in MCF-7/ADR breast cancer cells, and by inhibiting ERK signaling, MMP1 expression both blocks the degradation of type I collagen and reduces the invasive capacity of MCF-7/ADR cells (17). In the near future, we will investigate the gene–gene interactions among *MMP1* and other genes, for instance other *MMP* members or tissue inhibitors of MMPs, in order to reveal the possible contribution of *MMP1* genotypes to subtypes of breast cancer, such as triple-negative breast cancer.

In conclusion, our study indicated that the heterozygous 1G/2G or homozygous 1G/1G genotype at *MMP1* promoter 1607 does not seem to serve as a predictive marker for breast cancer susceptibility.

Acknowledgements

The Authors declare no conflicts of interest with regard to this study. They appreciate the Tissue-bank of China Medical University Hospital and Min-Chun Chao for their excellent data collection and technical assistance. This study was supported partially by research grant from Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW105-TDU-B-212-133019).

References

1 Su CH, Hsiao CL, Chang WS, Liu LC, Wang HC, Tsai CW, Li LY, Tsai CH and Bau DT: Evaluation of the contribution of cyclooxygenase 2 genotypes to breast cancer in Taiwan. Anticancer Res *34*: 6711-6716, 2014.

2 Hsieh YC, Lee CH, Tu SH, Wu CH, Hung CS, Hsieh MC, Chuang CW, Ho YS and Chiou HY: *CHRNA9* polymorphisms and smoking exposure synergize to increase the risk of breast cancer in Taiwan. Carcinogenesis *35*: 2520-2525, 2014.

3 Huang CY, Chang WS, Shui HA, Hsieh YH, Loh CH, Wang HC, Ji HX, Hsiao CL, Hsu CM, Tsai CW and Bau DT: Evaluation of the contribution of methylenetetrahydrofolate reductase genotypes to Taiwan breast cancer. Anticancer Res *34*: 4109-4115, 2014.

4 Su CH, Liu LC, Hsieh YH, Wang HC, Tsai CW, Chang WS, Ho CY, Wu CI, Lin CH, Lane HY and Bau DT: Association of alpha B-crystallin (CRYAB) genotypes with breast cancer susceptibility in Taiwan. Cancer Genomics Proteomics *8*: 251-254, 2011.

5 Liu LC, Su CH, Wang HC, Tsai CW, Chang WS, Ho CY, Wu CI, Li FJ, Lin CH, Lane HY and Bau DT: Significant association of caveolin-1 (*CAV1*) genotypes with breast cancer in Taiwan. Anticancer Res *31*: 3511-3515, 2011.

6 Wu MH, Chu CH, Chou YC, Chou WY, Yang T, Hsu GC, Yu CP, Yu JC and Sun CA: Joint effect of peroxisome proliferator-activated receptor gamma genetic polymorphisms and estrogen-related risk factors on breast cancer risk: results from a case–control study in Taiwan. Breast Cancer Res Treat *127*: 777-784, 2011.

7 Visse R and Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res *92*: 827-839, 2003.

8 Zimowska M, Brzoska E, Swierczynska M, Streminska W and Moraczewski J: Distinct patterns of MMP-9 and MMP-2 activity in slow and fast twitch skeletal muscle regeneration *in vivo*. Int J Dev Biol *52*: 307-314, 2008.

9 Wang W, Pan H, Murray K, Jefferson BS and Li Y: Matrix metalloproteinase-1 promotes muscle cell migration and differentiation. Am J Pathol *174*: 541-549, 2009.

10 Woessner JF Jr.: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. Faseb J *5*: 2145-2154, 1991.

11 Murphy G and Docherty AJ: The matrix metalloproteinases and their inhibitors. Am J Respir Cell Mol Biol *7*: 120-125, 1992.

12 Singh RD, Haridas N, Patel JB, Shah FD, Shukla SN, Shah PM and Patel PS: Matrix metalloproteinases and their inhibitors: correlation with invasion and metastasis in oral cancer. Indian J Clin Biochem *25*: 250-259, 2010.

13 Sharrocks AD, Brown AL, Ling Y and Yates PR: The ETS-domain transcription factor family. Int J Biochem Cell Biol 29: 1371-1387, 1997.

14 Westermarck J, Seth A and Kahari VM: Differential regulation of interstitial collagenase (*MMP1*) gene expression by ETS transcription factors. Oncogene *14*: 2651-2660, 1997.

15 Zhou J, Brinckerhoff C, Lubert S, Yang K, Saini J, Hooke J, Mural R, Shriver C and Somiari S: Analysis of matrix metalloproteinase-1 gene polymorphisms and expression in benign and malignant breast tumors. Cancer Invest *29*: 599-607, 2011.

16 Przybylowska K, Kluczna A, Zadrozny M, Krawczyk T, Kulig A, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J and Blasiak J: Polymorphisms of the promoter regions of matrix metalloproteinases genes *MMP1* and *MMP9* in breast cancer. Breast Cancer Res Treat *95*: 65-72, 2006.

17 Tower GB, Coon CI and Brinckerhoff CE: The 2G single nucleotide polymorphism (SNP) in the *MMP1* promoter contributes to high levels of *MMP1* transcription in MCF-7/ADR breast cancer cells. Breast Cancer Res Treat *82*: 75-82, 2003.

18 Hughes S, Agbaje O, Bowen RL, Holliday DL, Shaw JA, Duffy S and Jones JL: Matrix metalloproteinase single-nucleotide polymorphisms and haplotypes predict breast cancer progression. Clin Cancer Res *13*: 6673-6680, 2007.

19 Przybylowska K, Zielinska J, Zadrozny M, Krawczyk T, Kulig A, Wozniak P, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J and Blasiak J: An association between the matrix metalloproteinase 1 promoter gene polymorphism and lymph node metastasis in breast cancer. J Exp Clin Cancer Res *23*: 121-125, 2004.

20 McColgan P and 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 *125*: 1473-1478, 2009.

21 Zhou P, Du LF, Lv GQ, Yu XM, Gu YL, Li JP and Zhang C: Current evidence on the relationship between four polymorphisms in the matrix metalloproteinases (MMP) gene and breast cancer risk: a meta-analysis. Breast Cancer Res Treat *127*: 813-818, 2011.

22 Bau DT, Yang MD, Tsou YA, Lin SS, Wu CN, Hsieh HH, Wang RF, Tsai CW, Chang WS, Hsieh HM, Sun SS and Tsai RY: Colorectal cancer and genetic polymorphism of DNA double-strand break repair gene *XRCC4* in Taiwan. Anticancer Res *30*: 2727-2730, 2010.

23 Bau DT, Wang HC, Liu CS, Chang CL, Chiang SY, Wang RF, Tsai CW, Lo YL, Hsiung CA, Lin CC and Huang CY: Single-nucleotide polymorphism of the *EXO1* gene: association with gastric cancer susceptibility and interaction with smoking in Taiwan. Chin J Physiol *52*: 411-418, 2009.

24 Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010.

25 Kurahara S, Shinohara M, Ikebe T, Nakamura S, Beppu M, Hiraki A, Takeuchi H and Shirasuna K: Expression of MMPS, MT-MMP, and TIMPs in squamous cell carcinoma of the oral cavity: correlations with tumor invasion and metastasis. Head Neck *21*: 627-638, 1999.

26 Fan H, Jiang W, Li H, Fang M, Xu Y and Zheng J: MMP-1/2 and TIMP-1/2 expression levels, and the levels of collagenous and elastic fibers correlate with disease progression in a hamster model of tongue cancer. Oncol Lett *11*: 63-68, 2016.

27 Liu D, Guo H, Li Y, Xu X, Yang K and Bai Y: Association between polymorphisms in the promoter regions of matrix metalloproteinases (MMPs) and risk of cancer metastasis: a meta-analysis. PLoS One *7*: e31251, 2012.

Received May 2, 2016
Revised June 2, 2016
Accepted June 3, 2016