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Impacts of Matrix Metalloproteinase-2 Promoter Genotypes on Breast Cancer Risk

CHIH-CHIANG HUNG[1*], CHUNG-LIN TSAI[2*], YU-TING CHIN[3,4*], YUN-CHI WANG[3,4], CHIA-HUA LIU[1], MENG-LIANG LIN[5], SHIH-SHUN CHEN[6], JIE-LONG HE[7], CHIA-WEN TSAI[3,4], CHEN-HSIEN SU[4], DA-TIAN BAU[3,4,8] and WEN-SHIN CHANG[3,4]

[1]*Division of Breast Surgery, Department of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;*
[2]*Division of Cardiac and Vascular Surgery, Cardiovascular Center, Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[4]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[5]*Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan, R.O.C.;*
[6]*Department of Medical Laboratory Science and Biotechnology, College of Medical and Health Science, Asia University, Taichung, Taiwan, R.O.C.;*
[7]*Department of Post-Baccalaureate Veterinary Medicine, Asia University, Taichung, Taiwan, R.O.C.;*
[8]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: Matrix metalloproteinase-2 (MMP-2) has been implicated in the pathogenesis of breast cancer (BC). However, there is limited research on the role of MMP-2 genotypes in BC risk. This study aimed to investigate the associations between two MMP-2 promoter polymorphisms, rs243865 and rs2285053, and BC risk. Materials and Methods: MMP-2 genotypes were analyzed using PCR-based RFLP methodology in a cohort comprising 1,232 BC cases and 1,232 controls. Results: Genotypic frequencies of MMP-2 rs243865 and rs2285053 in controls were consistent with Hardy-Weinberg equilibrium (p=0.3702 and 0.2036, respectively). There were no significant differences in the distribution of rs243865 and rs2285053 genotypes between BC cases and controls (p for trend=0.1602 and 0.2170, respectively). Variant genotypes at rs243865 and rs2285053 appeared to confer a protective effect, although not statistically significant (all p>0.05). Similarly, the variant T allele at rs243865 and rs2285053 showed a non-significant trend towards decreased BC risk (OR=0.84 and 0.89, 95%CI=0.69-1.02 and 0.78-1.02, p=0.0811 and 0.1043, respectively). There was no interaction observed between MMP-2 rs243865 or rs2285053 genotypes and age. Stratified analysis did not reveal significant associations between MMP-2 rs243865 or rs2285053 genotypes and triple-negative breast cancer (TNBC) (p=0.6458 and 0.8745, respectively). Among both TNBC and non-TNBC cases, none of the variant genotypes at rs243865 or rs2285053 showed significant associations with TNBC (all p>0.05). Conclusion: MMP-2 rs243865 and rs2285053 genotypes appear to have a minimal impact on individual susceptibility to BC or TNBC.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau and Wen-Shin Chang, Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 (Ext. 5805), e-mail: artbau2@gmail.com (Bau), halittlemelon@hotmail.com (Chang)

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Breast cancer (BC) represents the most prevalent malignancy among women, profoundly impacting their physical and mental well-being in clinical settings. In 2020 alone, there were an alarming 2.26 million new cases of BC globally, surpassing lung cancer to become the leading cancer worldwide (1, 2). BC, as the foremost cause of cancer-related deaths in women, exhibits varied effects on quality of life and survival (3). Triple-

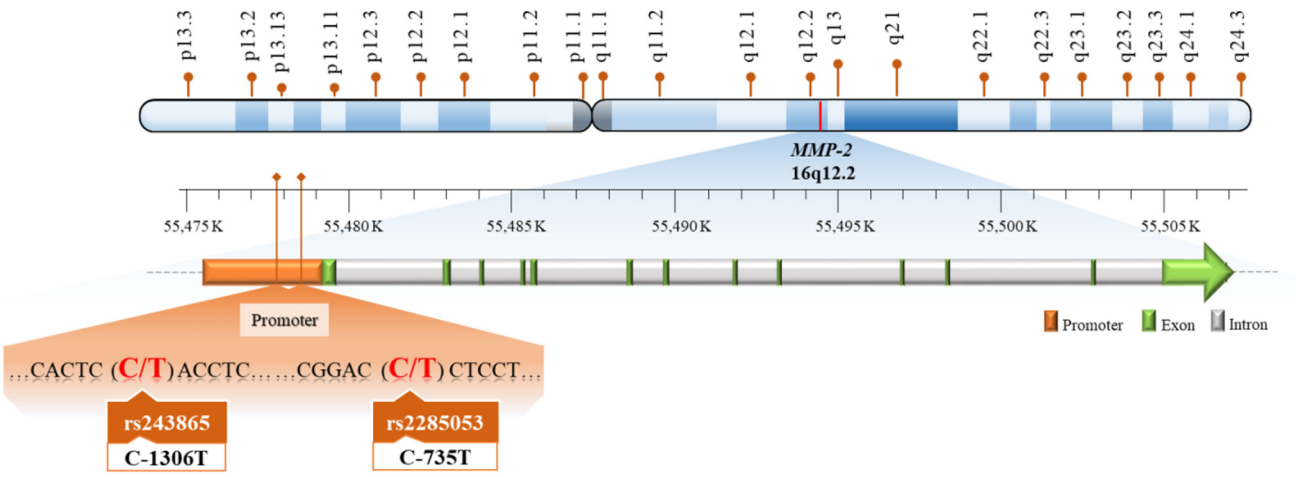

Figure 1. *Physical map of matrix metalloproteinase-2 (MMP-2) rs243865 and rs2285053 polymorphic sites.*

negative breast cancer (TNBC), comprising 15-20% of BC cases, is characterized by high relapse rates, aggressive metastatic behavior, and low 5-year survival rates (4, 5). Lack of practical biomarkers results in a high recurrence rate of 50% among early-stage (stage I~III) TNBC patients, with 37% mortality within 5 years post-surgery (6). Recently, translational scientists have focused on identifying potential biomarkers for BC, particularly TNBC (7-9).

Due to their collagenase activity, matrix metalloproteinases (MMPs) play a crucial role in the degradation of connective tissue components and basement membranes, thereby influencing processes, such as angiogenesis and metastasis (10, 11). Elevated expression levels of MMPs, including MMP-2 and MMP-9, have been associated with accelerated tumor growth and initiation of invasive and metastatic behaviors (12, 13). The literature consistently links elevated MMP expression levels with BC etiology and metastasis (14, 15). However, conflicting reports suggest lower active MMP-2 levels in BC patients compared to non-cancerous subjects (16, 17).

MMP-2, located on chromosome 16, encodes gelatinase A and targets substrates, such as gelatin, collagen V, and collagen VI (18). Numerous studies have explored the impact of specific *MMP-2* polymorphisms on BC risk, particularly focusing on the association with *MMP-2* rs243865, though findings have been controversial (19-29). Located at -1,306 in the promoter region, this polymorphic site's variant T allele has been reported to reduce MMP-2 expression levels (30). Conversely, the biological significance of the variant allele at another promoter polymorphic site, *MMP-2* rs2285053 (-735 position), remains unclear. In Tunisian and Iranian populations, the variant T allele of *MMP-2* rs2285053 appears to confer a protective effect against BC risk (28, 31).

Previously, we have reported associations between *MMP-2* rs243865 genotypes and factors, such as smoking, alcohol consumption, and *Helicobacter pylori* infection status, influencing individual susceptibility to gastric cancer (32). Both variant TT genotypes at *MMP-2* rs243865 and rs2285053 were observed at non-significantly lower frequencies in patients with childhood leukemia (33), prostate cancer (34), and pterygium (35) patients, compared to respective controls. These findings, along with the aforementioned data, suggest that *MMP-2* genotype may play a role in BC risk determination. Therefore, our study aimed to evaluate the influence of *MMP-2* rs243865 and rs2285053 genotypes (the physical location of *MMP-2* genotypes is illustrated in Figure 1) on BC risk specifically within a Taiwanese cohort comprising 1,232 BC cases and 1,232 non-cancerous controls. Additionally, we sought to investigate the potential predictive role of *MMP-2* genotypes in TNBC risk.

Materials and Methods

BC and non-cancerous control population. A total of 1,232 patients diagnosed with breast cancer (BC) were recruited from the outpatient clinics of the Department of General Surgery at China Medical University Hospital in Taiwan for this study. All participants were of Taiwanese descent, and detailed procedures, exclusion and inclusion criteria were previously documented (9, 36). Clinical characteristics, including histological details, were determined by expert surgical teams. Breast cancer tissue slides were independently reviewed and scored by at least two pathologists. Estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu immunoassay positivity was defined by nuclear staining in at least 10% of neoplastic cells. A Ki67 labeling index exceeding 30% was considered positive. HER-2/neu results adhered to guidelines set by the American Society of Clinical Oncology and the College of American Pathologists (37). All patients voluntarily

Table I. *Demographics of the 1,232 breast cancer patients and the 1,232 healthy controls.*

Characteristic	Controls (n=1,232)			Patients (n=1,232)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (yrs)							
<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 (yr)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at birth of first child (yr)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (yr)			48.8 (1.8)			49.3 (2.0)	0.59[b]
TNBC cases							
Yes				194	15.7%		
No				1,038	84.3%		

[a]Chi-square or [b]unpaired Student's *t*-test; SD: standard deviation; yr: years; TNBC Triple-negative breast cancer.

participated, completed a self-administered questionnaire, and provided peripheral blood samples. As controls for the study, 1,232 age-matched healthy volunteers were randomly selected initially from the Health Examination Cohort of the hospital. Exclusion criteria for the control group included previous malignancies, metastatic cancers of other or unknown origins, and any familial or genetic diseases. Both groups completed a brief questionnaire that included lifestyle habits. Our study was approved by the Institutional Review Board of China Medical University Hospital (DMR-99-IRB-108), and written informed consent was obtained from all participants. Both cases and controls were of Taiwanese origin, and key demographic characteristics of the population are summarized in Table I.

MMP-2 genotyping methodology. Peripheral blood samples were collected from all participants and their DNA was extracted within 24 h, following our established protocol (38-40). Genotypes for *MMP-2* rs243865 and rs2285053 were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. Primer sequences specific to *MMP-2* genotyping were designed and optimized by the Terry Fox Cancer Research Lab (35), and PCR conditions were set according to previously published protocols (32, 41). PCR products were digested with *Xsp* I and *Hinf* I overnight for *MMP-2* rs243865 and rs2285053, respectively, and genotyping profiles were identified by 3% agarose gel electrophoresis. Each sample's genotyping was independently and blindly repeated by at least two researchers, with all procedures yielding consistent results showing 100% concordance.

MMP-2 statistical analyzing methodologies. The Hardy-Weinberg equilibrium in the non-cancerous control group was assessed using a chi-square test for goodness-of-fit. The age distribution differences between the BC cases and non-cancerous control groups were presented as mean±standard deviation (SD), and statistical comparisons were performed using unpaired Student's *t*-test. Pearson's chi-square test with Yates' correction (when all analyzing cells >5) or Fisher's Exact test (when any analyzing cell ≤5) was utilized to evaluate the differential distribution of *MMP-2* genotypes. Associations between *MMP-2* genotypes and BC risk were assessed through odds ratios (ORs) with corresponding 95% confidence intervals (CIs) across various stratification analysis models. Statistical significance was considered when *p*-values were ≤0.05. All statistical analyses were performed using SPSS version 16.0 software (SPSS, Inc., Chicago, IL, USA).

Results

The demographic comparisons of the Taiwan BC and non-cancerous population. Table I presents the comparison of age, age at menarche, age at first childbirth, age at menopause, and TNBC status between 1,232 BC cases and 1,232 non-cancerous controls. Initially, no significant differences were detected between the case and control groups regarding age, age at menarche, age at first childbirth, and age at menopause (all $p>0.05$). Among the 1,232 BC cases, 194 were identified as TNBC cases (Table I).

The genotypic distributions of MMP-2 genotypes among Taiwan BC and non-cancerous subjects. Table II presents the genotypic distributions of *MMP-2* rs243865 and rs2285053 among 1,232 non-cancerous controls and 1,232 BC cases. Initially, the frequencies of *MMP-2* rs243865 and rs2285053 genotypes among the non-cancerous controls were consistent with Hardy-Weinberg equilibrium ($p=0.3702$ and 0.2036, respectively). Regarding *MMP-2* rs243865, no significant differential distribution was observed between the BC and non-cancerous control groups (p for trend=0.1602). Specifically, carriers of the *MMP-2* rs243865 heterozygous variant CT and homozygous variant TT genotypes exhibited 0.87- and 0.55-fold risks for BC, respectively, compared to those carrying the wild-type CC genotype (95%CI=0.71-1.07 and 0.24-1.24, $p=0.2137$ and 0.2062, respectively). Additionally, individuals with the CT or TT genotype showed an 0.85-fold risk for BC compared to those with CC genotypes in the dominant model (95%CI=0.69-1.04, $p=0.1292$). Regarding *MMP-2* rs2285053, similarly, no

Table II. *Matrix metalloproteinase-2 rs243865 and rs2285053 genotypes among the 1,232 patients with breast cancer and 1,232 non-cancerous healthy controls.*

Genotypes	Controls, n (%)	Cases, n (%)	OR (95%CI)	*p*-Value[a]
Promoter -1306 rs243865				
CC	994 (80.7)	1,024 (83.1)	1.00 (Reference)	
CT	222 (18.0)	199 (16.2)	0.87 (0.71-1.07)	0.2137
TT	16 (1.3)	9 (0.7)	0.55 (0.24-1.24)	0.2062
CT+TT	238 (19.3)	208 (16.9)	0.85 (0.69-1.04)	0.1292
p_{trend}				0.1602
p_{HWE}				0.3702
Promoter -735 rs2285053				
CC	745 (60.5)	787 (63.9)	1.00 (Reference)	
CT	416 (33.8)	379 (30.8)	0.86 (0.73-1.02)	0.0993
TT	71 (5.7)	66 (5.3)	0.88 (0.62-1.25)	0.5302
CT+TT	487 (39.5)	445 (36.1)	0.87 (0.73-1.02)	0.0885
p_{trend}				0.2170
p_{HWE}				0.2036

OR: Odds ratio; CI: confidence interval; [a]data based on Chi-square test with Yates' correction; p_{trend}: *p*-value based on trend analysis; p_{HWE}: *p*-value based on Hardy-Weinberg Equilibrium.

variant genotype was associated with altered BC risk in any of the examined models (Table II).

The allelic frequency distributions of MMP-2 among Taiwan BC and non-cancerous subjects. Allelic frequency distribution analyses were conducted to validate the findings presented in Table II for *MMP-2* rs243865 and rs2285053. Consistently, the variant T allele of both *MMP-2* rs243865 and rs2285053 showed a non-significant association with altered risk for BC (OR=0.84 and 0.89, 95%CI=0.69-1.02 and 0.78-1.02, *p*=0.0811 and 0.1043, respectively, Table III).

The correlation of MMP-2 genotypes with onset ages in determining BC risk. Genotyping results for *MMP-2* rs243865 and rs2285053 were further stratified by age among both cases and controls to investigate the interaction between *MMP-2* rs243865 and rs2285053 genotypes and age in relation to BC risk (Table IV and Table V). Heterozygous and homozygous variant genotypes of *MMP-2* rs243865 showed no significant association with BC risk in individuals aged both less than and greater than 55 years (OR=0.86, 0.44, 0.89, and 0.76, 95%CI=0.68-1.10, 0.15-1.29, 0.59-1.33, and 0.20-2.86, *p*=0.2687, 0.1985, 0.6316, and 0.7468, respectively, Table IV). Similarly, *MMP-2* rs2285053 genotypes did not exhibit altered BC risk in individuals aged younger or older than 55 years (OR=0.85, 0.96, 0.88, and 0.70, 95%CI=0.70-1.04, 0.64-1.44, 0.63-1.23, and 0.35-1.38, *p*=0.1355, 0.9163, 0.5157, and 0.3849, respectively, Table V).

Association of MMP-2 genotypes with TNBC risk. While *MMP-2* rs243865 and rs2285053 genotypes did not appear to be associated with BC risk, we explored their potential as biomarkers for predicting TNBC risk. To address this, BC patients were stratified into TNBC and non-TNBC subgroups. The findings indicated no significant association between *MMP-2* rs243865 or rs2285053 genotypes and TNBC (*p*=0.6458 and 0.8745). Among both TNBC and non-TNBC cases, none of the *MMP-2* rs243865 or rs2285053 variant genotypes showed a significant association with TNBC (all *p*>0.05) (Table VI and Table VII).

Discussion

As discussed in the introduction, numerous studies have explored the relationship between clinicopathological features of BC and MMP-2 expression. Several studies have reported that MMP-2 expression levels correlate with BC prognosis (42-47). Conversely, a few studies have observed higher MMP-2 expression levels in BC compared to adjacent non-cancerous tissues (48-50). However, Somiari *et al*. reported on multiple occasions that the active form of MMP-2 exhibits lower expression levels in BC patients compared to non-cancerous controls (16, 17). While proteomic investigations have not conclusively determined the precise role of MMP-2 in BC etiology, it is evident that MMP-2 plays a significant role in BC development and may represent a potential target for anti-tumor therapy. Given the hereditary nature of BC and the critical role of MMP-2 in epithelial-mesenchymal transition (EMT) and metastatic

Table III. *Allelic frequencies for matrix metalloproteinase-2 rs243865 and rs2285053 among the 1,232 patients with breast cancer and 1,232 non-cancerous healthy controls.*

Genotypes	Controls, n (%)	Cases, n (%)	Odds ratio (95% Confidence internal)	*p*-Value[a]
rs243865				
Allele C	2,210 (89.7)	2,247 (91.2)	1.00 (Reference)	
Allele T	254 (10.3)	217 (8.8)	0.84 (0.69-1.02)	0.0811
rs2285053				
Allele C	1,906 (77.4)	1,953 (79.3)	1.00 (Reference)	
Allele T	558 (22.6)	511 (20.7)	0.89 (0.78-1.02)	0.1043

[a]Data based on Chi-square test with Yates' correction.

Table IV. *Matrix metalloproteinase-2 rs243865 genotypes in breast cancer risk after stratification by age.*

Genotype	Younger (≤55), n		OR (95%CI)[a]	*p*-Value	Elder (>55), n		OR (95%CI)	*p*-Value[a]
	Controls	Cases			Controls	Cases		
CC	743	760	1.00 (ref)		251	264	1.00 (ref)	
CT	163	144	0.86 (0.68-1.10)	0.2687	59	55	0.89 (0.59-1.33)	0.6316
TT	11	5	0.44 (0.15-1.29)	0.1985	5	4	0.76 (0.20-2.86)	0.7468
Total	917	909			315	323		
p_{trend}				0.1667				0.7869

OR: Odds ratio; CI: confidence interval; TNBC: triple negative breast cancer; Ref: reference; p_{trend}, *p*-value for trend analysis; [a]data based on Chi-square test with Yates' correction (n≥5) or Fisher's exact test (n<5).

Table V. *Matrix metalloproteinase-2 rs2285053 genotypes in breast cancer risk after stratification by age.*

Genotype	Younger (≤55), n		OR (95%CI)[a]	*p*-Value	Elder (>55), n		OR (95%CI)	*p*-Value[a]
	Controls	Cases			Controls	Cases		
CC	560	585	1.00 (ref)		185	202	1.00 (ref)	
CT	307	274	0.85 (0.70-1.04)	0.1355	109	105	0.88 (0.63-1.23)	0.5157
TT	50	50	0.96 (0.64-1.44)	0.9163	21	16	0.70 (0.35-1.38)	0.3849
Total	917	909			315	323		
p_{trend}				0.3034				0.4973

OR: Odds ratio; CI: confidence interval; TNBC: triple negative breast cancer; Ref: reference; p_{trend}: *p*-value for trend analysis; adata based on Chi-square test with Yates' correction.

behaviors of cancer cells, investigating the contribution of *MMP-2* genotypes to BC susceptibility is warranted.

Several research teams have explored the association between *MMP-2* genotypes and BC risk, yielding conflicting and inconclusive results. In 2004, Zhou *et al*. reported a reduced BC risk among variant CT or TT carriers of the rs243865 polymorphism in *MMP-2* within a cohort of 462 BC patients and 509 non-cancerous controls from Beijing, China (19). Subsequently, Roehe *et al*. conducted a similar investigation among individuals in Brazil in 2007, finding no significant association (21). This lack of association was further supported by Lai *et al*. in a Swedish cohort the same year (20). In 2008, Delgado-Enciso *et al*. provided supportive evidence for Zhou's findings in a smaller study involving 90 BC patients and 96 controls from Mexico (22). Notably, these studies focused solely on the genotypic profiles of the single polymorphic site rs243865 in *MMP-2*. In 2009, Beeghly-Fadiel *et al*. expanded their investigation

Table VI. *Association of matrix metalloproteinase-2 rs243865 genotypes with breast cancer risk stratified with triple-negative breast cancer (TNBC), non-TNBC, or healthy controls.*

Genotype	Control	Non-TNBC	OR, 95%CI	*p*-Value[a]	TNBC	OR, 95%CI	*p*-Value[a]
CC	994	866	1.00 (Ref)		158	1.00 (Ref)	
CT	222	164	0.85 (0.68-1.06)	0.1600	35	0.99 (0.67-1.47)	0.9675
TT	16	8	0.57 (0.24-1.35)	0.2779	1	0.39 (0.05-2.99)	0.4949
Total	1,232	1,038			194		
p_{trend}				0.1623			0.6458

OR: Odds ratio; CI: confidence interval; Ref: reference; p_{trend}: *p*-value for trend analysis; adata based on Chi-square test with Yates' correction (n≥5) or Fisher's exact test (n<5).

Table VII. *Association of matrix metalloproteinase-2 rs2285053 genotypes with breast cancer risk stratified with triple-negative breast cancer (TNBC), non-TNBC, or healthy controls.*

Genotype	Control	Non-TNBC	OR, 95%CI	*p*-Value[a]	TNBC	OR, 95%CI	*p*-Value[a]
CC	745	667	1.00 (Ref)		120	1.00 (Ref)	
CT	416	317	0.85 (0.71-1.02)	0.0866	62	0.93 (0.67-1.29)	0.7045
TT	71	54	0.85 (0.59-1.23)	0.4392	12	1.05 (0.55-1.99)	0.8831
Total	1,232	1,038			194		
p_{trend}				0.1793			0.8745

OR: Odds ratio; CI: confidence interval; Ref: reference; p_{trend}: *p*-value for trend analysis.

to 39 *MMP-2* polymorphisms among 6,066 women from Shanghai, China, identifying associations with two SNPs (rs11644561 and rs11643630), while rs243865 showed no association (23). In 2013, Zagouri *et al*. analyzed *MMP-2* rs243865 in relation to BC risk in subgroups, finding no overall association but observing a favorable association between the rs243865 T allele and disease-free survival and overall survival in a limited sample of 113 cases and 124 controls (25). Yari *et al*. attracted attention in 2014 by suggesting that TT genotypes of *MMP-2* rs2285053 might serve as a novel protective marker for BC risk based on their study involving 98 BC cases and 135 healthy subjects in Iran (31). In our current study, our primary objective was to evaluate the intrinsic effects of *MMP-2* on BC risk within a representative cohort from Taiwan. We found no significant associations between the genotypes of two polymorphic sites, *MMP-2* rs243865 and rs2285053, and BC risk (Table II and Table III). Our study benefits from a relatively large sample size but warrants validation in diverse populations of various ethnic backgrounds.

Despite the inclusion of a representative sample size in this case-control study, several limitations merit concise discussion. Firstly, longer follow-up periods are necessary to investigate the contributions of *MMP-2* rs243865 and rs2285053 genotypes to BC prognosis, including survival duration, metastasis, and recurrence. For example, Zagouri *et al*. reported that the *MMP-2* rs243865 T allele may indicate longer survival periods in a Southern European population (25). Secondly, the lack of measurements for MMP-2 expression profiles at RNA or protein levels hinders genotype-phenotype correlation analyses. Thirdly, the potential involvement of other *MMP-2* polymorphisms, such as rs11644561 and rs11643630, in BC risk determination cannot be excluded. Fourthly, understanding the combined effects of *MMP-2* with the expression levels of other genes, such as ER and/or TIMP-2, could provide a more comprehensive understanding of BC etiology. TIMP-2, for instance, acts as both an endogenous MMP-2 inhibitor and suppressor of BC cell proliferation and metastasis (51, 52). Notably, in the context of TNBC, Peeney *et al*. reported that TIMP-2 suppressed TNBC tumor cell proliferation and metastasis (53). In a previous study, we demonstrated that *TIMP-2* rs8179090 genotypes were significantly associated with BC risk among younger (≤55 years old) women but not among older (>55 years old) individuals. Additionally, *TIMP-2* rs8179090 genotypes were associated with TNBC risk (9). Lastly, similar to the fourth point mentioned above, the effects of genotypes of other *MMP* and *TIMP* family members should be considered. Positive associations have been reported for *MMP-7* A-181G (54), *TIMP-1* rs4898 (55), and negative associations for *MMP-1* rs1799705 ([2G]-1607[1G]) (56), *MMP-8* -799C/T, Val436Ala, Lys460Thr (57), *TIMP-1* rs6609533, and rs2070584 (55).

In summary, our findings do not provide convincing evidence supporting an association between *MMP-2* rs243865 or rs2285053 genotypes and altered susceptibility to BC. Furthermore, MMP-2 may not serve prominently as a predictive marker for TNBC. Importantly, although *MMP-2* rs243865 and rs2285053 genotypes did not appear to be significant risk factors for BC or TNBC in the Taiwanese population, further studies across diverse populations are warranted to clarify their potential utility in anti-cancer therapy.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Research design: Chin YT, Hung CC and Tsai CL; patient and questionnaire summaries: Liu CH, Hung CC and Su CH; experimental work: Wang YC, Chin YT, Chang WS, CH SU and Tsai CW; statistical analysis: Lin MC, He JL, Chen SS, and Tsai CL; data clearance and validation: Chin YT, Wang YC, Tsai CW and Chang WS; article writing: Hung CC, Tsai CL, Bau DT and Chang WS; correction of manuscript: Chin YT, Hung CC, Tsai CL, Wang YC, Liu CH, Lin ML, review and revision: Hung CC, Chang WS and Bau DT.

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