Matrix Metalloproteinase-2 Polymorphisms and Breast Cancer Susceptibility

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Abstract

Matrix metalloproteinase-2 (MMP-2) is a well-known mediator of cancer metastasis but is also thought to be involved in several aspects of cancer development, including cell growth and inflammation. We comprehensively characterized genetic variation across the *MMP-2* gene and evaluated associations with breast cancer risk using a two-phase (phase 1 and phase 2) study design. A total of 39 polymorphisms were genotyped among 6,066 Chinese women participating in the Shanghai Breast Cancer Study, a population-based case-control study. Two *MMP-2* promoter polymorphisms were found to have consistent results between phase 1 and phase 2 participants, and to be significantly associated with breast cancer risk among all genotyped participants. Minor allele homozygotes for *rs11644561* (*G*/*A*) were found to have a decreased risk of breast cancer [odds ratio (OR), 0.6; 95% confidence interval (CI), 0.3-1.0] compared with major allele homozygotes, as were minor allele homozygotes for *rs11643630* (*T*/*G*) compared with major allele homozygotes (OR, 0.8; 95% CI, 0.7-1.0). When analyzed together, a rare haplotype (4.4%) with both *rs11644561 A* and *rs11643630 G* was found to have a significantly reduced risk of breast cancer (OR, 0.6; 95% CI, 0.4-0.8). In addition, rare allele homozygotes for *rs243865* (*–1306 C*/*T*) tended to have an increased risk of breast cancer (OR, 1.4; 95% CI, 0.9-2.4). Together, these findings support a role for *MMP-2* genetic variation in breast cancer susceptibility. (Cancer Epidemiol Biomarkers Prev 2009; 18(6):1770–6)

Introduction

As the enzyme capable of cleaving type 4 collagen, the major structural component of the epithelial basement membrane, matrix metalloproteinase-2 (MMP-2, gelatinase A), is well-known to be integral for cancer cell invasion and metastasis (1-3). With many additional extracellular matrix (ECM) and non-ECM substrates, MMP-2 is also involved in a variety of other, potentially pathologic processes, including inflammation, angiogenesis, and cellular proliferation (1-5). Oncogene-mediated cellular transformation was found to induce MMP-2 expression, causing altered cell growth and increased capacity for malignant progression (6, 7). *In vitro* assays of breast cancer cells stably transfected with *MMP-2* showed increased invasive properties, whereas accelerated tumor growth, enhanced metastatic colonization, and increased tumor burden was seen after the transfected cells were injected into mice (8, 9). On the other hand, *MMP-2* deficient mice were found to have reduced tumor-induced angiogenesis, significantly slower tumor growth rates, and decreased metastatic colonization of the lung after implantation of either melanoma or lung carcinoma cells (10).

In humans, normal breast tissue and benign breast lesions were rarely found to express *MMP-2*, whereas expression has been detected in both tumor and surrounding stromal cells (11-15). Furthermore, compared with adjacent breast tissue, a gradual increase in *MMP-2* expression was seen from noninvasive to invasive cancers, whereas MMP-2 activity has also been found to be significantly higher in malignant breast tissue compared with other breast tissues (16, 17). In addition, breast cancer patients were found to have significantly higher circulating MMP-2 levels compared with control volunteers (18).

Genetic variation that modulates *MMP-2* expression may contribute to individual differences in cancer susceptibility. Two single nucleotide polymorphisms (SNP) in the *MMP-2* promoter have been shown to affect expression *in vitro*; *C* to *T* transitions at *–1306* (*rs243865*) and *–735* (*rs2285053*) both result in lower transcriptional activities (19-21). These two SNPs are reported to be in high linkage disequilibrium (LD) and have an interactive effect on *MMP-2* transcription (21). Several epidemiologic studies have evaluated these promoter polymorphisms in relation to cancer risk with inconsistent results. To date, only a few studies have evaluated genetic variation in *MMP-2* in relation to breast cancer susceptibility, and all included only one polymorphism (*rs243865*; refs. 22-25). Preliminary results from a small study (89 cases and 100 controls) among Latin American women indicated that there was no association (22), whereas a small study among Mexican women (90 cases and 96 controls) found a significantly increased risk of breast cancer associated with *–1306 CC* (23). A larger study (462 cases and 509

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controls) among Chinese women found a significantly decreased risk of breast cancer for *T* allele carriers (24), whereas the largest study to date (959 cases and 952 controls), conducted among Swedish women, found no association (25).

As MMP-2 has been shown to contribute not only to cancer invasion and metastasis, but also to cellular transformation and tumor growth, this study was undertaken to comprehensively characterize genetic variation across the *MMP-2* gene and evaluate associations of *MMP-2* polymorphisms and breast cancer susceptibility.

Materials and Methods

Study subjects were participants of the Shanghai Breast Cancer Study (SBCS), a population-based case-control study among Chinese women; detailed information on the study design and data collection procedures have been previously described (26). Briefly, phase 1 cases were women diagnosed with breast cancer between August 1996 and March 1998, 25 to 64 y of age, without a previous cancer diagnosis, and alive at the time of interview. Recruitment for phase 2 occurred between April 2002 and February 2005, and eligibility criteria were expanded to include women 20 to 70 y of age (27, 28). All cases were identified via the population-based Shanghai Cancer Registry; diagnoses were confirmed by two senior pathologists. Controls were randomly selected from the general population using the Shanghai Resident Registry, a population registry of adult residents in urban Shanghai; women with previous cancer diagnoses were excluded. Structured questionnaires were administered by trained interviewers, and were used to obtain detailed information on demographic, reproductive, and behavioral factors; height and weight were also measured. Of eligible participants, 1,459 (91.1%) cases and 1,556 (90.3%) controls in phase 1 and 1,989 cases (83.7%) and 1,989 controls (70.4%) in phase 2, completed in-person interviews. In phase 1, 1,193 cases (81.8%) and 1,310 controls (84.2%) donated blood samples. In phase 2, 1,932 (97.1%) cases and 1,857 (93.4%) controls donated either blood or buccal cell samples. Genomic DNA was extracted using Puregene's DNA Purification kits (Gentra Systems) or Qiagen's DNA Purification kits (Qiagen) according to manufacturers' instructions. Laboratory staff was blinded to the case-control status of these subjects for all subsequent genotyping described.

Haplotype-tagging SNPs (htSNPs) were selected by searching Han Chinese data from the HapMap Project (29) using the Tagger program (30). htSNPs were selected to cover polymorphisms with minimum minor allele frequency (MAF) of 0.05 in the *MMP-2* gene ± 5 kb with an r^2 of 0.90 or greater. Selection of htSNPs was completed in December 2005 using HapMap Release 19. Genotyping assays for 19 htSNPs were completed for 2,131 phase 1 participants in 2006 using a Targeted Genotyping System (Affymetrix) based on an advanced Molecular Inversion Probe method (31). Blinded (n = 39) and HapMap samples (n = 12) were also included; consistency rates averaged 99.6%.

Two SNPs with promising results in phase 1 (*rs1116195* and *rs243865*) were selected for additional genotyping among phase 2 participants. Furthermore, a polymorphism reported to be functional that was not genotyped in phase 1 (*rs2285053*) was also selected. These 3 SNPs were genotyped among 2,932 phase 2 participants using the Sequenom iPLEX MassARRAY platform (Sequenom, Inc.). PCR and extension primers were designed using Sequenom Assay Design software. PCR and extension reactions were done according to manufacturer's instructions as previously described (32). Allele-specific extension products were determined by using matrix assisted laser desorption/ionization time-of-flight mass spectrometry. Blinded duplicate samples and negative controls were included in each 96-well plate; concordance rates between duplicate samples were 100% for all three polymorphisms genotyped by this method.

Recently, we completed genotyping for 4,157 cases and controls (2,213 phase 1 and 1,944 phase 2 participants) using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix), that includes 906,602 SNPs. From this, data for 19 additional SNPs that are located in *MMP-2* ± 10 kb were included in the current study to increase the density of genetic coverage and the statistical power of our analysis.

Hardy-Weinberg equilibrium was tested by comparing the observed and expected genotype frequencies of the controls (χ^2 test). Characteristics of cases and controls were compared with the χ^2 test or t test for categorical or continuous variables, respectively. Covariates considered included age at diagnosis, age at menarche, age at first live birth among parous women, age at menopause among postmenopausal women, history of breast fibroadenomas, body mass index, and leisure physical activity in the decade preceding diagnosis. Associations with breast cancer risk were evaluated by computing odds ratios (OR) and corresponding 95% confidence intervals (95% CI) by logistic regression. Additive models of effect were applied to all SNPs; tests for trend were conducted by coding for the number of variant alleles and reporting the P value for the β coefficient. Dominant or recessive effect models and P values were also calculated when suggested to be appropriate for particular SNPs. LD between polymorphisms was assessed by Haploview (33). Associations between haplotypes and breast cancer risk were analyzed with HAPSTAT software (34); additive, dominant, and recessive models of effect were also evaluated. Study phase was adjusted for in analyses that included participants from both phases. All statistical tests were two tailed, and P values of ≤0.05 were interpreted as statistically significant.

Results

A total of 6,066 Chinese women were included in the present study: 2,291 phase 1 participants and 3,775 phase 2 participants. Women in phase 2 were slightly older, and tended to participate in more regular physical activity than phase 1 women, but were generally comparable. As expected, breast cancer cases were found to differ from controls with regard to demographic, reproductive, and other known breast cancer risk factors (Table 1). Cases were more likely to have an earlier age at menarche, older age at first live birth, a history of breast fibroadenomas, higher body mass index and waist-to-hip ratio, and less likely to participate in regular physical activity than controls.

A total of 39 *MMP-2* SNPs were genotyped as follows: 19 htSNPs among phase 1 participants, 1 SNP selected

from the literature among phase 2 participants, and 19 additional SNPs among participants of both study phases. Of the *MMP-2* SNPs genotyped, 3 had MAFs of <1% in our study population, and were excluded from further analyses (*rs16955194*, *rs7189232*, and *rs11541998*). The remaining SNPs were found to have MAFs between 10.7 and 48.9% among genotyped controls; none were found to deviate from Hardy-Weinberg equilibrium. MAFs among SBCS controls were generally comparable with the MAF found among Han Chinese genotyped in HapMap, with 2 exceptions: SBCS controls had more A alleles (44.6%) for *rs1005912* than HapMap (38%), and fewer G alleles (40.4%) for *rs243867* than HapMap (46%), although neither of these differences was statistically significant. Associations with breast cancer susceptibility were initially assessed using additive models of effect. When the same SNP was genotyped by two methods, the data source with the higher number of genotyped participants was used. One SNP (*rs243865*) was genotyped by both Sequenom and Affymetrix 6.0 methods for 1,098 samples; the concordance rate between these methods was 100%. Seven SNPs (*rs243865*, *rs1477017*, *rs865094*, *rs1053605*, *rs243847*, *rs243839*, and *rs11639960*) were genotyped by both Affymetrix Targeted Genotyping and the Affymetrix 6.0 Genome Wide platform for ~2,000 participants; concordance rates for these samples ranged between 98.96% and 99.90%, and averaged 99.65%.

SNP information, genotyping method, study population genotyped, and associations with breast cancer risk for the 36 polymorphic *MMP-2* SNPs are detailed in Table 2. All available genotyped participants are included in the analyses; when study phases 1 and 2 are listed, the estimate of effect is pooled. Two promoter SNPs were found to confer significant reductions in breast cancer risk among rare allele homozygotes. *rs11644561 AA* participants were ~40% less likely to be breast cancer cases than those with *GG*, whereas *rs11643630 AA* participants were ~20% less likely to be breast cancer cases than those with the *TT* genotype. Additionally, one SNP, *rs1005912*, was associated with a small increased risk among heterozygotes, although rare allele homozygotes did not exhibit a stronger positive association.

Table 3 includes associations with breast cancer stratified by SBCS phase; included are the two SNPs above as well as two polymorphisms that had promising results after our initial targeted phase 1 genotyping. Among phase 1 participants, *rs1116195* was significantly associated with an increased risk of breast cancer for minor allele homozygotes (OR, 1.3; 95% CI, 1.0-1.7), whereas minor allele homozygotes for *rs243865* tended to have an increased risk (OR, 1.8; 95% CI, 0.8-4.1). Additional genotyping among 1,491 cases and 1,437 controls from phase 2 did not confirm an association between *rs1116195* and breast cancer risk. However, results from phase 2 for *rs243865* were similar to those from phase 1. In both phases, minor allele homozygotes for *rs243865* tended to have a moderate increased risk of breast cancer, although even in combined analysis, this did not reach statistical significance. The two SNPs with significant results among all genotyped participants (*rs11644561* and *rs11643630*) had consistent results when stratified by SBCS phase. Minor allele homozygotes compared with major allele homozygotes were significantly less likely to be breast cancer cases for *rs11644561* (OR, 0.6; 95% CI, 0.3-1.0) and for *rs11643630* (OR, 0.8; 95% CI, 0.7-1.0).

The LD structure of the 36 polymorphic *MMP-2* SNPs was constructed by combining all available genotyping data from 3,027 controls (Fig. 1). The two SNPs with significant risk reductions for homozygotes (*rs11644561* and *rs11643630*) were not found to be in high LD (D = 0.21; $r^2 = 0$), and so haplotype analysis was done to assess the effects of these SNPs in concert (Table 4). Compared with the common reference haplotype with both major alleles (H1: *GT*, 48.5%), the haplotype with minor alleles for both *rs11644561* and *rs11643630* (H4: *AG*, 4.4%) was associated with a significantly reduced risk of breast cancer in both additive and dominant models (OR, 0.6; 95% CI, 0.4-0.8). As single SNP analysis resulted in significant risk

Table 1. Characteristics of patients genotyped for *MMP-2*, by study phase the SBCS

Characteristics	Phase 1 (n = 2,291)			Phase 2 (n = 3,775)		
	Cases (n = 1,114)	Controls (n = 1,177)	P	Cases (n = 1,925)	Controls (n = 1,850)	P
Demographic factors						
Age, mean (y)	47.6 ± 8.0	47.2 ± 8.6	0.280	50.9 ± 8.3	51.7 ± 8.4	**0.003**
Age, range (y)	(28-64)	(25-64)	NA	(20-70)	(23-70)	NA
Education (less than middle school)	138 (12.4%)	171 (14.5%)	0.134	312 (16.2%)	209 (11.3%)	**<0.001**
Reproductive risk factors						
Age at menarche (y)	14.5 ± 1.6	14.7 ± 1.7	**<0.001**	14.4 ± 1.7	14.7 ± 1.8	**<0.001**
Premenopausal	745 (66.9%)	758 (64.4%)	0.213	1,084 (56.3)	934.(50.5%)	**0.001**
Age at menopause (y)*	48.1 ± 4.7	47.4 ± 5.0	**0.031**	48.5 ± 4.4	48.3 ± 4.6	0.196
Age at first live birth (y)†	26.8 ± 4.1	26.2 ± 3.8	**0.001**	26.2 ± 3.6	25.7 ± 3.8	**<0.001**
Used oral contraceptives	244 (21.9%)	354 (21.6%)	0.852	341 (17.7%)	356 (19.2%)	0.226
Used estrogen replacement therapy	29 (2.6%)	30 (2.6%)	0.929	89 (4.6%)	60 (3.2%)	**0.029**
Additional risk factors						
First-degree relative with breast cancer	37 (3.3%)	30 (2.6%)	0.273	104 (5.4%)	57 (3.1%)	**<0.001**
Ever had breast fibroadenomas	107 (9.6%)	58 (4.9%)	**<0.001**	192 (10.0%)	105 (5.7%)	**<0.001**
Body mass index (kg/m^2)	23.6 ± 3.4	23.2 ± 3.4	**0.013**	23.7 ± 3.3	23.4 ± 3.2	**0.004**
Waist-to-hip ratio	0.81 ± 0.06	0.80 ± 0.06	**0.002**	0.83 ± 0.05	0.82 ± 0.06	**<0.001**
Regular physical activity	214 (19.2%)	300 (25.5%)	**<0.001**	562 (34.4%)	636 (34.4%)	**<0.001**

NOTE: Continuous variables; mean values ± SD, P value from t tests; Categorical variables: numbers and percentages, P values from χ^2 test. Bold values considered to be significant at P value of ≤0.05.
*Age at menopause among postmenopausal women.
†Among parous women.

Table 2. *MMP-2* SNPs and breast cancer risk, the SBCS

SNP	Alleles*	Region	Method	Study phase†	MAF (%)‡	Breast cancer risk§		
						AB OR (95% CI)	BB OR (95% CI)	*P*
rs1005912	T/A	Promoter	Affy 6.0	1 & 2	45.3	**1.2 (1.0-1.3)**	1.1 (0.9-1.3)	0.207
rs1116195	A/T	Promoter	Targeted	1 & 2	44.6	1.0 (0.9-1.2)	1.2 (1.0-1.4)	0.075
rs11644561	G/A	Promoter	Affy 6.0	1 & 2	13.0	0.9 (0.8-1.1)	**0.6 (0.3-1.0)**	0.098
rs243867	A/G	Promoter	Affy 6.0	1 & 2	40.4	1.1 (0.9-1.2)	1.1 (0.9-1.3)	0.403
rs11643630	T/G	Promoter	Affy 6.0	1 & 2	42.9	1.0 (0.8-1.1)	**0.8 (0.7-1.0)**	**0.046**
rs243866	G/A	Promoter	Affy 6.0	1 & 2	11.0	1.0 (0.9-1.2)	1.2 (0.7-2.1)	0.602
rs243865	C/T	Promoter	Targeted	1 & 2	11.5	0.9 (0.8-1.1)	1.4 (0.9-2.4)	0.776
rs243864	T/G	Promoter	Affy 6.0	1 & 2	10.7	1.0 (0.9-1.2)	1.1 (0.6-2.0)	0.782
rs2285053	C/T	Promoter	Targeted	Phase 2	23.4	1.2 (1.0-1.4)	0.9 (0.6-1.2)	0.436
rs1477017	A/G	Intron 2	Both	1 & 2	27.4	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.833
rs865094	A/G	Intron 2	Both	1 & 2	29.1	0.9 (0.8-1.0)	1.1 (0.9-1.4)	0.838
rs11646643	A/G	Intron 3	Affy 6.0	1 & 2	15.5	1.0 (0.8-1.1)	1.1 (0.7-1.6)	0.726
rs1053605	C/T	Exon 5	Both	1 & 2	13.0	1.1 (0.9-1.2)	0.8 (0.4-1.3)	0.862
rs9302671	G/T	Intron 5	Affy 6.0	1 & 2	15.3	1.0 (0.8-1.1)	1.1 (0.8-1.6)	0.936
rs2241145	G/C	Intron 5	Targeted	Phase 1	48.9	1.0 (0.8-1.2)	0.9 (0.8-1.2)	0.613
rs2241146	G/A	Intron 5	Targeted	Phase 1	21.8	1.1 (0.9-1.3)	1.0 (0.7-1.5)	0.632
rs243849	C/T	Exon 7	Affy 6.0	1 & 2	18.8	0.9 (0.8-1.1)	1.1 (0.8-1.6)	0.816
rs12599775	G/C	Intron 7	Targeted	Phase 1	11.2	1.1 (0.9-1.4)	0.9 (0.4-1.9)	0.453
rs243847	T/C	Intron 7	Both	1 & 2	42.3	1.1 (0.9-1.2)	1.0 (0.8-1.2)	0.881
rs2192852	A/G	Intron 7	Targeted	Phase 1	38.1	1.0 (0.8-1.2)	0.9 (0.7-1.2)	0.546
rs12923011	C/T	Intron 7	Targeted	Phase 1	16.4	0.9 (0.7-1.1)	0.7 (0.4-1.3)	0.118
rs243845	G/A	Intron 8	Affy 6.0	1 & 2	31.1	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.945
rs243844	G/A	Intron 8	Targeted	Phase 1	30.7	1.0 (0.8-1.2)	1.1 (0.8-1.5)	0.604
rs2287074	G/A	Exon 9	Targeted	Phase 1	27.4	1.0 (0.8-1.2)	0.8 (0.5-1.1)	0.276
rs243842	T/C	Intron 9	Affy 6.0	1 & 2	31.9	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.882
rs183112	G/A	Intron 9	Targeted	Phase 1	18.7	1.0 (0.8-1.2)	0.9 (0.6-1.5)	0.874
rs243839	A/G	Intron 9	Both	1 & 2	41.1	1.0 (0.9-1.1)	1.0 (0.8-1.2)	0.924
rs9923304	C/T	Intron 9	Affy 6.0	1 & 2	27.1	1.0 (0.9-1.2)	0.9 (0.7-1.2)	0.983
rs11639960	A/G	Intron 10	Both	1 & 2	28.8	1.0 (0.9-1.1)	1.1 (0.8-1.3)	0.889
rs243831	T/G	3′ FR‖	Targeted	Phase 1	13.6	0.8 (0.7-1.0)	0.8 (0.4-1.6)	0.113
rs12930259	T/C	3′ FR‖	Targeted	Phase 1	33.6	1.0 (0.9-1.2)	1.0 (0.7-1.3)	0.899
rs2192853	A/G	3′ FR‖	Targeted	Phase 1	35.8	0.9 (0.8-1.1)	1.0 (0.7-1.3)	0.607
rs1583587	G/C	3′ FR‖	Affy 6.0	1 & 2	35.7	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.796
rs8053806	C/A	3′ FR‖	Affy 6.0	1 & 2	23.4	1.1 (1.0-1.3)	1.1 (0.9-1.5)	0.139
rs12708952	G/C	3′ FR‖	Affy 6.0	1 & 2	36.0	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.874
rs1583585	G/A	3′ FR‖	Affy 6.0	1 & 2	23.1	1.1 (0.9-1.2)	1.1 (0.9-1.5)	0.192

NOTE: Bold values considered to be significant at a *P* value of ≤0.05.
*Major/minor alleles as determined by allele frequency among genotyped controls.
†Genotyping Method and SBCS Study Phase: Affymetrix Targeted Genotyping among 1,062 cases and 1,069 controls from SBCS phase 1 and/or Sequenom Targeted Genotyping among 1,495 cases and 1,437 controls from SBCS phase 2 (Targeted), or Affymetrix 6.0 genotyping among 1,104 cases and 1,109 controls from phase 1, and 965 cases and 971 controls from SBCS phase 2 (Affy 6.0), or genotyped by both methods (Both); study phases 1 &2 show pooled estimates.
‡MAF among all genotyped controls.
§Risk of breast cancer, adjusted for age, education, and study phase (when appropriate); AA, major allele homozygotes (reference group); AB, heterozygotes; BB, minor allele homozygotes; *P* value for trend from additive models.
‖3′ FR, downstream flanking region, 3′of the MMP-2 gene.

reductions only for the homozygotes of these variants, and haplotype analysis indicated that it was the two SNPs in combination that best captured a decreased risk of breast cancer, these two SNPS were analyzed further. In logistic regression models that included both *rs11644561* and *rs11643630*, homozygotes for both SNPs were found to have significantly reduced risks; furthermore, an interaction term for the two polymorphisms was not found to

Table 3. Selected MMP-2 SNPs and breast cancer risk, by study phase the SBCS

SNP	Alleles*	Method†	MAF (%)‡	Phase 1 OR (95% CI)§			Phase 2 OR (95% CI)§			Combined OR (95% CI)§		
				AB	BB	*P*	AB	BB	*P*	AB	BB	*P*
rs1005912	T/A	Affy 6.0	45.3	1.2 (1.0-1.5)	1.2 (0.9-1.5)	0.187	1.1 (0.9-1.4)	1.0 (0.9-1.4)	0.642	**1.2 (1.0-1.3)**	1.1 (0.9-1.3)	0.207
rs1116195	A/T	Targeted	44.6	1.1 (0.9-1.4)	**1.3 (1.0-1.7)**	**0.038**	1.0 (0.8-1.2)	1.1 (0.9-1.3)	0.574	1.0 (0.9-1.2)	1.2 (1.0-1.4)	0.075
rs11644561	G/A	Affy 6.0	13.0	0.9 (0.8-1.2)	0.5 (0.2-1.1)	0.189	0.9 (0.7-1.1)	0.6 (0.3-1.4)	0.262	0.9 (0.8-1.1)	**0.6 (0.3-1.0)**	0.098
rs11643630	T/G	Affy 6.0	42.9	0.9 (0.7-1.1)	0.8 (0.7-1.1)	0.155	1.0 (0.9-1.3)	0.8 (0.6-1.0)	0.191	1.0 (0.8-1.1)	**0.8 (0.7-1.0)**	**0.046**
rs243865	C/T	Targeted	11.5	0.9 (0.7-1.1)	1.8 (0.8-4.1)	0.512	1.0 (0.8-1.2)	1.3 (0.7-2.5)	0.824	0.9 (0.8-1.1)	1.4 (0.9-2.4)	0.776

NOTE: Bold values considered to be significant at a *P* value of ≤0.05.
*Major/minor alleles as determined by allele frequency among genotyped controls.
†Genotyping Method: Affymetrix Targeted Genotyping among 1,062 cases and 1,069 controls from SBCS phase 1 and Sequenom Targeted Genotyping among 1,495 cases and 1,437 controls from SBCS Phase 2 (Targeted), or Affymetrix 6.0 genotyping among 1,104 cases and 1,109 controls from phase 1, and 965 cases and 971 controls from SBCS phase 2 (Affy 6.0).
‡Minor allele frequency among all genotyped controls.
§Breast Cancer Risk ORs and 95% CIs from additive models, adjusted for age, education, and study phase (when appropriate), *P* value for trend from additive effect models.

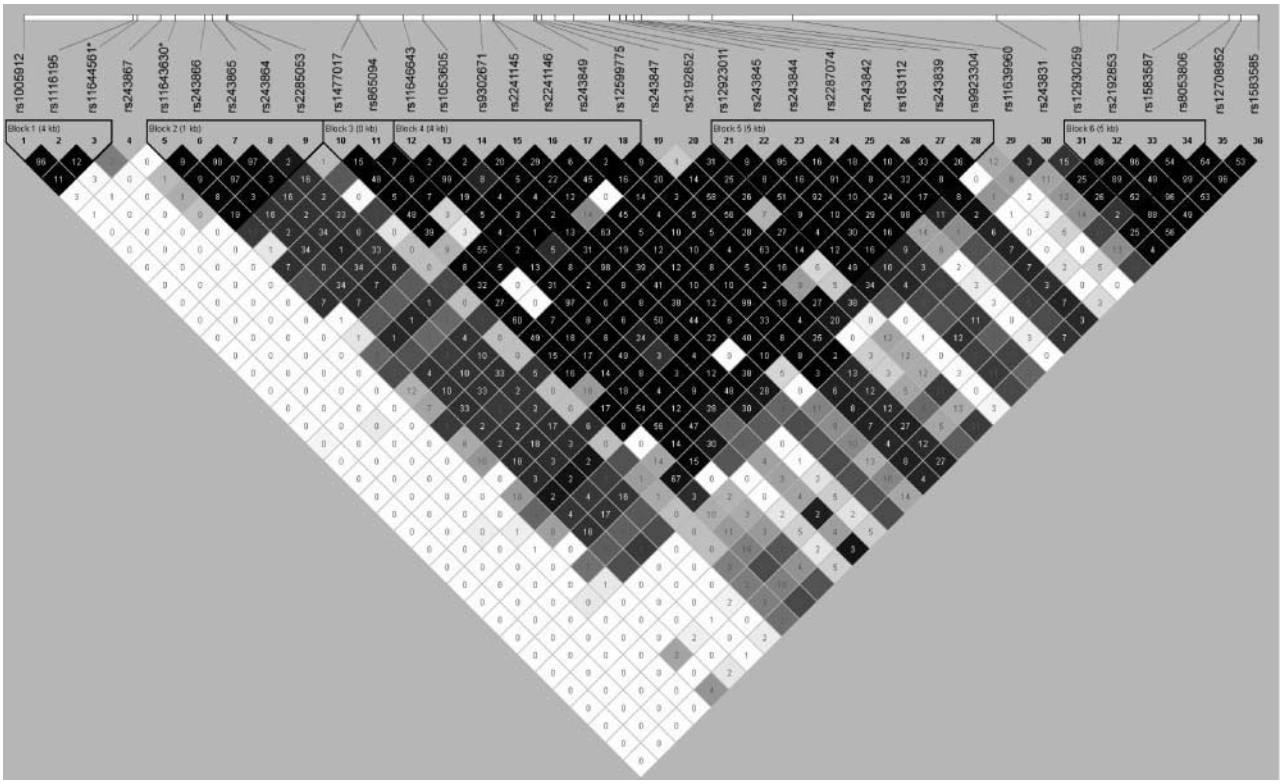

Figure 1. LD structure of 36 *MMP-2* SNPs among 3,027 SBCS controls; value shown is r^2. Two SNPs of interest, *rs11644561* and *rs11643630*, are marked (*), and are in positions 3 and 5, respectively.

reach statistical significance. Finally, haplotype analysis was also conducted for the two *MMP-2* SNPs previously reported in the literature; no significant haplotype effects for *rs243865* or *rs2285053* were observed.

Discussion

A two-phase case-control study was conducted to first comprehensively evaluate *MMP-2* genetic variants in relation to breast cancer risk, and then to validate any promising associations among a second independent sample population. Two *MMP-2* SNPs (*rs11644561* and *rs11643630*) were found to have associations with breast cancer risk that were consistent between phase 1 and phase 2 study populations as well as significant in combined analyses. Although the effects of these SNPs were found to be independent, a rare haplotype that included both minor alleles was associated with significant risk reduction. To the best of our knowledge, neither of these two *MMP-2* polymorphisms have been previously evaluated for associations with cancer susceptibility.

Table 4. Haplotype analysis of selected *MMP-2* polymorphisms, the SBCS

Haplotype*	Frequency†	Additive models		Dominant models		Recessive models	
		OR‡ (95% CI)	*P*	OR‡ (95% CI)	*P*	OR‡ (95% CI)	*P*
SNPs with replicated results: rs11644561 and rs11643630							
H1: GT	48.5	1.0 (Reference)		1.0 (Reference)		1.0 (Reference)	
H2: G**G**	38.5	0.9 (0.9-1.0)	0.302	1.0 (0.9-1.1)	0.670	1.0 (0.8-1.1)	0.534
H3: **A**T	8.6	1.0 (0.8-1.2)	0.929	1.0 (0.9-1.3)	0.641	0.9 (0.5-1.6)	0.830
H4: **AG**	4.4	**0.6 (0.4-0.8)**	**0.002**	**0.6 (0.4-0.8)**	**0.003**	0.4 (0.1-2.8)	0.341
Literature SNPs: rs243865 and rs2285053							
H1: CC	65.2	1.0 (Reference)		1.0 (Reference)		1.0 (Reference)	
H2: C**T**	23.4	1.1 (0.9-1.2)	0.347	1.1 (1.0-1.3)	0.176	0.9 (0.7-1.2)	0.527
H3: **T**C	11.4	1.0 (0.9-1.1)	0.999	1.0 (0.9-1.1)	0.674	1.2 (0.9-1.7)	0.218

NOTE: Bold values considered to be significant at $P \leq 0.05$.
*Bold letters indicate less common alleles.
†Frequency of haplotype among genotype controls.
‡Estimates of effect adjusted for age, education, and study phase.

Two promoter SNPs, *rs243865* (*–1306*) and *rs2285053* (*–735*), have been previously reported to affect *MMP-2* transcription *in vitro*; both *C* to *T* transitions result in reduced expression due to the ablation of specificity protein (Sp)1 transcription factor binding sites (19-21). Only one of these SNPs (*rs243865*) has been previously evaluated in relation to breast cancer risk, and results have been inconsistent. Two studies found no effect (22, 25), whereas two studies (n = 186 and n = 971) found significantly decreased risks of breast cancer associated with the *T* allele (23, 24). In contrast, in the current study, *rs243865 TT* homozygotes tended to have an increased risk of breast cancer, although this effect was not significant. Similarly, results from this study for *rs2285053* were not compelling; although heterozygotes had a marginally significant increased risk of breast cancer, homozygotes tended to have a diminished risk. Notably, these 2 SNPs were not found to be in high LD in this study population (D = 1; r^2 = 0.03), and no haplotype effects on breast cancer risk were found.

Although MMP-2 has traditionally been thought of as a mediator of metastasis, a growing body of evidence has connected MMP-2 to earlier aspects of carcinogenesis, including cell growth, inflammation, and angiogenesis (1-5). In addition to a wide range of ECM substrates, including gelatin, elastin, fibronectin, laminin, and collagens, MMP-2 has many non-ECM substrates that include growth factors modulators and cytokines (1, 2, 4). For example, hydrolysis of membrane bound fibroblast growth factor receptor type 1 by MMP-2 releases the soluble ectodomain of the active receptor, thereby influencing the mitogenic and angiogenic activities of FGF (4, 35). Additionally, MMP-2 was shown to contribute to the inflammatory response by being an alternative activator of pro-interleukin 1-β in the absence of the cytokines' favored activator caspace-1 (36). Finally, degradation of ECM substrates may also contribute to cancer development and progression, as MMP-2 cleavage of the proteoglycan decorin releases transforming growth factor-β1 from its extracellular reservoir (37).

Studies of the *MMP-2* promoter have identified several putative regulatory regions and transcription factor binding sites within 2 kb of the transcription initiation site, including those for Sp1, p53, S1, S2, activator protein, AP-2, Ets-1, CAAT/enhancer binding protein, cAMP-responsive element binding protein, GCN-His, and Pea3 (38, 39). Some of these elements have been shown to be critical for *MMP-2* expression in different cell types or due to different chemical or oncogenic stimuli (38-40). To our knowledge, further upstream *MMP-2* promoter sequences do not seem to have been previously characterized. In the current study, *rs11644561* and *rs11643630* were both found to confer decreased risk for homozygotes; these SNPs are located ~4 and 2.6 kb upstream of the MMP-2 transcription initiation site, respectively. Using available bioinformatics tools, we tried to evaluate these regions further, but our analysis was uninformative. Therefore, with current evidence, we cannot determine whether these loci represent novel functional SNPs that may affect MMP-2 expression, or else, if together, they best tag another, as yet ungenotyped, variation. As their effects were found to be independent, they may each tag this other loci to different degrees, explaining why the haplotype with both SNPs resulted in capturing the risk reduction more than the recessive effects of either haplotype with only one of the alleles alone.

In summary, we identified two *MMP-2* promoter polymorphisms that were associated with modest decreases in breast cancer risk. Homozygotes of the minor alleles for *rs11644561* and *rs11643630* were 40% and 20% less likely to have breast cancer, respectively. Although a two-phase study design was used to reduce type I error, we cannot rule out the possibility that our findings could be due to chance. Furthermore, neither association remains significant after adjusting for the number of SNPs evaluated. However, this is the largest and most comprehensive analysis of *MMP-2* polymorphisms conducted to date, and our results are consistent with both *in vitro* and *in vivo* evidence that show a role for *MMP-2* in breast cancer development. Therefore, additional studies to evaluate these *MMP-2* polymorphisms in population studies are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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