Null Results in Brief

No Association between *Matrix Metalloproteinase (MMP)-1*, *MMP-3*, and *MMP-7* SNPs and Endometrial Cancer Risk

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Introduction

The matrix metalloproteinases (MMP) function as regulators of the dynamic tissue remodeling that occurs in the endometrial lining of the uterus during the normal human menstrual cycle; dysregulation of the MMPs is thought to contribute to the development of both endometriosis and endometrial cancer (1). MMP-1 expression was found to be significantly higher in endometriotic lesions than in surrounding endometrium (2, 3), whereas MMP-3 levels have been reported to be both lower (4) and higher (5) in women with endometriosis compared with women without. MMP-7 expression was found to be significantly higher in endometrial hyperplasia and adenocarcinomas than normal endometrium (6) and, further, was associated with higher grade endometrial tumors (7), and both myometrial (6) and lymph node invasion (8). These three *MMP* genes are located on the negative strand of chromosome 11, and functional polymorphisms that influence their respective transcription levels have been identified for each (9-12). However, previous studies on *MMP* SNPs and endometrial cancer are sparse; two studies were found to have evaluated a single *MMP-1* single nucleotide polymorphism (SNP) and results were inconsistent. Therefore, this comprehensive study of individual genetic variation across *MMP-1*, *MMP-3*, and *MMP-7* was undertaken to evaluate associations with endometrial cancer susceptibility.

Materials and Methods

The Shanghai Endometrial Cancer Study (SECS) is a large, population-based case-control study that has been previously described (13, 14). Briefly, cases were women diagnosed with endometrial cancer between January 1997 and December 2003, ages 30 to 69 y, identified from the Shanghai Cancer Registry. Controls were randomly selected from the Shanghai Resident Registry and frequency matched to cases in 5-year intervals. Of 1,458 identified eligible cases, in-person interviews were completed for 1,204 (82.6%). Reasons for nonparticipation included refusal (n = 137, 9.4%), death before interview (n = 66, 4.5%), inability to be located (n = 37, 2.5%), and health or communication problems (n = 14, 1.0%). Of eligible controls identified (1,629), in-person interviews were completed for 1,212 (74.4%). Reasons for nonparticipation included refusal (n = 340, 20.9%), absence during the study period (n = 61, 3.7%), and health or communication problems (n = 16, 1.1%). Institutional review board approval was granted by relevant institutions in both China and the United States. Informed consent was obtained from each included participant. DNA samples were provided and available for 87.3% of cases (n = 1,052) and 87.3% (n = 1,058) of controls.

Haplotype-tagging SNPs were selected from Han Chinese data from the HapMap Project (15) using the Tagger program (16) to capture SNPs with a minimum minor allele frequency (MAF) of 0.05 in either *MMP-1, MMP-3*, or *MMP-7* (± 5 kb) with an r^2 of 0.90 or greater. Known or potentially functional SNPs were forced into the haplotype-tagging SNP selection process. For *MMP-1*, 17 SNPs were selected, with 14 successfully genotyped. For *MMP-3*, seven SNPs were selected, with six successfully genotyped. For *MMP-7*, 12 SNPs were selected, with 11 successfully genotyped. Genotyping was conducted using the Affymetrix Targeted Genotyping System (Affymetrix; ref. 17) for 1,037 cases (98.6%) and 1,018 controls (96.2%).

Hardy-Weinberg equilibrium was applied to test the observed and expected genotype frequencies for cases and controls (χ^2 test). Associations between SNPs and covariates were evaluated with the χ^2 test or t test when appropriate. Covariates considered included age at diagnosis, education, age at menarche, age at menopause among postmenopausal women, menopausal status, number of pregnancies, oral contraceptive use, body mass index, waist-to-hip ratio (WHR), physical activity in the preceding decade, and first-degree family history of breast, colorectal, or endometrial cancer. Odds ratios and

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corresponding 95% confidence intervals were determined by logistic regression using additive models that included adjustment for age and education. Dominant and recessive models were additionally used when appropriate. Linkage disequilibrium was assessed by Haploview (18). All statistical tests were two-tailed, and *P* values were considered to be statistically significant when ≤0.05.

Results

Consistent with previous SECS analyses (13, 14) and other epidemiologic studies, cases, and controls included in the current study differed with regard to age at menarche, age at menopause, menopausal status, number of pregnancies, use of oral contraceptives, body mass index and WHR, physical activity, and first-degree family history of cancer (data not shown). SNPs included in this study are listed in Table 1; their order corresponds to the open reading frames of the genes on the negative strand of chromosome 11. Of the 31 polymorphisms genotyped, one was found not to be polymorphic in this study population (*MMP-7 rs11568819*) and thus was not included in our analyses. No SNPs were found to deviate from Hardy-Weinberg equilibrium. Associations with endometrial cancer risk were calculated in additive effect models that included adjustment for age and education; further adjustment for body mass index, number of pregnancies, menopausal status, or family history of cancer did not appreciably alter the effect estimates. No significant associations were observed. Two *MMP-7* SNPs, *rs17098318* and *rs11568818*, both tended to confer an increased, but nonsignificant, risk of endometrial cancer for homozygotes, in both additive and recessive models. Furthermore, no SNPs were found to have effects that significantly differed by menopausal status. The linkage disequilibrium structure of these 30 SNPs is shown in Fig. 1, and includes six haplotype blocks. Similar to single SNP analysis, no significant effects were observed in haplotype analysis of these MMP polymorphisms (data not shown).

Discussion

Promoter polymorphisms in the *MMP-1*, *MMP-3*, and *MMP-7* genes have been associated with altered susceptibility to cancer in human populations (17, 19-30), although studies on endometrial cancer risk and MMP SNPs have been lacking. Two small studies evaluating *MMP-1 -1607 1G/2G* (*rs1799750*) and *MMP-3 -1171*

Table 1. MMP-3, MMP-1, and MMP-7 SNPs and endometrial cancer risk, evaluated among 1,037 cases and 1,018 controls, the SECS

Gene, SNP	Region	Alleles*	MAF†	HWE	Endometrial cancer risk, OR (95% CI)‡		
				P§	AB	BB	*P*
MMP-3							
rs645419	Promoter	G/A	32.6%	0.573	1.1 (0.9-1.3)	1.0 (0.8-1.4)	0.628
rs632478	Promoter	C/A	33.0%	0.766	1.1 (0.9-1.3)	1.0 (0.8-1.4)	0.717
rs522616	Promoter	A/G	36.2%	0.700	1.0 (0.8-1.2)	1.2 (0.9-1.6)	0.321
rs679620	Exon 2	G/A	32.9%	0.754	1.1 (0.9-1.3)	1.0 (0.8-1.4)	0.765
rs650108	Intron 8	A/G	40.2%	0.905	1.0 (0.9-1.3)	1.1 (0.8-1.4)	0.624
rs655403	Intron 8	C/T	7.2%	0.552	1.0 (0.8-1.3)	1.5 (0.4-5.3)	0.832
MMP-1							
rs484915	Promoter	A/T	34.2%	0.311	1.0 (0.8-1.2)	0.9 (0.7-1.3)	0.777
rs1155764	Promoter	T/G	22.0%	0.765	0.9 (0.7-1.0)	1.1 (0.7-1.7)	0.397
rs509332	Promoter	A/G	13.4%	0.124	1.1 (0.9-1.4)	0.8 (0.4-1.5)	0.639
rs470206	Promoter	G/A	13.4%	0.114	1.1 (0.9-1.4)	0.8 (0.5-1.5)	0.672
rs2075847	Promoter	T/C	24.0%	0.253	1.1 (0.9-1.3)	1.2 (0.8-1.6)	0.369
rs498186	Promoter	A/C	44.0%	0.966	1.0 (0.8-1.2)	0.9 (0.7-1.2)	0.507
rs475007	Promoter	T/A	34.0%	0.702	0.9 (0.8-1.1)	1.1 (0.9-1.5)	0.626
rs996999	Intron 4	C/T	49.1%	0.291	1.1 (0.9-1.3)	1.0 (0.7-1.2)	0.731
rs470558	Exon 5	G/A	12.4%	0.642	0.9 (0.7-1.1)	1.0 (0.5-2.2)	0.421
rs7125062	Intron 6	C/T	30.6%	0.478	1.0 (0.8-1.2)	0.8 (0.6-1.1)	0.472
rs1938901	Intron 8	T/C	42.4%	0.691	1.1 (0.9-1.3)	1.1 (0.9-1.4)	0.440
rs2071231	Intron 9	T/G	20.4%	0.607	1.1 (0.9-1.3)	1.2 (0.8-1.8)	0.173
rs7945189	3′ FR‖	C/T	8.8%	0.250	0.9 (0.7-1.1)	1.3 (0.4-4.3)	0.411
rs1470504	3′ FR‖	G/A	13.9%	0.663	0.9 (0.7-1.1)	0.9 (0.4-1.7)	0.328
MMP-7							
rs880197	Promoter	A/T	38.7%	0.755	0.9 (0.7-1.1)	0.9 (0.7-1.1)	0.230
rs17098318	Promoter	G/A	7.9%	0.309	1.0 (0.8-1.2)	2.2 (0.7-7.2)	0.782
rs11568818	Promoter	A/G	8.0%	0.281	1.0 (0.7-1.2)	2.0 (0.6-6.5)	0.970
rs11225307	Intron 3	A/G	26.5%	0.461	1.1 (0.9-1.3)	1.1 (0.8-1.6)	0.325
rs17352054	Intron 5	A/C	12.0%	0.682	1.2 (1.0-1.5)	1.1 (0.6-2.3)	0.112
rs495041	3′ FR‖	C/T	49.5%	0.508	0.9 (0.8-1.2)	1.0 (0.8-1.2)	0.741
rs10895304	3′ FR‖	A/G	24.5%	0.853	1.0 (0.8-1.2)	1.0 (0.7-1.5)	0.865
rs7935378	3′ FR‖	T/C	23.0%	0.941	0.9 (0.8-1.1)	1.1 (0.7-1.6)	0.655
rs12184413	3′ FR‖	C/T	29.5%	0.577	0.9 (0.8-1.1)	1.0 (0.7-1.4)	0.636
rs11225297	3′ FR‖	A/T	20.7%	0.629	1.0 (0.8-1.2)	0.9 (0.5-1.4)	0.572

Abbreviations: OR, odds ratio; CI, confidence interval.
*Major and minor alleles as determined by the distribution among SECS controls.
†Minor Allele Frequency (MAF) among SECS controls.
‡Odds ratio and 95% confidence interval for the risk of endometrial cancer, age and education adjusted; AA, major allele homozygous; BB, minor allele homozygous, AB heterozygous; *P* value for trend.
§Hardy-Weinberg equilibrium test, *P* value among SECS controls.
‖3′ Flanking region, downstream of the coding region.

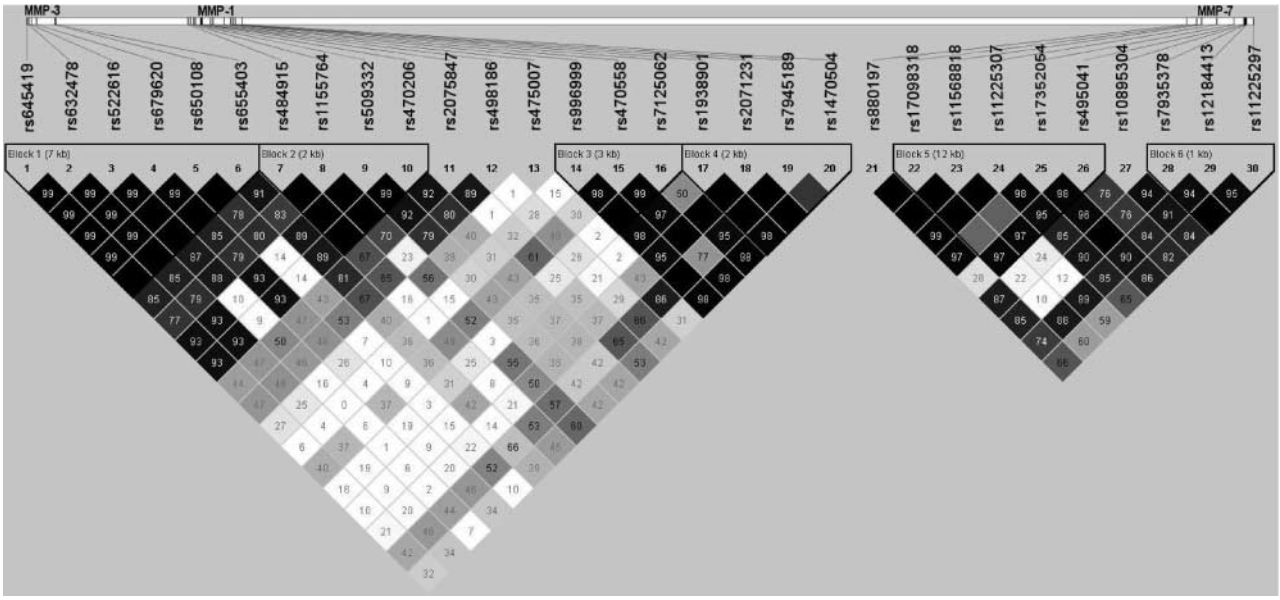

Figure 1. Linkage disequilibrium structure of 30 MMP-3, MMP-1, and MMP-7 SNPs, on chromosome 11 among 1,018 controls from the SECS; value shown in D′.

5A/6A (*rs35068180* and *rs3025059*) and endometriosis found mixed results. No association for either SNP was seen among 56 cases and 71 controls (31), whereas the *MMP-1 2G* allele was found to confer an increased risk of endometriosis among 100 cases and 150 controls (32). Similarly, the *MMP-1 -1607 2G* allele was found to confer an increased risk of endometrial adenocarcinoma among 100 cases and 150 controls (33), whereas no difference was seen between 107 cases and 213 controls (34). Unfortunately, neither of these functional SNPs were genotyped in the current study. However, *MMP-3 rs679620* was genotyped and shares moderate linkage disequilibrium with *MMP-1 rs1799750* (D = 0.79; r^2 = 0.60; ref. 35); no association with endometrial cancer risk was observed. To our knowledge, no previous studies of *MMP-3* or *MMP-7* SNPs and endometrial cancer risk have been conducted. In this study, both of the functional promoter *MMP-7* SNPs were genotyped. Although *MMP-7 -153 C/T* (*rs11568819*) was not found to be polymorphic in this population, *MMP-7 -181 A/G* (*rs11568818*) and another promoter SNP in high linkage disequilibrium (*rs17098318*; D = 1.0; r^2 = 0.99) both seemed to confer an increased risk of endometrial cancer in homozygote carriers of the rare allele. This is similar to our findings for breast cancer risk among premenopausal women (17), and may indicate a real, but low prevalence association that the current study lacked adequate power to detect under recessive models. Given the size of our study population, this analysis had only 31% power to detect a recessive effect of an odds ratio of 2.0 for a gene with a MAF of only 8%. However, for additive associations, we had >92% power to detect an odds ratio of 1.4 for a SNP with a MAF of 10%, >93% power to detect an odds ratio of 1.3 for a SNP with a MAF of 20%, and >77% power to detect an odds ratio of 1.2 for a SNP with a MAF of 30%. In summary, 30 haplotype tagging polymorphisms in *MMP-1*, *MMP-3*, and *MMP-7* were evaluated among 1,037 endometrial cancer cases and 1,018 controls; none were found to be significantly associated with endometrial cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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