Polymorphisms in the *CYP19A1* (Aromatase) Gene and Endometrial Cancer Risk in Chinese Women

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Abstract

Aromatase, encoded by the *CYP19A1* gene, is a key enzyme in estradiol biosynthesis, which catalyzes the conversion of androstenedione and testosterone to estrone and estradiol, respectively. Given the critical role of estrogen in the development of endometrial cancer risk, we evaluated genetic polymorphisms of the *CYP19A1* gene, including rs1065779, rs700519, rs28566535, rs752760, and rs1870050, in association with endometrial cancer in a population-based case-control study conducted in Shanghai, China. Genotypes of 1,040 incident endometrial cancer cases and 1,031 frequency-matched controls were included in the study. We applied a logistic regression model to derive adjusted odds ratios (OR) and their 95% confidence intervals (95% CI). Six common haplotypes with a frequency ≥5% were estimated; the highest frequency haplotype was GCACA (27.8% in cases and 26.2% in controls). We observed an inverse association between *CYP19A1* haplotype TCATC and endometrial cancer in our population (OR, 0.76; 95% CI, 0.62-0.92). An inverse association was found between endometrial cancer and single nucleotide polymorphism rs1870050 in the promoter region with ORs of 0.81 (95% CI, 0.68-0.97) and 0.58 (95% CI, 0.42-0.80) for the AC and CC genotypes, respectively. We observed a multiplicative interaction between single nucleotide polymorphism rs700519 and body mass index among postmenopausal women (P = 0.01), with stronger associations between rs700519 genotypes and endometrial cancer risk among heavier (body mass index, ≥25) postmenopausal women. In summary, our data show that polymorphisms in the *CYP19A1* gene may contribute to endometrial carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2007;16(5):943–9)

Introduction

Estrogens stimulate the proliferation of endometrial cells (1). Both prospective and retrospective epidemiologic studies have shown an increased risk of endometrial cancer with higher levels of estrogen, most frequently with estrone and total estradiol (2-6).

Before menopause, the ovary is the principal source of estrogen, whereas during and after menopause, estrogen is mainly produced in extragonadal sites (primarily in adipose tissue; ref. 7). Encoded by the *CYP19A1* gene, aromatase is a key enzyme in the biosynthesis of estradiol, catalyzing the conversion of androstenedione and testosterone to estrone and estradiol, respectively (8). The conversion of androstenedione to estrone increases as a function of aging and obesity (9). Previous studies have detected aromatase protein and mRNA in endometrial cancer tissue, whereas aromatase expression was undetectable or low in normal endometrial tissue and endometrial hyperplasia (10, 11). *In vitro* studies also have shown an increase in the aromatase activity of endometrial neoplastic cells, but not in normal endometrial cells (12), and a positive correlation has been found between enzyme activity and *CYP19A1* mRNA in endometrial cancer tissue (13). Treatment of *in vitro* endometrial cancer tissues with aromatase inhibitors has shown that depletion of *in situ* tumor estrogen results in decreased cell proliferation of endometrial carcinoma cells (14). Given the important role of aromatase in the pathogenesis of endometrial cancer, we hypothesized that functional polymorphisms in the *CYP19A1* gene may be involved in endometrial carcinogenesis.

The *CYP19A1* gene, located at 15q21.1, comprises 10 exons, and transcription begins in exon II. The multiple nontranslated exon I controls gene expression in a tissue-specific manner and under complex hormonal regulation (15). Polymorphisms in *CYP19A1* have been evaluated for their association with breast cancer risk (16-23) and, to a lesser extent, with endometrial (24, 25) and prostate cancer (26, 27) and endometriosis (28). The *CYP19A1* gene is highly polymorphic (16). Two of the polymorphisms, the tetranucleotide repeat polymorphism (*[TTTA]n*) in intron 4 and $Arg^{264}Cys$ (rs700519) in exon 7, have been frequently studied for breast cancer associations with inconsistent results (18-22). Identified in a Japanese population, the variant allele of the $Trp^{39}Arg$ (rs2236722) polymorphism was found to abolish or reduce aromatase activity (29). However, this polymorphism was inconsistently associated with breast cancer risk in two small-scale Japanese populations (17, 19). Located in the first exon, single nucleotide polymorphism (SNP) rs28566535 is close to the promoter I.4. Paynter et al. (24) found no association between rs28566535 polymorphism and risk of endometrial cancer.

In the present study, we evaluate *CYP19A1* genetic polymorphisms, including rs1065779, rs700519, rs28566535, rs752760, rs1870050, in relation to endometrial cancer risk in a case-control study conducted in Shanghai, China.

Materials and Methods

The Shanghai Endometrial Cancer Study is a population-based case-control study. Eligible cases were identified from the Shanghai Cancer Registry, which is the largest and oldest

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population-based registry in China and captures >85% of incident cancer patients in urban Shanghai (30). Eligible cases included all permanent residents of urban Shanghai between the ages of 30 and 69 years who were diagnosed with endometrial cancer between January 1997 and December 2003. A total of 1,458 eligible endometrial cancer cases were identified and in-person interviews were completed for 1,204 (82.6%). The major reasons for nonparticipation were refusals (137 cases, 9.4%), death before interview (66 cases, 4.5%), inability to locate the subject (37 cases, 2.5%) or absence during the study period (12 cases, 0.8%), and health or communication problems (14 cases, 1.0%).

Controls were randomly selected from the Shanghai Resident Registry, which registers all permanent residents of urban Shanghai, and were frequency matched to cases by age (5-year intervals). The number of controls in each age-specific stratum was determined in advance according to the age distribution of incident endometrial cancer cases in 1996. Women who had had a hysterectomy (identified during survey) were not eligible (n = 36). Interviews were completed for 1,212 (74.4%) eligible women (n = 1,629). Reasons for nonparticipation of controls included refusal (340 controls, 20.9%), absence during the study period (61 controls, 3.7%), and other health or communication problems (16 controls, 1.1%).

A standardized structured questionnaire was administered to participants and covered demographic factors, menstrual and reproductive history, hormone use, usual dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight history, and family history of cancer. All study participants were interviewed in person by trained retired nurses and physicians and their current weight, circumferences of the waist and hips, and sitting and standing heights were measured using a standard protocol. All measurements were taken twice. The averages of the measurements were used to calculate body mass index (BMI; kg/m^2) and waist-to-hip circumference ratio. Menopause was defined as a cessation of the menstrual cycle for 12 months or longer, excluding periods of menstrual cessation due to pregnancy or breast-feeding. The study protocols were approved by the Institutional Review Boards of all institutes involved in the study, and written informed consent was obtained from all participants.

The polymorphisms included in the study were chosen based on literature review and their potential functionality. Of the four polymorphisms [*Arg*^{264}*Cys* (rs700519), *Trp*^{39}*Arg* (rs2236722), rs28566535, and *TTTA* repeat] in the *CYP19A1* gene that have been investigated previously in relation to cancer at the time we initiated our study, three were included. The Trp^{39}Arg polymorphism has a low minor allele frequency in the Chinese population and thus was excluded from the study. Genotyping of the *TTTA* repeat polymorphism is still ongoing and results will be reported in the future. In addition, we searched SNP databases and included in the study two SNPs in the promoter region/untranslated exon (rs752760 and rs1870050) and one SNP in the intron-exon boundary (rs1065779) regions that have a minor allele frequency >10% in the Chinese population. Thus, five polymorphisms were included in the study: three SNPs (rs752760, rs1870050, and rs28566535) in the promoter/untranslated exon regions, one SNP (rs1065779) in the intron-exon boundary, and one SNP (rs700519) in exon 7 codon 264.

Genomic DNA was extracted from buffy coat fractions or buccal cells by using a QIAmp DNA Mini kit (Qiagen) following the manufacturer's protocol. Except for the rs700519 polymorphism, allelic discrimination of the *CYP19A1* polymorphisms was assessed with the ABI Prism 7900 Sequence Detection System (Applied Biosystems, Inc.) using the Taqman genotyping assay with primers and probes obtained from Applied Biosystems. The assay IDs were as follows: C_8234755_10 for rs1065779, C_1664178_10 for rs28566535, C_798312_10 for rs752760, and C_11672268_20 for rs1870050. The Taqman assay method has been described previously (31). Briefly, the final volume for each reaction was 5 μL, consisting of 2.5 μL Taqman Universal PCR Master Mix, 0.6 μL of each primer, 0.2 μL of each Taqman probe, and 5 ng genomic DNA. The PCR profile consisted of an initial denaturation step at 95°C for 10 min and 40 cycles of 92°C for 15 s and 60°C for 1 minute. The fluorescence level was measured with the ABI Prism 7900HT sequence detector (Applied Biosystems). Allele frequencies were determined by ABI Sequence Detection System software.

We were not able to design a Taqman assay for the *CYP19A1* rs700519 (*Arg*^{264}*Cys*) polymorphism. This SNP was genotyped using the MGB Eclipse (3′ hybridization triggered fluorescence reaction) assay (Epoch Biosciences) following the method described in SNP500Cancer.[5] The primers were 5′-GGATTTGAAAGATGCCATAGAAG-3′ and 5′-CAACT-CAGTGGCAAAGTCCATA-3′. The MGB Eclipse probes were AAAGACGCAGGAT-FAM and AAAAGATGCAGGA-TET. Five nanograms of lyophilized sample DNA were used to do a 5 μL MGB Eclipse assay. Reactions were set up using 2.35 μL of the 2× Jumpstart Master Mix (Sigma-Aldrich), 0.15 μL of 2.5 units/μL JumpStart Taq Polymerase (Sigma-Aldrich), 0.25 μL primers, and 0.25 μL probes. All reactions were set up in a 384-well plate. The PCR profile consisted of an initial denaturation step at 95°C for 2 min and 45 cycles of 95°C for 5 s, 58°C for 20 s, and 76°C for 20 s. The end point dissociation (melting) curves were generated on the ABI 7900HT Sequence Detection System by monitoring fluorescence while heating the reactions from 30°C to 80°C at a 10% ramp rate. Dissociation curves of the first derivative of fluorescence and raw fluorescent values were then exported from the ABI Sequence Detection System software in text format for further analysis using the MGB Eclipse melt curve macro (Epoch Biosciences) for genotype scoring.

The laboratory staff was blind to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 384-well plate contained four water, eight CEPH 1347-02 DNA, eight blinded quality control DNA, and eight unblinded quality control DNA samples. The concordance rates for the quality control samples were 98.7% for rs700519, 97.4% for rs75276 and rs1870050, and 100% for rs1065779 and rs28566535. In addition, we genotyped rs752760 and rs1870050 in 45 DNA samples of the Chinese participants used in HapMap and 24 DNA samples used in Perlegen as an additional quality control. The genotypes of the samples generated from our study were compared with data downloaded from HapMap[6] and/or Perlegen[7]. The concordance rate between the data generated in our laboratory and the data from the above databases was 100%.

Among those who provided a buccal cell sample, 189 cases and 198 controls provided samples using a mouthwash method; 93 cases and 88 controls provided samples using a buccal swab method. Due to a very low DNA yield of the buccal swab method, we did not include buccal swab DNA samples in the genotyping. In addition, DNA from blood samples donated by 19 control subjects were not available because of their use in previous studies. Thus, DNA samples from 1,046 (86.9%, 857 blood and 189 buccal cell) cases and 1,035 (85.4%, 837 blood and 198 buccal cell) controls were included in the genotyping study. Genotyping data were obtained from 1,040 cases and 1,031 controls; a success rate of 98.1% and 99.6%, respectively.

The χ^2 test was used to compare the distributions of *CYP19A1* alleles and genotypes between cases and controls.

[5] http://snp500cancer.nci.nih.gov/
[6] http://www.hapmap.org
[7] http://genome.perlegen.com

The exact χ^2 goodness-of-fit test was used to test for Hardy-Weinberg equilibrium of the genotypes. Haplotypes were estimated with PHASE software using a Bayesian method (32). Unconditional logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) for associations with endometrial cancer risk. Interactions between genotypes and BMI (<25 and ≥25) and menopausal status (premenopausal/postmenopausal) were evaluated by constructing a multiplicative term in the logistic regression model. All analyses were adjusted for age. Potential confounding effects from other demographic factors and known endometrial cancer risk factors, such as educational level, BMI, age at menarche, age at menopause, parity, and oral contraceptive use, were also examined and no appreciable confounding was observed.

Results

The distribution of selected demographic characteristics and major risk factors for endometrial cancer among cases and controls are presented in Table 1. Compared with controls, cases had an earlier age at menarche, later age at menopause, and longer duration of menstruation. Cases were also more likely than controls to have a higher educational level, a higher BMI or waist-to-hip circumference ratio, and a family history of colorectal, endometrial, or breast cancer among first-degree relatives, to be nulliparous, and to have never used oral contraceptives.

The allele distribution of *CYP19A1* genetic polymorphisms among cases and controls is summarized in Table 2. All SNPs were consistent with Hardy-Weinberg equilibrium among both cases and controls ($P > 0.05$). The frequencies of the common alleles for SNPs rs1065779, rs700519, rs28566535, rs752760, and rs1870050 among controls were 54.8%, 86.7%, 66.6%, 61.8%, and 68.6%, respectively. The allele frequencies of SNPs rs700519, rs28566535, and rs752760 were similar among cases and controls, and slightly more cases carried the rs1065779 *G* allele than controls (57.7% and 54.8% for case and controls, respectively). For the SNP rs1870050, the allele frequencies for cases (*A*, 74.6%; *C*, 25.4%) were significantly different from controls (*A*, 68.6%; *C*, 31.4%; $P < 0.01$). Based on observed genotype data, the estimated common haplotype (≥5%) frequencies for the SNPs are shown in Table 2. The estimated frequency of the *CYP19A1* haplotypes was very different between cases and controls ($P = 0.02$).

The associations between *CYP19A1* genotypes and endometrial cancer *risk* are presented in Table 3. The *TT* genotype of SNP rs1065779 was associated with a reduced risk of endometrial cancer compared with the GG genotype (OR, 0.78; 95% CI, 0.62-0.99). Compared with the TT genotype of SNP rs752760, the TC genotype was related to elevated risk (OR, 1.27; 95% CI, 1.07-1.51), whereas the CC genotype was unrelated to risk (OR, 1.11; 95% CI, 0.86-1.42). For SNP rs1870050, the AC genotype (OR, 0.81; 95% CI, 0.68-0.97) and CC genotype (OR, 0.58; 95% CI, 0.42-0.80) were both related to reduced risk compared with the AA genotype ($P_{trend} < 0.01$), and the inverse associations were observed among both premenopausal and postmenopausal women. We observed no associations with endometrial cancer for the SNPs rs700519 and rs28566535 in our population.

Because the main organ site of estrogen synthesis differs by menopausal status (i.e., ovary during the premenopausal period and fat tissue after menopause), we further analyzed

Table 1. Comparison of cases and controls on demographics and selected endometrial cancer risk factors

	Cases (n = 1,204)	Controls (n = 1,212)	P
Age (mean ± SD)	54.9 ± 8.5	55.1 ± 8.5	0.75*
Education (%)			
No formal education	7.9	11.0	
Elementary education	14.1	12.9	
Middle and high school	62.9	63.3	
Professional, college and above	15.1	12.8	0.03†
Marital status (%)			
Unmarried	1.5	1.1	
Married or cohabiting	87.6	87.6	
Separated/divorced/widowed	10.9	11.3	0.63†
Family income in previous year, yuan (%)			
<10,000	11.6	11.2	
10,000-14,999	21.7	21.3	
15,000-19,999	19.9	18.5	
20,000-29,999	21.9	25.6	
≥30,000	24.9	23.4	0.33†
No. pregnancies (%)			
Never	7.4	3.6	
1	16.9	13.8	
2	25.2	26.0	
3	22.3	24.9	
4	16.9	17.6	
≥5	11.3	14.1	<0.01†
Cancer among first-degree relatives (%)‡	8.5	4.4	<0.01†
Natural menopause (%)	97.0	99.1	<0.01†
Oral conceptive use (%)	18.5	24.9	<0.01†
Hormone replacement therapy (%)	4.4	4.0	0.66†
Regular exercise (%)	28.7	33.5	0.01†
Age at menarche§	14.0 (13.0, 16.0)	15.0 (13.0, 16.0)	<0.01*
Age at menopause (among postmenopausal women)§	50.3 (48.6, 52.5)	49.6 (47.3, 51.1)	<0.01*
Years of menstruation§	33.3 (30.1, 36.1)	31.5 (28.2, 34.4)	<0.01*
BMI§	25.2 (22.9, 28.1)	23.5 (21.4, 26.0)	<0.01*
Waist-to-hip circumference ratio§	0.84 (0.81, 0.87)	0.81 (0.78, 0.85)	<0.01*

NOTE: Subjects with missing values were excluded from the analysis.
*Nonvariable test on continuous variables.
†χ^2 test was used to compare the distribution of categorical variables.
‡Family history of colorectal, endometrial, or breast cancer among first-degree relatives.
§Median (25th, 75th percentile) is presented.

Table 2. Allele frequencies (%) of the *CYP19A1* gene and haplotype distribution of *CYP19A1* polymorphisms, the Shanghai Endometrial Cancer Study

Alleles	Cases (%)	*P* (HWE)	Controls (%)	*P* (HWE)	*P*
SNP					
rs1065779					
G	1,192 (57.7)	0.08	1,129 (54.8)	0.34	0.06
T	874 (42.3)		931 (45.2)		
rs700519					
C	1,783 (87.0)	0.51	1,780 (86.7)	0.60	0.83
T	267 (13.0)		272 (13.3)		
rs28566535					
A	1,350 (64.9)	0.77	1,374 (66.6)	0.42	0.24
C	730 (35.1)		688 (33.4)		
rs752760					
T	1,238 (60.3)	0.29	1,230 (61.8)	0.14	0.32
C	816 (39.7)		760 (38.2)		
rs1870050					
A	1,515 (74.6)	0.91	1,382 (68.6)	0.57	<0.01
C	515 (25.4)		632 (31.4)		
Estimated frequency of *CYP19A1* haplotypes (in the order of SNP 1-2-3-4-5* based on their chromosome positions)					
GCACA	27.8		26.2		
TCATC	12.3		15.6		
GCCTA	16.0		14.0		
TTCTA	8.8		7.6		
GCATC	8.8		9.6		
TCACA	8.5		8.3		
Others	18.5		17.7		
Overall χ^2 test			$P = 0.02$		

NOTE: The number of participants does not add up to the total number of women because of missing data on genotypes for some participants. Common allele of the SNP is in boldface type.
*SNP1, rs1065779; SNP2, rs700519; SNP3, rs28566535; SNP4, rs752760; and SNP5, rs1870050.

gene-disease associations stratifying by menopausal status and BMI. We observed some variation in the risk estimates by menopausal status, but none of the tests for multiplicative interaction reached statistical significance. Similarly, no multiplicative interactions between genotypes and BMI were noted (data not shown). In addition, we analyzed gene-disease associations among postmenopausal women and stratified by BMI (Table 4). The rs1065779 TT genotype was associated with a significantly reduced risk of endometrial cancer risk only in postmenopausal women (Table 3) and among women with a

Table 3. Associations between *CYP19A1* genotypes and endometrial cancer risk

	All subjects			Premenopausal women			Postmenopausal women		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
rs1065779	(n = 1,033)	(n = 1,030)							
G/G	330	317	1.0	139	128	1.0	191	189	1.0
G/T	532	495	1.07 (0.90-1.27)	227	190	1.08 (0.82-1.43)	305	305	1.04 (0.84-1.31)
T/T	171	218	0.78 (0.62-0.99)	79	77	0.94 (0.65-1.37)	92	141	0.68 (0.50-0.93)
P_{trend}			0.06			0.91			0.02
$P_{interaction}$ = 0.15									
rs700519	(n = 1,025)	(n = 1,026)							
C/C	773	774	1.0	338	300	1.0	435	474	1.0
C/T	237	232	1.03 (0.84-1.26)	102	87	1.05 (0.76-1.44)	135	145	1.01 (0.78-1.31)
T/T	15	20	0.76 (0.39-1.49)	6	6	0.92 (0.29-2.90)	9	14	0.70 (0.30-1.62)
P_{trend}			0.84			0.91			0.74
$P_{interaction}$ = 0.73									
rs28566535	(n = 1,040)	(n = 1,031)							
A/A	436	452	1.0	187	172	1.0	249	280	1.0
A/C	478	470	1.08 (0.90-1.27)	208	191	1.03 (0.78-1.34)	270	279	1.10 (0.88-1.36)
C/C	126	109	1.22 (0.92-1.61)	56	32	1.62 (1.01-2.60)	70	77	1.03 (0.72-1.46)
P_{trend}			0.24			0.16			0.69
$P_{interaction}$ = 0.40									
rs752760	(n = 1,027)	(n = 995)							
T/T	365	391	1.0	153	148	1.0	212	243	1.0
T/C	508	448	1.27 (1.07-1.51)	217	171	1.29 (0.98-1.71)	291	277	1.25 (1.00-1.55)
C/C	154	156	1.11 (0.86-1.42)	76	63	1.18 (0.80-1.74)	78	93	1.00 (0.71-1.39)
P_{trend}			0.32			0.38			0.72
$P_{interaction}$ = 0.62									
rs1870050	(n = 1,015)	(n = 1,007)							
A/A	566	478	1.0	249	182	1.0	317	296	1.0
A/C	383	426	0.81 (0.68-0.97)	160	154	0.85 (0.64-1.12)	223	272	0.81 (0.65-1.01)
C/C	66	103	0.58 (0.42-0.80)	29	47	0.49 (0.30-0.81)	37	56	0.65 (0.42-1.01)
P_{trend}			<0.01			<0.01			0.01
$P_{interaction}$ = 0.54									

NOTE: The number of participants does not add up to the total number of women because of missing data on genotypes for some participants.
*Unconditional logistic model adjusted for age.

Table 4. Associations between *CYP19A1* genotypes and endometrial cancer risk among postmenopausal women

	BMI < 25			BMI ≥ 25		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
rs1065779						
G/G	91	113	1.0	100	79	1.0
G/T	129	181	0.92 (0.67-1.25)	176	124	1.17 (0.84-1.62)
T/T	40	91	0.57 (0.37-0.87)	52	50	0.86 (0.54-1.35)
P_{trend}			0.02			0.47
$P_{interaction}$ = 0.27						
rs700519						
C/C	210	286	1.0	225	188	1.0
C/T	47	89	0.72 (0.49-1.06)	88	56	1.32 (0.91-1.92)
T/T	2	10	0.28 (0.06-1.27)	7	4	1.46 (0.42-5.06)
P_{trend}			0.03			0.15
$P_{interaction}$ = 0.01						
rs28566535						
A/A	121	174	1.0	128	106	1.0
A/C	116	165	0.99 (0.73-1.35)	154	114	1.16 (0.84-1.60)
C/C	25	48	0.74 (0.44-1.24)	45	29	1.34 (0.80-2.23)
P_{trend}			0.45			0.34
$P_{interaction}$ = 0.22						
rs752760						
T/T	86	138	1.0	126	105	1.0
T/C	137	183	1.1 7 (0.87-1.60)	154	94	1.47 (1.05-2.04)
C/C	36	53	1.07 (0.67-1.59)	42	40	0.94 (0.58-1.52)
P_{trend}			0.53			0.85
$P_{interaction}$ = 0.76						
rs1870050						
A/A	141	177	1.0	176	119	1.0
A/C	97	169	0.73 (0.54-1.00)	126	103	0.92 (0.66-1.27)
C/C	17	35	0.62 (0.34-1.14)	20	21	0.71 (0.38-1.36)
P_{trend}			0.03			0.13
$P_{interaction}$ = 0.69						

NOTE: The number of participants does not add up to the total number of women because of missing data on genotypes for some participants.
*Unconditional logistic model adjusted for age.

lower BMI (Table 4). However, tests for multiplicative interaction between this SNP and menopausal status or BMI were not significant. For SNP rs700519, the association between the TT genotype and endometrial cancer risk was in opposite directions for lean (BMI, <25) versus overweight (BMI, >25) postmenopausal women, and a test for multiplicative interaction was significant ($P_{interaction}$ = 0.01). We did not observe multiplicative interactions between SNPs rs28566535, rs752760, and rs1870050 and BMI among postmenopausal women.

To evaluate the relationship between common haplotypes (≥5%) and endometrial cancer risk, a likelihood ratio test was done using the most common haplotype, GCACC, as the reference group, which contained the less common allele C at SNP rs752760 and common alleles for other SNPs, and pooling all haplotypes with frequencies <5% into a single category (Table 5). We found that haplotype TCATC containing common alleles at SNPs rs28566535 and rs752760 was inversely associated with endometrial cancer (OR, 0.76; 95% CI, 0.62-0.92). The associations showed little change after stratification by menopausal status. The haplotype GCATC was related to reduced risk (OR = 0.64, 95% CI = 0.44-0.93) among premenopausal women but unrelated to risk among postmenopausal women. We also evaluated the effect of the *CYP19A1* haplotype by BMI status for all women and further stratified by menopausal status. We found no indication of an interaction between haplotypes and BMI status (data not shown).

Discussion

Given the importance of estrogens in the etiology of endometrial cancer, it is conceivable that polymorphisms in the *CYP19A1* (aromatase) gene, a gene critical to control of the enzyme activity that converts androgen to estrogen (8), might serve as a potential determinant of endometrial cancer risk. In this large scale, population-based case-control study, we found that several SNPs and a haplotype of the *CYP19A1* gene were associated with the risk of endometrial cancer.

Table 5. Associations between haplotyes of *CYP19A1* and endometrial cancer risk

Haplotypes	All subjects			Premenopausal women			Postmenopausal women		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
GCACA †	588	550	1.0	256	211	1.0	332	339	1.0
TCATC	296	367	0.76 (0.62-0.92)	126	134	0.79 (0.58-1.07)	170	233	0.75 (0.58-0.96)
GCCTA	373	331	1.05 (0.87-1.27)	161	124	1.07 (0.79-1.44)	212	207	1.05 (0.82-1.34)
TTCTA	193	166	1.09 (0.89-1.38)	84	62	1.11 (0.76-1.62)	109	104	1.07 (0.79-1.46)
GCATC	150	168	0.83 (0.65-1.07)	61	80	0.64 (0.44-0.93)	89	88	1.04 (0.74-1.44)
TCACA	183	173	0.99 (0.78-1.26)	89	67	1.06 (0.74-1.54)	94	106	0.91 (0.66-1.24)
Others	301	315	0.90 (0.74-1.09)	125	114	0.90 (0.66-1.23)	176	201	0.89 (0.69-1.15)
P_{trend}			0.75			0.64			0.88

NOTE: The number of participants does not add up to the total number of women because of missing data on genotypes for some participants.
*Unconditional logistic model adjusted for age.
†In the order of SNP 1-2-3-4-5 based on their chromosome position (SNP1, rs1065779; SNP2, rs700519; SNP3, rs28566535; SNP4, rs752760; and SNP5, rs1870050).

The *CYP19A1* gene comprises nine coding exons (II–X) with several untranslated first exons regulated by tissue-specific promoters (15). Elevated levels of aromatase expression have been observed in breast tumor tissue relative to normal breast tissue. The increased aromatase expression is accompanied by a switch in *CYP19A1* promoter utilization, from the primary adipose tissue promoter I1.4 to promoter II, which drives aromatase expression mainly in the ovary, and up-regulation of the endothelial-type promoter I.7 and promoter I1.3, which is minimally used in adipose tissue (33). Similarly, compared with normal endometrium, elevated aromatase protein and mRNA also have been detected in endometrial cancer tissue (10, 11). This hints at a possible alternative in promoter utilization in endometrial cancer. However, genetic regulation of aromatase has not been extensively studied in endometrial carcinoma, in contrast to its role in breast cancer (34). SNPs rs1870050 and rs752760 are both located in the first exon, close to promoter I.1, the major promoter for the placenta. Our study found inverse associations between the AC and CC genotypes of SNP rs1870050 and endometrial cancer risk and a marginal positive association between the heterozygote of the SNP rs752760 and endometrial cancer. Within intron 9, SNP rs1065779 is 53 bp upstream of exon 10. Compared with the GG genotype, we observed an inverse association of endometrial cancer with the TT genotype of this polymorphism. To our knowledge, our study is the first to investigate associations between these SNPs and endometrial cancer risk. The biological mechanisms underlying these associations are unknown. We cannot exclude a chance finding as a possible explanation. More studies are needed to investigate the potential role of these polymorphisms in endometrial cancer etiology and their biological functions.

The nonsynonymous SNP rs700519 occurs within the coding region and results in amino acid changes. Earlier functional studies have produced conflicting results, one finding no effect of the polymorphism and another study finding reduced aromatase enzyme activity (35, 36). We did not observe an association between the $Arg^{264}Cys$ (rs700519) genotype and endometrial cancer. Previous studies on the relationship between the $Arg^{264}Cys$ genotype and breast cancer risk have had inconsistent results (16, 19, 22, 23). The *Cys* allele has also been studied in association with prostate cancer, and inconsistent results were reported in Japanese populations (27, 37). The frequency distribution of this SNP differs substantially among ethnic groups. In our study, the frequency of the rare (*Cys*) allele (13.3%) was lower than that found in Korean (39%; ref. 23) and Japanese (26.7-54%) women (16, 19, 36). The frequency of the *Cys* allele was very low (<10%) among Caucasians (16, 22).

During and after menopause, endogenous estrogen is produced primarily in adipose tissue (7). Thus, it is possible that *CYP19A1* polymorphisms may have a stronger effect among postmenopausal women, particularly those with higher BMI. Our data suggest that associations between rs700519 genotypes and endometrial cancer risk among postmenopausal women may be modified by BMI. Further studies are needed to confirm our finding and to elucidate the underlying biological mechanisms.

Consistent with one prior investigation (24), we observed no association between SNP rs28566535 and endometrial cancer. In a study of postmenopausal Caucasian women not using postmenopausal hormones, Paynter et al. (24) reported that the rs28566535 *C* allele (5.1% of that study population) was not associated with risk of endometrial cancer nor with plasma steroid hormone levels. The minor *C* allele of rs28566535 has a much higher frequency (34.3%) in our study, which agrees with the frequency of this allele reported in a population-based case-control study of breast cancer we conducted in Shanghai (38).

We derived six common haplotypes at ≥5% estimated frequency with genotype information at five SNPs in *CYP19A1*. The highest frequency haplotype (27.8% in cases and 26.2% in controls) comprised the less common allele at rs752760 and the common alleles at other loci. Our results suggested an inverse association between endometrial cancer risk and the *CYP19A1* haplotype TCATC (OR, 0.76; 95% CI, 0.62-0.92). The *CYP19A1* haplotype TCATC contains rare alleles of rs1065779 and rs1870050 and common alleles for other loci. Haplotype GCATC was inversely associated with the risk of endometrial cancer only among premenopausal women. We did not observe any differences in the haplotype-disease associations when stratifying by BMI.

Strengths of this study include the population-based study design and a large sample size, which minimized the selection bias and led to relatively stable risk estimation. The detailed exposure information enabled an evaluation of gene-environment interactions. Nevertheless, the statistical power in subgroups of our study remained limited due to low frequencies of the variant alleles, which limited our ability to identify weak associations. The study applied the candidate gene approach and primarily focused on potential common genetic variance (>5%) and polymorphisms with amino acid changes. We cannot rule out the possibility that SNPs of *CYP19A1* other than those included in our study may be related to risk of endometrial cancer. Given that multiple genes are involved in estrogen biosynthesis and metabolism (39), the confounding and/or modifying effects of other genes also cannot be excluded.

In summary, we found some evidence that common polymorphism of *CYP19A1* genes plays an important role in the development of endometrial cancer among Chinese women.

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