RESEARCH COMMUNICATION

Interaction Between *CYP19A1* Polymorphisms and Body Mass Index in the Risk of Endometrial Cancer in Postmenopausal Japanese Women

Satoyo Hosono[1], Keitaro Matsuo[1,2]*, Hidemi Ito[1], Miki Watanabe[1], Kaoru Hirose[3], Toru Nakanishi[4], Kazuo Tajima[5], Hideo Tanaka[1,2]

Abstract

Extra-ovarian sex hormone production plays an important role in endometrial cancer in postmenopausal women. Aromatase, which is encoded by *CYP19A1*, is a key enzyme in estrogen biosynthesis after menopause. To examine the association between polymorphisms in *CYP19A1* and endometrial cancer risk among postmenopausal Japanese women, we conducted a hospital-based case control study in 48 patients with histologically diagnosed incident endometrial cancer and 253 non-cancer control subjects. Information on lifestyle factors was obtained from a self-administered questionnaire. Twenty-five tag SNPs (single nucleotide polymorphisms) of *CYP19A1* were examined by TaqMan methods and haplotype blocks were identified by LD analysis. Associations were assessed by an unconditional logistic regression model adjusted for potential confounders. We found no significant association between *CYP19A1* genotypes and haplotypes and endometrial cancer risk. However, among women with a BMI (body mass index) >23, significantly positive associations were observed for rs2899473, rs1865803, rs16964220, rs2008691, rs17647707, rs17647719, rs1902586, rs936306, and rs1004982, while negative associations were seen for rs1902585, rs752760 and rs2445768. These showed significant interactions with BMI. Further, of the six haplotype blocks identified, the haplotype CTT of block 1, GATA of block 5 and CA of block 6 showed statistically significant interactions with BMI. These results suggest that *CYP19A1* polymorphisms might play an important role in the etiology of endometrial cancer, and that the effect of these polymorphisms might be influenced by BMI.

Keywords: Case-control study - *CYP19A1* polymorphisms - endometrial cancer - Japan

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Introduction

Endometrial cancer is an increasingly common gynecological cancer in Japanese women, with the age-standardized incidence rate increasing from 1.4 in 1975 to 7.3 in 2005 (Matsuda et al., 2011). Women aged 40 years or older account for 94.1% of cases (Matsuda et al., 2011), showing that endometrial cancer is a tumor of the postmenopausal period. Estrogen in postmenopausal women is synthesized from adrenal androgens in adipose tissue by the cytochrome P450 enzyme aromatase. This enzyme, which is encoded by the *CYP19A1* gene, converts C19 androgens to C18 estrogens. Several studies have investigated the association between *CYP19A1* single nucleotide polymorphisms (SNPs) and haplotypes and endometrial cancer risk (Berstein et al., 2001; Paynter et al., 2005; Olson et al., 2007; Tao et al., 2007; Setiawan et al., 2009). In our previous study, we showed that several tag SNPs and haplotypes in *CYP19A1* were associated with serum estrone and testosterone levels among postmenopausal Japanese women (Kidokoro et al., 2009), suggesting the possibility that these are potential susceptibility markers of endometrial cancer.

Here, we conducted a case-control study to examine the association between polymorphisms and haplotypes in *CYP19A1* and endometrial cancer risk among postmenopausal Japanese women. We also explored whether these associations differed by BMI (body mass index).

Materials and Methods

Subjects

The subjects were 48 postmenopausal patients who were newly and histologically diagnosed with endometrial carcinoma between January 2001 and November 2005 at Aichi Cancer Center Hospital (ACCH) in Japan. All cases were endometrioid adenocarcinoma. Controls (n=253) were randomly selected from 11,814 postmenopausal women diagnosed as cancer-free at ACCH between

*[1]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, [2]Department of Epidemiology, Nagoya University Graduate School of Medicine, [3]Department of Planning and Information, Aichi Prefectural Institute of Public Health, [4]Department of Gynecologic Oncology, Aichi Cancer Center Hospital, [5]Director, Aichi Cancer Center Research Institute, Nagoya, Japan *For correspondence: kmatsuo@aichi-cc.jp*

January 2001 and June 2006. Subjects currently receiving any kind of hormonal therapy were excluded. All subjects were recruited within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), as described elsewhere (Tajima et al., 2000; Hamajima et al., 2001; Hosono et al., 2008). In brief, information on lifestyle factors was collected using a self-administered questionnaire from all first-visit outpatients aged 20 to 79 at ACCH who were enrolled in HERPACC between January 2001 and June 2006. Patients were also asked about their lifestyle when healthy or before the current symptoms developed and were requested to provide a blood sample. Responses were checked by trained interviewers. Our previous study showed that the lifestyle patterns of first-visit outpatients accorded with those in a randomly selected sample of the general population of Nagoya City (Inoue et al., 1997). All participants gave written informed consent and the study was approved by Institutional Ethical Committee of Aichi Cancer Center.

Exposure data

Smoking and drinking habits were entered in the three categories of never, former, and current. Former smokers and drinkers were included as ever-smokers and ever-drinkers with current smokers and drinkers for analysis. BMI at enrollment was calculated from self-reported height and weight as BMI (kg/m^2) = (weight in kg)/(height in m)^2. In this study, subjects who reported an exercise frequency of at least once a month were defined as those who exercised.

SNP selection, genotyping and analysis for linkage disequilibrium (LD)

The SNP loci examined in this study were basically selected by SNP browser ver. 3.5 (Applied Biosystems, Foster City, CA, USA) (De La Vega et al., 2006) on the basis of HapMap database for Japanese residing in the Tokyo metropolitan area (build 36) (Consortium, 2003, Thorisson et al., 2005). Selection criteria for each locus were a minor allele frequency (MAF) greater than 30% and a haplotype R^2-value greater than 0.95. Based on these criteria, 25 tag SNPs were finally selected from 169 candidate SNPs.

DNA was extracted from the buffy coat fraction in each subject using a Blood Mini Kit (Qiagen KK, Tokyo, Japan) and analyzed using the polymerase chain reaction (PCR) TaqMan method (Livak, 1999) with the 7500 real-time PCR System (Applied Biosystems). The probes used were specially designed for rs12148604 (SNP 1), rs2899473 (SNP 2), rs12900487 (SNP 3), rs1865803 (SNP 4), rs10459592 (SNP 5), rs12591359 (SNP 6), rs767199 (SNP 7), rs16964220 (SNP 8), rs2008691 (SNP 9), rs11856927 (SNP 10), rs4441215 (SNP 11), rs4545755 (SNP 12), rs3889391 (SNP 13), rs11632903 (SNP 14), rs17647707 (SNP 15), rs17647719 (SNP 16), rs1902586 (SNP 17), rs936306 (SNP 18), rs999480 (SNP 19), rs2470152 (SNP 20), rs3751591 (SNP 21), rs1004982 (SNP 22), rs1902585 (SNP 23), rs752760 (SNP 24) and rs2445768 (SNP 25). Genotyping was conducted in duplicate in cases where accordance with Hardy-Weinberg equilibrium (HWE) was violated.

LD was evaluated by means of LD coefficient (D'). Haplotype blocks were defined as D' > 0.80 and haplotype frequency as > 0.01 using Haploview 4.2 (Daly Lab at the Broad Institute, Cambridge, USA) (Barrett et al., 2005).

Statistical analysis

To assess the strength of associations between polymorphisms and risk of endometrial cancer, odds ratios (ORs) with 95% confidence intervals (CIs) per minor allele were estimated using unconditional logistic models adjusted for potential confounders. Potential confounders considered in the multivariate analyses were age; smoking habit (never or ever); drinking habit (never or ever); current BMI (< 23 or ≥ 23 kg/m^2); regular exercise (yes or no); age at menarche (≤ 12, 13 - 14, or ≥ 15); age at menopause (≤ 39, 40 - 54, or ≥ 55); parity (0, 1 - 2, ≥ 3); history of diabetes (yes or no); history of hypertension (yes or no); and contraceptive use (yes or no). Differences in categorized demographic variables between cases and controls were tested by the chi-squared test. Age, BMI, age at menarche, age at menopause, and parity were compared by the Mann-Whitney test. To explore the interaction between current BMI and each locus and haplotypes, additional analyses were performed with stratification by BMI (≤ 23 or > 23 kg/m^2, based on median of current BMI among controls).

Genotypes were included as scores. A retrospective profile-likelihood method was applied in logistic regression to estimate haplotype effects using the "haplologit" command (Marchenko et al., 2008). Haplotypes were constructed using unphased information of loci that were in LD on chromosome 15.

P-values less than 0.05 were considered statistically significant. All analyses were conducted using STATA version 10.1 (Stata Corp., College Station, TX), and LD estimates were calculated using Haploview.

Results

Baseline characteristics of the 48 endometrial cancer patients and 253 controls are shown in Table 1. Median age of cases was lower than that of controls. Current BMI among cases was significantly higher than that among controls ($P = 0.004$). Smoking and drinking status did not differ between the two groups. Regarding reproductive factors, low parity was more prevalent ($P = 0.001$) and

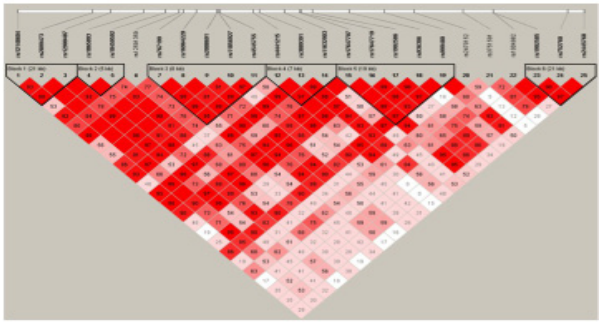

Figure 1. Numbers in the Panel Indicate Pair-wise D' Values. Increasing shades of red indicate a high degree of correlation.

Table 1. Characteristics of Study Subjects

Characteristic	Cases (n = 48)	(%)	Controls (n = 253)	(%)	P value
Histology					
endometrioid grade 1 (%)	17	(35.4)			
endometrioid grade 2 (%)	18	(37.5)			
endometrioid grade 3 (%)	9	(18.8)			
endometrioid grade unknown (%)	4	(8.3)			
Age (median,[min-max])	59 (35-79)		63 (48-79)		0.003
< 55 (%)	7	(14.6)	22	(8.7)	0.302
55 - 69	35	(72.9)	183	(72.3)	
≥ 70 (%)	6	(12.5)	48	(19.0)	
Smoking status					
Ever (%)	10	(20.8)	29	(11.5)	0.084
Never (%)	38	(79.2)	220	(87.0)	
Unknown (%)	0	(0)	4	(1.6)	
Drinking status					
Ever (%)	11	(22.9)	86	(34.0)	0.124
Never (%)	37	(77.1)	165	(65.2)	
Unknown (%)	0	(0)	2	(0.8)	
Current body mass index					
(median,[min-max])	24.2 (18.7-40.9)		22.3 (15.7-33.3)		0.004
< 23 kg/m2 (%)	19	(39.6)	152	(60.1)	0.011
≥ 23 kg/m2 (%)	28	(58.3)	100	(39.5)	
Unknown (%)	1	(2.1)	1	(0.4)	
Regular exercise					
No (%)	13	(27.1)	64	(25.3)	0.756
Yes (%)	34	(70.8)	187	(73.9)	
Unknown (%)	1	(2.1)	2	(0.8)	
Age at menarche					
(median,[min-max])	14 (11-18)		14 (11-20)		0.262
≤ 12 (%)	9	(18.8)	31	(12.3)	0.400
13 - 14 (%)	23	(47.9)	117	(46.3)	
≥ 15 (%)	15	(31.3)	97	(38.3)	
Unknown (%)	1	(2.1)	8	(3.2)	
Age at menopause					
(median,[min-max])	52 (28-57)		50 (38-63)		0.079
≤ 39 (%)	3	(6.3)	3	(1.2)	0.065
40 - 54 (%)	42	(87.5)	236	(93.3)	
≥ 55 (%)	2	(4.2)	13	(5.1)	
Unknown (%)	1	(2.1)	1	(0.4)	
Parity					
(median,[min-max])	2 (0-4)		2 (0-4)		0.009
0 (%)	9	(18.8)	14	(5.5)	0.001
1 - 2 (%)	31	(64.6)	151	(59.7)	
≥ 3 (%)	8	(16.7)	85	(33.6)	
Unknown (%)	0	(0)	3	(1.2)	
Diabetes history					
No (%)	40	(83.3)	234	(92.5)	0.032
Yes (%)	8	(16.7)	18	(7.1)	
Unknown (%)	0	(0)	1	(0.4)	
Hypertension history					
No (%)	34	(70.8)	197	(77.9)	0.290
Yes (%)	14	(29.2)	56	(22.1)	
Contraceptive use history					
No (%)	44	(91.7)	247	(97.6)	0.035
Yes (%)	4	(8.3)	6	(2.4)	

age at menopause was marginally higher among cases (P = 0.079). A history of diabetes and contraceptive use was more common among cases.

Regarding the genotype distribution of the 25 SNPs in *CYP19A1*, each locus among controls were in accordance with the Hardy-Weinberg equilibrium. In the per-allele model, we did not observe any significant associations for any loci examined. However, for SNP 2, SNP 4, SNP 8, SNP 9, SNP 15-19, and SNP 22, an inverse association was observed in patients with a BMI ≤ 23, whereas a positive association was seen with a BMI > 23. In contrast, for SNP 23, 24 and 25, a positive association was observed in patients with a BMI ≤ 23 but an inverse association with a BMI > 23. *P* values for these interactions were statistically significant under a case-only design.

Figure 1 shows the LD of each locus. We identified six haplotype blocks. Table 2 shows their impact on endometrial cancer and the interaction with current BMI.

Table 2. Impact of Haplotypes of *CYP19A1* on Endometrial Cancer Risk According to Current BMI

Haplotype	Haplotype freq (control)	Total Multivariate OR (95%CI)*	BMI ≤ 23 Multivariate OR (95%CI)*	BMI >23 Multivariate OR (95%CI)*	(case only) interaction P*
Block 1 (CYP19 SNP1, SNP2, and SNP3)					
TCC	0.365253	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
CCT	0.270938	0.89 (0.51-1.57)	1.37 (0.63-2.95)	0.50 (0.20-1.21)	0.130
CTT#	0.266252	1.01 (0.58-1.75)	0.33 (0.11-1.03)	1.66 (0.81-3.41)	0.030
TCT	0.082817	1.23 (0.56-2.68)	0.80 (0.22-2.95)	1.59 (0.57-4.43)	0.416
TTT	0.010428	NE	NE	NE	NE
Block 2 (CYP19 SNP4 and SNP5)					
TG	0.369565	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
CT	0.343873	1.17 (0.70-1.95)	0.58 (0.25-1.36)	1.73 (0.88-3.44)	0.057
TT	0.286561	0.95 (0.54-1.66)	1.13 (0.52-2.49)	0.73 (0.32-1.66)	0.594
Block 3 (CYP19 SNP7, SNP8, and SNP10)					
AGG	0.363593	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
GAT	0.347161	0.97 (0.58-1.64)	0.52 (0.21-1.29)	1.33 (0.67-2.63)	0.079
GGT	0.265443	0.71 (0.38-1.31)	1.05 (0.46-2.42)	0.39 (0.15-1.04)	0.183
GGG	0.013213	2.60 (0.74-9.11)	3.63 (0.61-21.56)	1.80 (0.31-10.55)	0.792
Block 4 (CYP19 SNP12, SNP13, and SNP14)					
GGC	0.568418	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
CAT	0.306116	1.16 (0.71-1.88)	1.45 (0.69-3.04)	1.07 (0.55-2.09)	0.543
GGT	0.065761	1.51 (0.68-3.35)	1.99 (0.61-6.51)	1.27 (0.43-3.78)	0.808
CGC	0.056056	1.18 (0.47-2.99)	0.95 (0.20-4.39)	1.68 (0.48-5.84)	0.698
Block 5 (CYP19 SNP16, SNP17, SNP18 and SNP19)					
AGCG	0.572982	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
GATA#	0.244449	0.97 (0.56-1.66)	0.34 (0.11-1.00)	1.62 (0.80-3.26)	0.012
GATG	0.065594	1.65 (0.77-3.55)	1.43 (0.45-4.55)	1.82 (0.64-5.17)	0.456
AGTG	0.051166	0.87 (0.29-2.61)	0.40 (0.05-3.16)	2.53 (0.78-8.21)	0.129
AATG	0.049249	1.31 (0.51-3.34)	0.38 (0.05-2.96)	1.71 (0.42-6.91)	0.308
Block 6 (CYP19 SNP23 and SNP25)					
GC	0.503107	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	
CA#	0.362790	0.97 (0.60-1.57)	2.02 (0.97-4.20)	0.96 (0.41-2.26)	0.024
GA	0.127328	1.15 (0.60-2.21)	1.58 (0.53-4.66)	0.59 (0.30-1.17)	0.710

*Multivariate models adjusted for age, current BMI, smoking, drinking, regular exercise, age at menarche, age at menopause, parity, diabetes history, and history of oral contraceptive use; #Significant interaction with current BMI; NE, not estimated

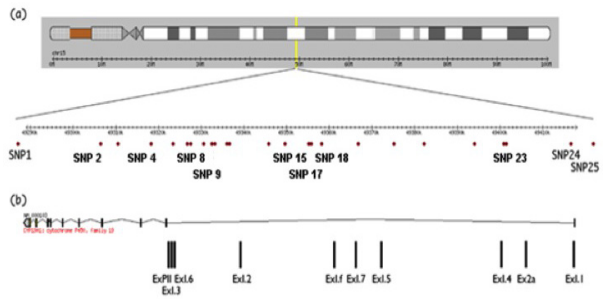

Figure 2. (a) Ideogram of *CYP19A1* and distribution of 25 tag SNPs. (b) Genetic structure of *CYP19A1*.

None of the haplotypes was associated with cancer risk. In contrast, haplotype CTT of block 1, GATA of block 5 and CA of block 6 showed significant interactions.

Discussion

In this study, we found no significant association between 25 *CYP19A1* tag SNPs and 6 haplotypes and endometrial cancer risk. However, several *CYP19A1* genotypes and haplotypes showed significant interactions with current BMI. These results provide useful information to our understanding of the etiology of endometrial cancer.

The *CYP19A1* gene is located on human chromosome 15q21.2 and is more than 120 kb long. The region encoding aromatase spans 30 kb and the upstream region contains a number of promoters (Figure 2) (Bulun et al., 2007). Aromatase is expressed not only in ovarian granulosa cells, the placental syncytiotrophoblast, and the testicular Leydig cells but also in extraglandular sites, including adipose tissue and skin fibroblasts. After menopause, adipose tissue becomes the major source of estrogen, with estrogenically weak estrone biosynthesized from adrenal androstenedione by aromatase (Grodin et al., 1973; Bulun and Simpson, 1994). Aromatase might therefore be involved in estrogen-dependent diseases, including breast cancer and endometrial cancer. Several molecular epidemiological studies have investigated the associations between *CYP19A1* polymorphisms and endometrial cancer risk (Berstein et al., 2001; Paynter et al., 2005; Olson et al., 2007; Tao et al., 2007; Setiawan et al., 2009). Seitawan et al. showed that common *CYP19A1* SNPs (rs749292 and rs727479; selected based on their previous study (Haiman et al., 2007)) were associated with risk, particular in older and obese women (Setiawan et al., 2009). Other studies also explored a number of potentially functional *CYP19A1* SNPs and suggested significant associations with endometrial cancer in different ethnic populations (Berstein et al., 2001; Paynter et al., 2005; Olson et al., 2007; Tao et al., 2007). These previous and our present results might therefore be inconsistent. The current paucity of evidence concerning the association

between *CYP19A1* SNPs and endometrial cancer warrants further evaluation in multiple ethnicities.

In this study, we examined a substantial number of tag SNPs to cover the region of the *CYP19A1* gene on the basis of known biological mechanisms with tag SNPs among Japanese. In our previous study, estrone levels differed with SNP 1 (rs12148604) and SNP 14 (rs11632903) among postmenopausal Japanese women (Kidokoro et al., 2009). Although we had expected that these SNPs would be associated with increased endometrial cancer risk, we found no significant association with *CYP19A1* genotypes and haplotypes (Table 2), and although we included age at first visit in the multivariate regression models, we could not completely adjust for the impact of the age difference between the cases and controls. In this regard, Hemsell et al. and Grodin et al. suggested that the conversion rate of plasma androstenedione to estrone in humans increases as a function of obesity and aging (Grodin et al., 1973, Hemsell et al., 1974; Cleland et al., 1985; Bulun et al., 2007). Further, our findings might be partially explained by the small sample size.

As obesity is an established risk factor for endometrial cancer and partially indicates the amount of adipose tissue, our exploration of the interaction between these SNPs and haplotypes included analyses stratified by current BMI (Table 2). We found significant interactions between many polymorphisms (SNP 2, SNP 4, SNP 8, SNP 9, SNP 15, SNP 16, SNP 17, SNP18, SNP 22, SNP 23, SNP 24 and SNP 25) and haplotypes (CTT of block 1, GATA of block 5 and CA of block 6 in Table 2) and current BMI, with statistical significance. Interactions between CYP19A1 polymorphisms and current BMI have also been observed in several other studies (Setiawan et al., 2009, Tao et al., 2007). These findings may be of value in future epidemiologic and biological studies, and the combination of this genetic information with findings concerning anthropometric and environmental factors may provide insights into the individualized prevention of endometrial cancer.

Several potential limitations of our study warrant consideration. First, although we aimed to identify *CYP19A1* loci which showed an association with endometrial cancer risk, significant associations with marker loci do not warrant the functional importance of the loci. Second, we did not examine the serum or intratumoral concentrations of sex steroid hormones. In addition to the importance of serum estrogen to the development and progression of endometrial cancer, an important role for the local biosynthesis of estrogen by aromatase within endometrial carcinomas has also recently come to be recognized (Berstein et al., 2004, Takahashi-Shiga et al., 2009). Third, we should interpret these results in consideration of multiple comparison. Finally, our study had a small sample size, and replication in a larger population is necessary.

In conclusion, we examined the association between 25 tag SNPs and 6 haplotypes of *CYP19A1* and endometrial cancer in a population of postmenopausal Japanese women. Although we found no significant association between *CYP19A1* genotypes and haplotypes and endometrial cancer risk, we did find significant interaction between *CYP19A1* loci and current BMI. Among women with a BMI > 23, significant positive associations were observed for SNP 2, SNP 4, SNP 8, SNP 9, SNP 15, SNP 16, SNP 17, SNP18, and SNP 22, whereas negative associations were seen for SNP 23, SNP 24 and SNP 25. The haplotype CTT of block 1, GATA of block 5 and CA of block 6 showed statistically significant interactions with current BMI. Further investigation of these findings is warranted.

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