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Original Article

Association of a single nucleotide polymorphism at 6q25.1, rs2046210, with endometrial cancer risk among Chinese women

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Abstract

A recent genome-wide association study identified a new susceptibility locus for breast cancer, rs2046210, which is a single nucleotide polymorphism (SNP) located upstream of the estrogen receptor α (*ESR1*) gene on chromosome 6q25.1. Given that endometrial cancer shares many risk factors with breast cancer and both are related to estrogen exposure and that rs2046210 is in close proximity to the *ESR1* gene, we evaluated the association of SNP rs2046210 with endometrial cancer risk among 953 cases and 947 controls in a population-based, case-control study conducted in Shanghai, China. Logistic regression models were used to derive odds ratios (ORs) and 95% confidence intervals (95% CIs) after adjusting for potential confounders. We found that the A allele of rs2046210, linked to an increased risk of breast cancer, was associated with increased but not statistically significant risk of endometrial cancer (OR = 1.16, 95% CI = 0.96–1.41 for the GA and AA genotypes compared with the GG genotype); the association was stronger among post-menopausal women (OR = 1.28, 95% CI = 1.00–1.65). The association tended to be stronger among women with higher or longer estrogen exposure than among women with relatively lower or shorter exposure to estrogen. Our study suggests that rs2046210 may play a role in the etiology of endometrial cancer. Additional studies are needed to confirm our findings.

Key words Endometrial cancer, polymorphism, genetic susceptibility, case-control study, estrogen

Introduction

In a genome-wide association study of breast cancer conducted among Chinese women, we identified a new susceptibility locus for breast cancer, the single nucleotide polymorphism (SNP) rs2046210[1]. SNP rs2046210 is located on chromosome 6q25.1, 29 kb upstream of the first untranslated exon and 180 kb upstream of the transcription start site in the first exon of the estrogen receptor α (*ESR1*) gene. Recently, we confirmed this association in other East-Asian populations and in women of European ancestry, but found no association in women of African ancestry. Endometrial cancer is the most common gynecologic cancer and is the fourth most common cancer among women in the United States[2]. It has been suggested conditions increasing exposure to unopposed estrogen increase the risk of endometrial cancer[3-7]. The signal transduction and biological effects of estrogen are mediated primarily through high-affinity binding to estrogen receptors (ERs). Given that endometrial and breast cancers are both hormone-related and share many risk factors[4-11] and that rs2046210 is in close proximity to the *ESR1* gene, we evaluated whether rs2046210 is associated with endometrial cancer risk employing data from a population-based, case-control study conducted in Shanghai, China.

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Patients and Methods

Study participants

Details of the methods used in the Shanghai Endometrial Cancer Study (SECS) have been published previously [12]. Briefly, through the population-based Shanghai Cancer Registry, we identified women who were 30 to 69 years of age and permanent residents of urban Shanghai and who were diagnosed with primary endometrial cancer between 1997 and 2003. The diagnosis of each case was confirmed by a medical chart review and a review of the available pathology slides by senior study pathologists. Controls were randomly selected from the general female population of urban Shanghai using the Shanghai Resident Registry and were frequency matched to cases on age (± 5 years) distribution. Women with a history of any cancer or hysterectomy were not eligible. A total of 1204 cases (response rate: 82.6%) and 1212 controls (response rate: 74.4%) participated in the study. In-person interviews were conducted to collect information on non-genetic risk factors, including demographic factors, menstrual and reproductive history, hormone use, usual dietary intake, prior disease history, tobacco and alcohol use, family history of cancer, and height and weight history. Anthropometrics including weight, height, and waist and hip circumference were measured using a standard protocol. The study protocol was approved by the Institutional Review Boards of all participating institutes, and written informed consent was obtained from all participants prior to interview.

Genotyping

DNA samples from 1037 cases (86.1%, 850 blood and 187 buccal cell) and 1020 controls (84.2%, 834 blood and 186 buccal cell) were included in the present study. SNP rs2046210 was genotyped employing a TaqMan assay (assay ID, C_12034236_10, Applied Biosystems) on an ABI 7900HT system. Laboratory staff was blinded to the identity of the subjects. Quality control (QC) samples were included in the genotyping assays. The concordance rate for the blinded samples was 98.9%. Genotyping data were obtained from 953 cases and 947 controls with a success rate of 91.9% and 92.8%, respectively. The major reason for incomplete genotyping was an insufficient quantity of DNA from blood and buccal cells.

Statistical analysis

Case-control differences for selected risk factors and rs2046210 genotype distributions were evaluated using χ^2 statistics for categorical variables and Wilcoxon tests for continuous variables. Logistic regression models were employed deriving odds ratios (ORs) and 95% confidence intervals (95% CIs) after adjusting for potential confounders. Interactive effects were investigated employing logistic regression analysis, applying the likelihood ratio test to compare the model including the main effects with the model including both the main effects and the interaction terms. Statistical significance was determined with a P value $\leqslant 0.05$, and all analyses were conducted by using SAS version 9.1 (SAS Institute).

Results

The distribution of selected demographic characteristics and major risk factors for endometrial cancer among cases and controls is presented in Table 1. There were no appreciable differences for these factors between cases included in the genotyping study and the entire study population. The same was true for the two control groups. Endometrial cancer cases and controls were comparable with respect to age and education. More cases had a family history of any cancer than did controls. Compared with controls, cases were more likely to have been younger at menarche, have been older at menopause, have had a longer duration of menstruation, have had fewer live births, and to currently have a higher body mass index (BMI) and higher waist-to-hip ratio (WHR), but were less likely to regularly drink alcohol, have ever used oral contraceptives, or to regularly participate in physical activity.

The genotype distribution of rs2046210 was consistent with Hardy-Weinberg equilibrium in both cases and controls. The minor allele frequency among controls was 0.34, similar to the minor allele frequency reported previously for a Chinese population [1]. Table 2 presents the association of rs2046210 with endometrial cancer risk. Compared with the GG genotype, women with the GA or AA genotype had an increased risk of endometrial cancer of borderline statistical significance (OR = 1.16, 95% CI = 0.96−1.41). This association was statistically significant among postmenopausal women (OR = 1.28, 95% CI = 1.00−1.65).

Analyses stratified by selected variables related to estrogen exposure showed that association of the A allele of rs2046210 with endometrial cancer risk was more pronounced among women with higher BMI, higher WHR, or who had a longer duration of menstruation (Table 3). None of the interactions, however, were statistically significant based on the

Table 1. Comparison of demographic characteristics and selected risk factors for 953 cases of endometrial cancer and 947 controls with genotyping data[a]

Subject characteristic	Cases	Controls	*P* value[b]
Age (years, mean ± SD)	54.8 ± 8.5	54.8 ± 8.5	0.907
⩾ Middle school education (%)	77.9	77.7	0.941
Regular cigarette smoking (%)	3.4	3.4	0.980
Regular alcohol consumption (%)	2.9	5.5	0.006
First degree relative with any cancer (%)	35.3	30.0	0.015
Age at menarche (mean ± SD)	14.5 ± 1.7	14.8 ± 1.8	< 0.001
Postmenopausal (%)	56.8	61.0	0.059
Age at menopause[c] (mean ± SD)	50.4 ± 3.6	49.0 ± 3.7	< 0.001
Years of menstruation (mean ± SD)	32.8 ± 4.9	30.7 ± 5.3	< 0.001
Number of live births (mean ± SD)	1.7 ± 1.2	1.8 ± 1.1	0.040
Ever used oral contraceptives (%)	18.1	24.4	< 0.001
Ever used hormone replacement therapy (%)	4.8	4.3	0.604
BMI (mean ± SD)	25.8 ± 4.1	23.8 ± 3.5	< 0.001
WHR (mean ± SD)	0.84 ± 0.05	0.82 ± 0.06	< 0.001
Engaged in regular physical activity (%)	28.3	34.1	0.007

[a] The Shanghai Endometrial Cancer Study, 1997–2003
[b] From the χ^2 test (categorical variables) or non-parametric Wilcoxon test (continuous variables).
[c] Only among postmenopausal women.
BMI, body mass index; WHR, waist-to-hip ratio; SD, standard deviation.

Table 2. Association of SNP rs2046210 with endometrial cancer risk between 953 cases and 947 controls

Genotypes	Cases	Controls	OR1 (95% CI)[a]	OR2 (95% CI)[b]
All participants				
GG	405	424	1.00 (Reference)	1.00 (Reference)
GA/AA	548	523	1.10 (0.91–1.31)	1.16 (0.96–1.41)
GA	431	402	1.12 (0.93–1.36)	1.18 (0.96–1.45)
AA	117	121	1.01 (0.76–1.35)	1.10 (0.81–1.50)
Pre-menopausal				
GG	164	146	1.00 (Reference)	1.00 (Reference)
GA/AA	248	223	1.00 (0.75–1.33)	1.05 (0.76–1.44)
GA	194	174	0.99 (0.73–1.34)	1.03 (0.74–1.45)
AA	54	49	1.01 (0.65–1.59)	1.09 (0.67–1.77)
Post-menopausal				
GG	241	278	1.00 (Reference)	1.00 (Reference)
GA/AA	300	300	1.15 (0.91–1.46)	1.28 (1.00–1.65)
GA	237	228	1.20 (0.93–1.54)	1.33 (1.02–1.75)
AA	63	72	1.01 (0.69–1.48)	1.12 (0.74–1.69)

[a] Adjusted for age.
[b] Adjusted for age, education level, height, body mass index, duration of menstruation, waist-to-hip ratio, menopausal status, and family history of any cancer.
Reference, reference group. OR, odds ratio; CI, confidence interval.

multiplicative model.

Discussion

Estrogen, by binding to estrogen receptors, plays an important role in endometrial and breast carcinogenesis [13,14]. There are two major classes of estrogen receptors, estrogen receptor alpha (ER-α), which is encoded by the *ESR1* gene and estrogen receptor beta (ER-β), which is encoded by the *ESR2* gene. ER-α is the predominant receptor responsible for the estrogenic effect. SNP rs2046210, located upstream of the *ESR1* gene, is associated with breast cancer risk

Table 3. Association of SNP rs2046210 with endometrial cancer risk, stratified by factors related to estrogen exposure

Stratified variable[a]	GG		GA/AA	
	Cases/Controls	OR (95% CI)	Cases/Controls	OR (95% CI)
BMI[b]				
⩽ 22.26	73/130	1.00 (Reference)	106/186	1.10 (0.75– 1.62)
22.27–25.10	122/146	1.31 (0.88– 1.94)	156/168	1.52 (1.04– 2.21)
> 25.10	208/147	2.04 (1.39– 2.99)	285/169	2.39 (1.65– 3.46)
WHR[c]				
⩽ 0.798	81/131	1.00 (Reference)	106/189	0.95 (0.65– 1.40)
0.799–0.840	117/138	1.13 (0.76– 1.67)	150/172	1.27 (0.88– 1.84)
> 0.840	205/155	1.62 (1.11– 2.36)	292/162	2.13 (1.48– 3.07)
Years of menstruation				
All study participants[d]				
⩽ 29.35	84/128	1.00 (Reference)	116/189	0.94 (0.65– 1.36)
29.36–33.20	106/136	1.17 (0.79– 1.73)	159/177	1.42 (0.98– 2.04)
> 33.20	212/158	1.99 (1.37– 2.88)	270/156	2.57 (1.78– 3.70)
Post-menopausal participants[e]				
⩽ 29.43	36/84	1.00 (Reference)	53/109	1.21 (0.71– 2.07)
29.44–33.37	66/92	0.83 (0.45– 1.52)	88/99	1.10 (0.61– 1.99)
> 33.37	138/102	0.90 (0.43– 1.89)	157/92	1.16 (0.56– 2.41)

[a] Categorized by tertile according to the values among controls.
[b] Adjusted for age, education level, duration of menstruation, waist-to-hip ratio, menopausal status, height, and family history of any cancer. *P* for interaction = 0.96.
[c] Adjusted for age, education level, body mass index, duration of menstruation, menopausal status, height, and family history of any cancer. *P* for interaction = 0.43.
[d] Adjusted for age, education level, body mass index, waist-to-hip ratio, menopausal status, height, and family history of any cancer. *P* for interaction = 0.46.
[e] Among post-menopausal women with natural menopause and adjusted for age, education level, body mass index, waist-to-hip ratio, height, and family history of any cancer. *P* for interaction = 0.96.
Reference, reference group. Other abbreviations as in Tables 1 and 2.

among both East-Asian women and women of European ancestry [1]. Given the importance of estrogens in the etiology of endometrial cancer and as endometrial cancer shares many risk factors with breast cancer, especially conditions related to high exposure to estrogen, it is possible that rs2046210 may also be a marker of endometrial cancer risk. In this large-scale, population based, case-control study, we found suggestive evidence that the A allele of rs2046210 may be associated with an increased risk for endometrial cancer. The association was more pronounced among post-menopausal women and women with longer duration of menstruation. The risk also appeared to be more evident among women with higher BMI and higher WHR, conditions that are related to higher endogenous estrogen exposure.

Although underlying biological mechanisms for the observed association are unknown, if SNP rs2046210 were related to an altered function of the *ESR1* gene, a stronger association would be expected among women with higher or longer exposure to endogenous estrogen. However, no evidence is available to support a direct relation between this SNP and *ESR1* function. An alternative explanation is that rs2046210 is a surrogate marker for un-identified causal SNP(s) determining the function of *ESR1* gene. Using data from a recently completed genome-wide association study (scanned using the Affymetrix SNP array 6.0) of 832 cases and 2049 controls, we carefully examined all SNPs within the 100 kb region surrounding rs2046210 for their association with endometrial cancer risk. No other SNPs were observed possessing a statistically significant association with endometrial cancer risk.

SNP rs2046210 is also in close proximity (6 kb downstream) to *C6orf97*, an open reading frame located on human chromosome 6. The function of *C6orf97* is unclear, and little information about *C6orf97* has been published. A BLAST database search using the *C6orf97* coding peptide as the query sequence found a structural maintenance of chromosomes (SMC) domain in the C-terminus of the C6orf97 protein. The C-terminus of human C6orf97 shares 22% identity at the amino acid

level with budding yeast protein Smc2p. Although no direct evidence links SMC proteins to cancer development in humans, they do appear to play a role in chromosome dynamics[15]. In yeast, Smc2p and Smc4p consist of a heterodimer which interacts with other proteins to form the chromosome condensing complex[16]. It has been shown that the condensing complex is required for chromosome condensation[15,16]. The yeast smc2-6 mutant shows a defect in chromosome segregation and partial chromosome decondensation in cells arrested during mitosis[17]. Further studies of the function of *C6orf97* and its association with cancer are certainly warranted.

The strengths of this study include the population-based, case-control study design and a relatively high response rate, minimizing the potential for selection bias. Detailed exposure information collected in this study enabled an evaluation of gene-environment interactions. In addition, Chinese women living in Shanghai are relatively homogeneous in ethnic background with over 98% classified into a single ethnic group (Han Chinese). Thus, potential confounding by ethnicity is not a major concern for our study. Although our study is one of the largest epidemiological studies of endometrial cancer, the size of our study population (953 cases and 947 controls) provided only 77% statistical power to detect an OR of 1.2 per risk allele (alpha = 0.05, two-sided) under the additive model, which is the magnitude of the association of this SNP with breast cancer risk that we observed. Statistical power in stratified analyses was further reduced. Therefore, additional studies are needed to confirm our findings.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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