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CYP19 gene polymorphisms and the susceptibility to breast cancer in Xinjiang Uigur women

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ABSTRACT. In this study, the relationship between *CYP19* gene polymorphisms and breast cancer in Xinjiang Uigur women was investigated. A case-control study was designed to compare 112 Uigur breast cancer patients with 139 Uigur healthy controls. Individuals were genotyped for the *CYP19* rs10046 polymorphism using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Accordingly, the relationship between the rs10046 polymorphism and the susceptibility of Xinjiang Uigur women to breast cancer was analyzed. Given that the allele at the rs10046 site varies between C and T within the *CYP19* gene, the frequency distribution of the C and T allele in breast cancer subjects were 48.2 and 51.8% respectively, and 47.5 and 52.5% in control cases. Moreover, the frequency distribution of the TC, CC, and TT genotype were 26.8, 42.9, and 30.4% in breast

cancer cases, but 18.0, 59.0, and 23.0% in control cases ($P < 0.05$). Risk factors within the Uigur population for breast cancer included an age ≥ 50 years old, a BMI ≥ 25 kg/m^2, and a parity ≥ 2. Conversely, an abortion and the *CYP19* rs10046 TC genotype were protective factors. Menopause was another independent risk factor for breast cancer in Uigur women after the correction for age, BMI, age at first parity, pregnancy, and breastfeeding. In conclusion, breast cancer in Xinjiang Uigur women is closely connected with the age, BMI, parity, abortion, and CYP19 rs10046 polymorphisms. The TC genotype and an abortion can reduce the risk of the breast cancer disease in Uigur women.

Key words: Breast cancer; CYP19 gene; Uigur; Gene polymorphisms

INTRODUCTION

Breast cancer is the most common malignant tumor in women, and hormone balance plays a key role in its development. Aromatase, which is encoded by the aromatase gene, *CYP19*, is a key enzyme in estrogen metabolism. *CYP19* gene polymorphisms have been known to cause changes in enzyme activity and have influenced the synthesis of estrogen. This suggests a possible role of *CYP19* gene polymorphisms in breast cancer initiation and progression. Our study selected breast cancer patients and healthy controls in Uigur women in Xinjiang and attempted to determine the association between *CYP19* rs10046 site polymorphism and its relation to susceptibility to breast cancer.

MATERIAL AND METHODS

Patients

One hundred and twelve Uigur women with newly diagnosed breast cancer and 139 healthy control cases visiting the Cancer Hospital Affiliated to Xinjiang Medical University from October 2010 to July 2012 were used for this study. The youngest breast cancer patients were 22 years old, and the oldest was 71, the average age was 44.45 ± 9.22 years old. With respect to control cases, the youngest control cases were 25 years old, and the oldest were 65, with the mean age being 45.30 ± 9.60 years old. The result had no significant difference ($P > 0.05$). The incidence of invasive ductal carcinoma in the patient group was 82 cases (73%). The 139 control patients were Uigur women that went through a health medical examination in the three-A General Hospital, and were all community residents in Urumchi. All general information for both groups was obtained at the same time, including the postoperative pathology, menopause, menstrual history, malignant tumor family history, pregnancy histories, and lactation history.

Selection

Inclusion criteria of case group

The following are the inclusion criteria for the breast cancer patients group: 1) the

primary breast cancer in Uigur women got histodiagnosis, 2) they had no endocrine, radiation, and/or chemical therapy prior to admission, 3) they had no other malignant tumor history, and no breast cancer metastasis, advanced breast cancer, or mental disease, 4) they provided informed consent, and signed the medical informed consent document, and 5) it was on the Ethics Committee approval.

Exclusion criteria of case group

The following are the exclusion criteria for the breast cancer patient group: 1) they had received hormone therapy prior to admission, 2) they had severe heart, lung, liver, kidney, and/or other organ dysfunction, and 3) they had lived in Xinjiang for less than 10 years.

Inclusion criteria of control group

The following are the inclusion criteria for the control group: 1) they were Uigur women that had a healthy medical examination, 2) they were ± 5 years matched to the case group, and 3) they were under the Ethics Committee approval, provided informed consent, and signed the medical informed consent document.

Exclusion criteria of control group

The following are the exclusion criteria for the control group: 1) they received hormone therapy, 2) they possessed heart, lung, liver, kidney, and/or other organ dysfunction previously, and 3) they had lived in Xinjiang less than 10 years, and 4) they were at gestation or breastfeeding period.

Detection in the laboratory

Materials and Reagents

Whole Blood Gene Extraction Kit was purchased from TIANGEN Biotech Co., Ltd (Beijing, China). The primers were synthesized by Shanghai Biological Technology Service Company. The restriction enzyme *Sdu*I (Bsp1286) (10 U/μL) was purchased from MBI Fermentas Co. (Canada).

Methods

Two milliliters of blood was collected in an EDTA vacutainer from patients as well as controls. Genomic DNA was extracted using a Whole Blood Gene Extraction Kit following the manufacturer protocol and stored at -20°C. Genotyping for the *CYP19* rs10046 (CYP19 E10 c. +19C>T; located in exon 10, 19 bp downstream from the amber stop codon) polymorphisms was performed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Alternatively, genotyping of the SNP rs10046 was performed by two separate allele-specific conventional PCRs with the following primers: forward, 5'-CTGGAACACTAGAGAAGGCTGGTCAGTGC-3'; and reverse,

5'-GTTCTCTGGTGTGAACAGGAGATGAC-3'. The PCRs were initiated with denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, followed by renaturation at 63°C for 30 s, elongation at 72°C for 30 s and elongation at 72°C for 5 min (40 cycles). PCR products were separated by electrophoresis on a 2% agarose gel under 110 V for 30 min, and the objective band was 202 bp. The PCR products were digested by the restriction enzyme *Sdu*I (*Bsp*1286) at 37°C overnight.

The arginine allele (CC) was identified by the presence of 172- and 30-bp fragments. Alternatively, the tryptophan allele (TT) was identified by 202-bp fragments, while the TC allele was identified with the 202-, 172-, and 30-bp fragments. Some *CYP19* rs10046 PCR products were selected to sequence.

Statistical analysis

Data were analyzed using SPSS16.0, and the genotype was measured according to Hardy-Weinberg (H-W). Allele frequency differences between the groups were examined by the χ^2 test. Breast cancer disease factors were analyzed by univariate and multivariate logistic regression analysis, and then by calculating the odds ratio (OR) and 95% confidence interval (CI). The variation allele frequency of the *CYP19* rs10046 site was analyzed by the χ^2 test (size of test $\alpha = 0.05$). A P value of <0.05 was considered to be significant.

RESULTS

Detection of genotype DNA

The genomic DNA was detected using UV spectrophotometry, and the average concentration of DNA for the 251 patients was 566.20 ng/μL.

PCR of *CYP19*

The PCR products of the *CYP19* fragment was 202 bp, which was located between the third band 300 bp and the forth band 200 (closer to the 200 bp band). If there was no band, we recorded the case and its number, and then repeated it one more time. Genomic DNA was extracted again when it was necessary (Figure 1).

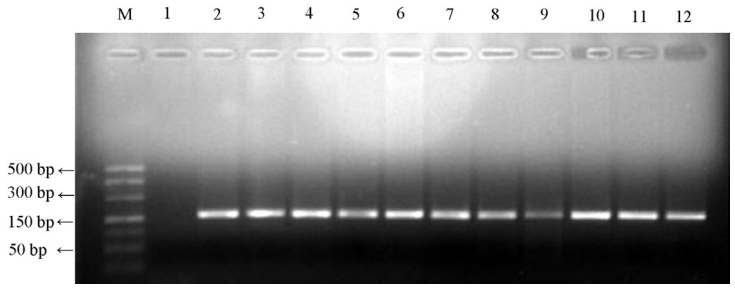

Figure 1. *CYP19* gene PCR products separated by gel electrophoresis. Lanes on the agarose gel are denoted: *lane M* = marker, and *lanes 1-12* = PCR products.

Restriction enzyme *Sdu*I (*Bsp*1286) digestion of *CYP19*

The arginine allele (CC) was identified by the presence of 172 and 30 bp fragments. Alternatively, the tryptophan allele (TT) was identified by 202-bp fragments, and the TC allele was identified by 202-, 172- and 30-bp fragments (Figure 2).

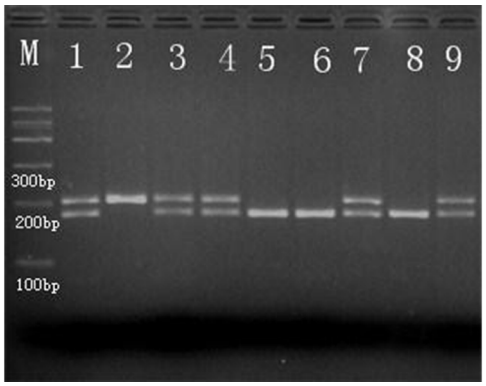

Figure 2. Restriction enzyme *Sdu*I (*Bsp*1286) digestion of *CYP19* gene mapping. Lanes on the agarose gel are denoted: *lane M* = marker, *lanes 1-9* = mutant TT, *lanes 1*, *3*, *4*, *7*, and *9* = heterozygote TC, and *lanes 5*, *6*, and *8* = wild-type CC.

Sequencing of PCR products

The *CYP19* rs10046 three genotype PCR products were randomly selected to sequence. The base changes in the sequence results were consistent with the results of enzyme digestion (Figure 3A-C).

H-W balanced detection

H-W balance analysis was made using the SPSS16.0 software, and in the breast cancer case group $\chi^2 = 2.464$ ($P > 0.05$; Table 1), and in the control group $\chi^2 = 2.092$ ($P > 0.05$; Table 2). The genotype of the breast cancer case group and control group were in accordance with H-W. It was shown that the expected and actual values tallied well for both genotype and allele. The randomness of samples was good, and they belonged to the same Mendelian population. The patients in the cases group could be said to represent the native breast cancer patients.

Comparison of the breast cancer-related risk factors

After stratified analysis of each factor, it was revealed that age was a risk factor in breast cancer for Xinjiang Uigur women. Compared with ≤35 year old women, the number of breast cancer cases for women above 50 years old was higher (OR = 6.618, 95%CI = 2.645-16.558). Similarly, the breast cancer risk of women in menopause was 5.864-fold higher than in non-menopause Uigur women (95%CI = 2.75-12.505). After stratified analysis of menarche age, it was not shown to have a relationship with the incidence of breast cancer in this population. The breast cancer risk was, however, found to be higher as the parity times increased.

For instance, the breast cancer risk of parity two times was 1.956-fold that of the parity one time in Uigur women (95%CI = 1.004-3.81), while the three times was 4.644 fold that of 1 time (95%CI = 2.149-10.037). Abortion was a protective factor against breast cancer for Uigur women (OR = 0.469, 95%CI = 0.282-0.78). There was no relationship between the childbearing age and breastfeeding time with breast cancer (Table 3).

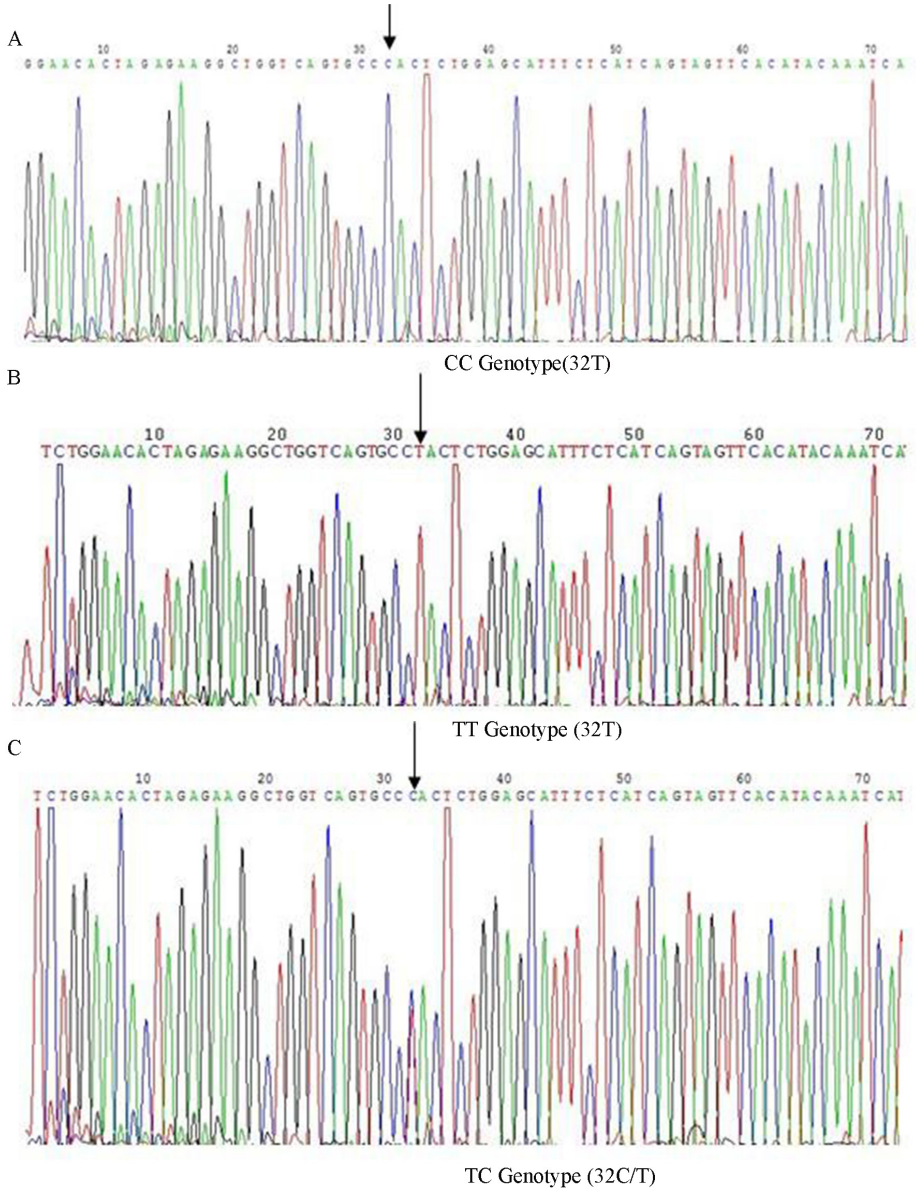

Figure 3. *CYP19* PCR amplification.

Table 1. Hardy-Weinberg balanced detection in the breast cancer group.

Genotype	Practical frequency (%)	Theoretical frequency (%)	χ^2	P
CC	301 (26.8)	20 (17.9)		
TC	48 (42.9)	52 (46.4)	2.46	
TT	34 (30.4)	40 (35.8)	4	0.266

Table 2. Hardy-Weinberg balanced detection in the control group.

Genotype	Practical Frequency (%)	Theoretical frequency (%)	χ^2	P
CC	25 (18.0)	30 (21.6)		
TC	82 (59)	70 (50.4)		
TT	32 (27.0)	39 (28.1)	2.092	0.351

Table 3. A comparison of breast cancer risk factors between the case and control groups.

Variable	Cases (%)	Control (%)	OR	95%CI	
Age (year)					
≤35	15 (13.39)	39 (28.06)	1		
36-49	69 (61.61)	89 (64.03)	1.831	0.936	3.579
≥50	28 (25.00)	11 (7.91)	6.618	2.645	16.558
Menarche age					
≥12	36 (32.14)	51 (36.69)	1		
13-16	63 (56.25)	75 (53.96)	1.19	0.629	2.047
≤17	13 (11.61)	13 (9.35)	1.417	0.588	3.413
Menopause					
No	77 (68.75)	129 (92.81)	1		
Yes	35 (31.25)	10 (7.19)	5.864	2.75	12.505
Menopause age					
≤40	4 (11.43)	2 (20.00)	1		
41-49	18 (51.43)	4 (40.00)	2.250	0.3	16.854
≥50	13 (37.14)	4 (40.00)	1.625	0.213	12.422
Fertility					
No	8 (7.14)	15 (10.79)	1		
Yes	104 (92.86)	124 (89.21)	1.573	0.641	3.855
Parity					
1	18 (17.31)	44 (35.48)	1		
2	48 (46.15)	60 (48.39)	1.956	1.004	3.812
≥3	38 (36.54)	20 (16.13)	4.644	2.149	10.037
Age at first parity					
<18	9 (8.65)	4 (3.23)	1		
18-30	89 (85.58)	110 (88.71)	0.360	0.107	1.206
≥31	6 (5.77)	10 (8.06)	0.267	0.056	1.261
Breast-feeding					
No	18 (16.07)	19 (13.67)	1		
Yes	94 (83.93)	120 (86.33)	0.827	0.411	1.663
Breast-feeding times					
<12	17 (17.53)	18 (15.00)	1		
12-23	61 (62.89)	88 (73.33)	0.734	0.351	1.537
≥24	19 (19.59)	14 (11.67)	1.437	0.553	3.742
Abortion					
No	70 (62.50)	70 (43.88)	1		
Yes	42 (37.5)	69 (56.12)	0.469	0.282	0.781
Abortion times					
1	16 (38.10)	30 (43.48)	1		
2	23 (54.76)	26 (37.68)	1.659	0.726	3.793
≥3	3 (7.14)	13 (18.84)	0.433	0.107	1.745
Marriage					
No	7 (6.25)	11 (7.91)	1		
Yes	105 (93.75)	128 (92.09)	1.289	0.483	3.442
BMI (kg/m^2)					
<25	50 (44.64)	88 (63.31)	1		
≥25	62 (55.36)	51 (36.69)	2.142	1.288	3.555

Distribution of genotype

There were 30 cases of the CC genotype, 48 cases of the TC genotype, and 34 cases of the TT genotype in the case group, corresponding to 26.8, 42.9, and 30.4% of the measured population, respectively. In the control group, there were 25 cases of the CC genotype, 82 cases of the TC genotype, and 32 cases of the TT genotype, which corresponded to 18.0%, 59%, and 23.0% of the measured population, respectively. Two groups had statistical difference (χ^2 = 6.991, P < 0.05, Table 4). The C and T allele frequencies of *CYP19* were 48.2 and 51.8% in the breast cancer group, respectively, and 47.5 and 52.5% in the control group, respectively. There was no statistical difference between C and T allele frequencies (χ^2 = 0.027, P > 0.05, Table 4).

Table 4. Distribution of genotypes and alleles in the breast cancer and control groups.

	Genotype frequencies			χ^2	P	Allele frequencies		χ^2	P
	CC (%)	TC (%)	TT (%)			C (%)	T (%)		
Cases	30 (26.78)	48 (42.85)	34 (30.35)			108 (48.21)	116 (51.79)		
Control	25 (17.99)	82 (58.99)	32 (23.02)	6.991	0.030*	132 (47.48)	146 (52.52)	0.0027	0.471

*P < 0.05.

Genotype and breast cancer

Compared with the *CYP19* gene fragment that carried the CC genotype, the OR for the gene carrying the TC genotype was 0.468 (95%CI = 0.246-0.892; P < 0.05), which indicates that the *CYP19* gene carrying the TC genotype has a protective factor against Uigur breast cancer. The OR value of the gene carrying the TT genotype was 0.824, (95%CI = 0.401-1.692) and the TC + TT genotype was 0.570 (95%CI = 0.311-1.047). The OR value of the T allele carrier was 0.943 (95%CI = 0.663-1.342), when compared with the C allele of the *CYP19* gene (Table 5).

Table 5. Relationship between the *CYP19* rs10046 genotypes and alleles and breast cancer risk.

CYP19	Breast cancer (case)	Control (case)	OR	95% CI	P
Genotype					
CC	30	25	1		
TC	48	82	0.468	0.246-0.892	0.020*
TT	34	32	0.824	0.401-1.692	0.598
TC + TT	82	114	0.570	0.311-1.047	0.068
Allele					
C	108	132	1		
T	116	146	0.943	0.663-1.342	0.746

*P < 0.05.

Breast cancer is the most common malignant tumor in women worldwide, and the risk factors related to this disease include family or genetic factors, environmental genetic factors, lifestyle, race, fertility, hormone balance, BMI, and breastfeeding. This study revealed that the age, BMI, parity, fertility, and menopause had a relationship with incidence of breast cancer in Uigur women, which is consistent with previous results (Cheng et al., 2010). Aromatase, which is encoded by *CYP19*, is a key enzyme for transforming androgen into estrogen. Specifically, it catalyzes the transformation of androstendion and testosterone into estrone and

estradiol, respectively (Miyoshi et al., 2000; Lee et al., 2003; Song et al., 2006a), and thereby influences the synthesis of estrogen (Figure 4). Estrogen levels have a close relationship with breast cancer.

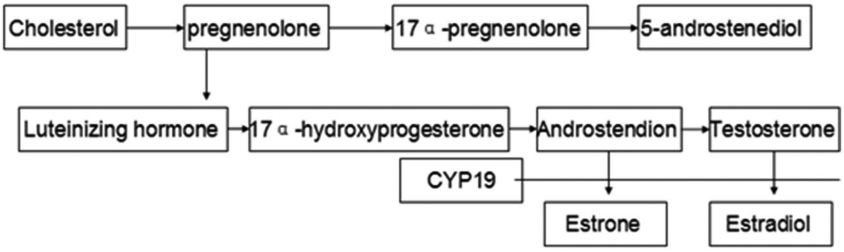

Figure 4. Estrogen synthetase cycle.

Zarrabeitia et al. (2004) showed that the variant contributing to P450 19 gene polymorphism had relationship with estrone synthesis, and revealed that there was relationship between this polymorphism and the susceptibility to breast cancer. Moreover, Rhiem et al. (2003) found that metastasis and the recurrence of breast cancer patients was higher than primary breast cancer significantly (57.6 *vs* 8.9%, $P < 0.01$) in *CYP19* gene polymorphism. Recently, it was reported that the SNP in the *CYP19* gene was related to the susceptibility of Uigur women to breast cancer. Our study selected the rs10046 site located in 3'-noncoding region, where there existed a C/T SNP. The study of the *CYP19* rs10046 genotype distribution in Shanghai (Zhang et al., 2009) and Tianjin (Song et al., 2006b) revealed that the *CYP19* rs10046 site polymorphism was related to an individual's susceptibility to developing breast cancer. Moreover, the *CYP19* TC genotype and *CYP19* T allele also had relationship with the development of breast cancer in Han carriers. This study also observed that the risk of breast cancer increased before menopause. *CYP19* with T allele genotype was a strong risk factor, whose risk rate improved the breast cancer significantly. CYP19 with T allele genotype and ER-αT genotype have a combined effect on breast cancer disease. The study on the Shanghai population indicated that CC, CT, and TT genotypes, and carriers of the T allele did not improve the risk of breast cancer significantly. This study revealed that *CYP19* rs10046 genotype frequency, in accordance with the H-W balance laws, and the two group genotype distributions were statistically different. Compared with the CC genotype, the TC genotype provided a protective factor of Uigur breast cancer, which conflicted with the results of the study on the domestic Han population. Furthermore, the relationship was not significant between *CYP19* TT genotype or T allele frequency and the susceptibility to breast cancer in Uigur women.

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