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A single nucleotide polymorphism of the TNRC9 gene associated with breast cancer risk in Chinese Han women

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ABSTRACT. A single nucleotide polymorphism (SNP) in the TNRC9 gene was identified as a breast cancer susceptibility genetic variant in recent genome-wide association studies of women of European ancestry. We investigated whether TNRC9 polymorphisms are associated with risk of breast cancer in Chinese women of the Han nationality. We genotyped the SNPs rs3803662, rs1362548, rs1123428 in 870 women, including 388 breast cancer patients and 482 healthy controls, via the PCR-single strand conformation polymorphism procedure and by sequence detection. We found that the T allele and the TT genotype of the SNP rs38033662 is significantly associated with risk for breast cancer in Chinese Han women; however, no significant association was found for rs1362548 or rs1123428. We

conclude that SNP rs3803662 is a putative risk factor for breast cancer in Chinese Han women.

Key words: Trinucleotide-repeat-containing 9; Breast cancer; SNP; Han nationality: PCR-SSCP

INTRODUCTION

Breast cancer is a multifactorial disease. Environment, genetics, and immunological defects are major factors in the etiology of breast cancer (Parkin et al., 2005). It has been reported that close relatives of patients have an approximately 2-fold increased risk of cancer, and the genetic factors believed to be most crucial have been revealed from twin studies (Peto and Mack, 2000; Pharoah et al., 2004).

TNRC9 (trinucleotide-repeat-containing 9), also termed TOX3, was first identified in a screen for transcripts containing trinucleotide (CAG) repeat expansions (Margolis et al., 1997). It is a gene located at chromosome 16q12, and has been identified as a risk factor for breast cancer through genome-wide association studies (GWAS) (Stacey et al., 2007). There is little further data on TNRC9, but it is known that a single nucleotide polymorphism (SNP) near its 5'-end appears to be strongly associated with breast cancer susceptibility (Easton et al., 2007; Huijts et al., 2007). Several polymorphisms have been identified in the TNRC9 gene: rs3803662, rs12443621, and rs8051542 (Huijts et al., 2007). GWAS indicated that TNRC9 rs3803662 exhibited a stronger association with breast cancer (Easton et al., 2007; Stacey et al., 2007; Thomas et al., 2009). SNP rs3803662 lies 8 kb upstream of TNRC9 and has been observed to be associated with an increased risk of breast cancer in both BRCA1 and BRCA2 mutation carriers (Antoniou et al., 2008). The rs3803662 polymorphism is either restricted to or more strongly associated with estrogen receptor (ER)-positive tumors than those ER-negative cancers (Stacey et al., 2007; Garcia-Closas et al., 2008; Dittmer et al., 2011). Several epidemiological studies have evaluated the association between TNRC9 polymorphisms and breast cancer risk, but the results remain inconclusive according to different ethnic groups (Zheng et al., 2009; Li et al., 2009; Ruiz-Narváez et al., 2010). In the present study, we investigated the rs3803662, rs1362548 and rs1123428 polymorphisms of TNRC9 associated with risk of breast cancer in Chinese Han women.

MATERIAL AND METHODS

Study population

Groups of 388 Chinese women of the Han nationality with breast cancer (patient group) and 482 healthy women of the same ethnic group (control group) were used in this study. All patients were diagnosed with a primary breast tumor in the Cancer Hospital of Hubei Province from May 2011 to June 2012. Clinical characteristics were recorded by tracing case-histories and interviews with all patients. ER and progesterone receptor (PR) status of breast cancers was established with an immunohistochemical (Liang et al., 2010), where the reaction was considered to be positive when the percentage of stained cells was equal to or greater than 10%. The demographic characteristics of patients and healthy controls are shown

in Table 1. The study was approved by the Ethics Committee of the Life Science School of Hubei University. All subjects gave their written informed consent.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood by the conventional proteinase K digestion/phenol-chloroform extraction method. TNRC9 SNPs (rs3803662, rs1362548 and rs1123428) were genotyped by PCR-SSCP and DNA sequencing. The assays were performed in a 5-μL reaction mixture containing 1X TaqMan PCR core reagents, 5 mM $MgCl_2$, 200 nM of each PCR primer, 100 nM MGB probes, 0.5 U AmpliTaq Gold, 0.2 U AmpErase UNG, and 5 ng genomic DNA. Approximately 1% of duplicated samples were used as internal controls. Discrepancies were not observed.

Statistical analysis

All data were analyzed with the SPSS13.0 statistical software (SPSS, Inc., Chicago, IL, USA). Differences between cases and controls based on demographic characteristics were calculated by χ^2 tests (for categorical variables) or the Student *t*-test (for continuous variables). Hardy-Weinberg equilibrium was assessed using a χ^2 test in each group. The allelic and genotypic frequencies were determined by direct counting, and statistical comparison was performed by the χ^2 test with Yates correction or the Fisher exact test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the disease in carriers of specific alleles. When P was less than 0.05, the difference was considered to be statistically significant.

RESULTS

The characteristics of healthy controls and breast cancer patients are shown in Table 1. The age range for the two groups matched quite well (P > 0.6). The age of first live birth, menopausal status, and number of pregnancies showed no significant difference between patient and control groups. The occurrence of breast cancer in first-degree relatives was markedly higher than in the control group (26.55 *vs* 19.29%, P = 0.011). The percentage of breast cancer patients who never breastfed was higher than for the controls, but not statistically significant (39.68 *vs* 33.76%, P = 0.07). ER and PR data showed a positive rate higher than a negative rate (55.93 *vs* 44.07% and 57.47 *vs* 42.53%, respectively).

Allelic frequencies of TNRC9 rs3803662, rs1362548 and rs1123428 between breast cancer patients and controls are summarized in Table 2. The frequency of the T allele of SNP rs3803662 in breast cancer patients was markedly higher than in controls (36 *vs* 31%, P = 0.041, OR = 1.23, 95%CI = 1.01-1.50). Allelic frequencies for the other two SNPs showed no statistical difference (P > 0.05).

Genotype frequencies of polymorphic variants of TNRC9 for patient and control groups are summarized in Table 3. Frequencies of the TT genotype for SNP rs3803662 was significantly different between the patient and control groups (P = 0.013). The TT genotype frequency of rs3803662 in patients was greater than controls (13 *vs* 8%, OR = 1.83, 95%CI = 1.15-2.92), suggesting that the TT genotype at rs3803662 is a breast cancer risk factor. For other genotypes, rs1362548 and rs1123428, patients and controls showed no significant difference (P > 0.05).

Table 1. Characteristics of healthy controls and breast cancer patients.

Variables	Patients (N = 388)	Controls (N = 482)	P value
Age [years range (mean ± SD)]	36-68 (52.23 ± 12.61)	22-65 (51.75 ± 11.87)	0.623[c]
Age at first live birth [year (mean ± SD)][a]	25.62 ± 3.25	23.67 ± 3.43	0.342[c]
Menopausal status			
Premenopausal	184 (47.42%)	223 (46.27%)	0.734[d]
Post-menopausal	204 (52.58%)	259 (53.73%)	
Family history of cancer			
Positive	103 (26.55%)	93 (19.29%)	0.011[d]
Negative	285 (73.45%)	389 (80.71%)	
Pregnancy[b]			
1	69 (17.78%)	97 (20.12%)	0.208[d]
2	110 (28.35%)	150 (31.75%)	
3	98 (25.26%)	93 (19.29%)	
≥4	101 (28.61%)	131 (28.84%)	
Breastfeeding[b]			
Ever	228 (60.32%)	312 (66.24%)	0.075[d]
Never	150 (39.68%)	159 (33.76%)	
ER			
Positive	217 (55.93%)		
Negative	171 (44.07%)		
PR			
Positive	223 (57.47%)		
Negative	165 (42.53%)		

[a]Age at first live birth information for 70 breast cancer cases (95.36%) and 459 controls (95.23%). [b]Pregnancy and breastfeeding were available in 378 breast cancer cases (97.42%) and 471 controls (97.72%). ER = estrogen receptor; PR = progesterone receptor. [c]P value between patients and controls, for the *t*-test. [d]P value between patients and controls, for the χ^2 test.

Table 2. Allele frequencies of polymorphic variants of the *TNRC9* gene in breast cancer patients and healthy controls.

SNP ID	Allele	Allelic frequency		Statistic		OR (95%CI)
		Patients [N (Freq.)]	Controls [N (Freq.)]	χ^2	P value	
rs3803662	T	280 (0.36)	303 (0.31)	4.174	0.041*	1.23 (1.01-1.50)
HWE (P)			0.10			
rs1362548	G	427 (0.55)	550 (0.57)	0.718	0.397	0.92 (0.76-1.11)
HWE (P)			0.14			
rs1123428	T	343 (0.44)	447 (0.46)	0.815	0.367	0.92 (0.76-1.11)
HWE (P)			0.17			

SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium; OR = odds ratios; 95%CI = 95% confidence intervals; N = number of alleles; Freq. = frequency. *P < 0.05.

Table 3. Genotype frequencies of polymorphic variants of the *TNRC9* gene in patients with breast cancer and healthy controls.

SNP sites	Genotype	Patients [N (Freq.)]	Controls [N (Freq.)]	P value	OR (95%CI)
rs3803662	CT	159 (0.41)	217 (0.45)		1
	CT	178 (0.46)	227 (0.47)	0.639	1.07 (0.81-1.42)
	TT	51 (0.13)	38 (0.08)	0.013*	1.83 (1.15-2.92)
rs1362548	CC	77 (0.30)	81 (0.41)		
	CG	195 (0.40)	252 (0.46)	0.27	0.81 (0.57-1.17)
	GG	116 (0.30)	149 (0.31)	0.32	0.82 (0.55-1.22)
rs1123428	AA	130 (0.34)	155 (0.32)		
	AT	173 (0.45)	207 (0.43)	0.98	0.99 (0.73-1.36)
	TT	85 (0.22)	120 (0.25)	0.36	0.85 (0.59-1.21)

SNP = single nucleotide polymorphism; OR = odds ratios; 95%CI = 95% confidence intervals; N = number of alleles; Freq. = frequency. *P < 0.05.

DISCUSSION

Our results demonstrating that the T allele and TT genotype of SNP rs3803662 are a risk factor is consistent with the GWAS mentioned above (Thomas et al., 2009; Zheng et al., 2009). Several epidemiological studies have evaluated the association between TNRC9 polymorphisms and breast cancer risk in Chinese women (Li et al., 2009; Liang et al., 2010); however, the results remain inconclusive, partially because of the possible negligible effect of polymorphism on breast cancer risk in different nationalities of China and the relatively small sample size used in the previously published studies. Our investigation showed that rs3803662 was associated with breast cancer risk in Chinese Han women, while rs1362548 and rs1123428 were unrelated. Whether rs3803662 has linkage disequilibrium with other SNPs in TNRC9 and haplotype associated with breast cancer requires further inquiry.

Little is known about the mechanism of TNRC9 in breast cancer, but some reports suggest its potential to play the role of a transcription factor and it has been implicated in breast cancer metastasis of the bone (Smid et al., 2006). The SNP appears to be strongly associated with breast cancer susceptibility (Huijts et al., 2007; Stacey et al., 2007). Several reports have demonstrated that genetic variants in TNRC9 are associated with risk of ER-positive breast cancer (Liang et al., 2010; Dittmer et al., 2011). In our study, both ER- and PR-positive patients were more susceptible to breast cancer than ER- or PR-negative patients (Table 1). The data supported the hypothesis that TNRC9 contributes to breast cancer occurrence through pathways related to estrogen and/or progesterone.

In conclusion, our results suggest that the T allele and the TT genotype of TNRC9 rs3803662 are significantly correlated with breast cancer risk and that no significant association exists with TNRC9 rs1362548 and rs1123428. Moreover, it is necessary to conduct larger sample studies considering gene-gene and gene-environment interactions.

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Conflicts of interest

The authors declare no conflict of interest.

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