RESEARCH ARTICLE

Single Nucleotide Polymorphisms of DNA Base-excision Repair Genes (APE1, OGG1 and XRCC1) Associated with Breast Cancer Risk in a Chinese Population

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Abstract

Altered DNA repair capacity can result in increased susceptibility to cancer. The base excision repair (BER) pathway effectively removes DNA damage caused by ionizing radiation and reactive oxidative species (ROS). In the current study, we analyzed the possible relation of polymorphisms in BER genes, including 8-oxoguanine DNA glycosylase (OGG1), apurinic/apyrimidinic endonuclease 1 (APE1), and X-ray repair cross-complementing group 1 protein (XRCC1), with breast cancer risk in Chinese Han women. This case-control study examined 194 patients with breast cancer and 245 cancer-free hospitalized control subjects. Single nucleotide polymorphisms (SNPs) of OGG1 (Ser326Cys), XRCC1 (Arg399Gln), and APE1 (Asp148Glu and -141T/G) were genotyped and analyzed for their association with breast cancer risk using multivariate logistic regression models. We found that XRCC1 Arg399Gln was significantly associated with an increased risk of breast cancer. Similarly, the XRCC1 Gln allele was significantly associated with an elevated risk in postmenopausal women and women with a high BMI (≥ 24 kg/m^2). The OGG1 Cys allele provided a significant protective effect against developing cancer in women with a low BMI (< 24 kg/m^2). When analyzing the combined effects of these alleles on the risk of breast cancer, we found that individuals with ≥ 2 adverse genotypes (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser) were at a 2.18-fold increased risk of breast cancer (P = 0.027). In conclusion, our data indicate that Chinese women with the 399Gln allele of XRCC1 have an increased risk of breast cancer, and the combined effects of polymorphisms of BER genes may contribute to tumorigenesis.

Keywords: Base excision repair - single nucleotide polymorphisms - breast cancer - genetic susceptibility

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Introduction

Breast cancer is the most frequently diagnosed cancer in women and the leading cause of cancer death in females worldwide (Jemal et al., 2011). Numerous factors have been associated with the increased risk of breast cancer, including ionizing radiation (IR), heterocyclic aromatic amines, alcohol, reactive oxygen radicals, bulky DNA adducts, oxidized DNA bases, and DNA strand breaks (Hu et al., 2002; Smith et al., 2003; Dumitrescu 2005). Mammalian cells utilize different pathways to repair diverse types of DNA damage, and thus conserve genome stability and integrity. Faulty DNA repair machinery might contribute to insufficient repair of damaged DNA and subsequent genomic instability, which may lead to deletions, amplifications, and/or mutations of crucial genes, leading to breast carcinogenesis (Parshad et al., 1996). However, data from previous studies suggest that proteins encoded by various polymorphic variants of DNA repair genes might vary in their properties and activities, and thus possibly link to individual differences in cancer susceptibility (Goode et al., 2002; Misra et al., 2003).

Identifying an association between single nucleotide polymorphisms (SNPs) in candidate genes and the risk of human cancers has been a popular area of research in molecular cancer epidemiology, and genes involved in DNA repair have been increasingly studied because of their critical roles in maintaining genomic integrity (Hoeijmakers, 2001; Wood et al., 2005). The efficient and potent base excision repair (BER) pathway plays a major role in maintaining integrity of the genome by correcting somatic mutations induced by endogenous free radicals produced during cellular metabolism or by exogenous exposure to chemicals and ionizing radiation (Nock et al., 2006).

Human BER pathway consists of at least 11 DNA damage specific glycosylases and additional core proteins, each with a specialized function. Among these proteins, 8-oxoguanine DNA glycosylase (OGG1), apurinic/apyrimidinic endonuclease 1 (APE1), and X-ray repair cross-complementing group 1 protein (XRCC1) are three key enzymes in this repair pathway (Hung et al., 2005;

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Table 1. Sequences of Primers Used in This Study

Target gene	Sequence of primers	Allele and size of PCR products (bp)
OGG1 Ser326Cys	F1: 50-CAGCCCAGACCCAGTGGACTC-30	For C allele (252 bp)
	R1: 50-TGGCTCCTGAGCATGGCGGG-30	
	F2: 50-CAGTGCCGACCTGCGCCAATG-30	For G allele (194 bp)
	R2: 50-GGTAGTCACAGGGAGGCCCC-30	
XRCC1 Arg399Gln	F1: 50-TCCCTGCGCCGCTGCAGTTTCT-30	For G allele (447 bp)
	R1: 50-TGGCGTGTGAGGCCTTACCTCC-30	
	F2: 50-TCGGCGGCTGCCCTCCCA-30	For A allele (222 bp)
	R2: 50-AGCCCTCTGTGACCTCCCAGGC-30	
APE1-141T/G	F1: 50-CTAACTGCCAGGGACGCCGA-30	For T allele (136 bp)
	R1: 50-ACACTGACTTAAGATTCTAACTA-30	
	F2: 50-ACTGTTTTTTTCCCTCTTGCACAG-30	For G allele (335 bp)
	R2: 50-TGAGCAAAAGAGCAACCCCG-30	
APE1 Asp148Glu	F1: 50-CCTACGGCATAGGTGAGACC-30	For G allele (167 bp)
	R1: 50eTCCTGATCATGCTCCTCC-30	
	F2: 50-TCTGTTTCATTTCTATAGGCGAT-30	For T allele (236 bp)
	R2: 50-GTCAATTTCTTCATGTGCCA-30	

Robertson et al., 2009). BER pathway activity begins with recognition and excision of a damaged base by the specific DNA glycosylase. For example, OGG1 recognizes and removes an oxidized 8-oxoguanine base by releasing the modified base, and thus creating an apurinic/apyrimidinic site. This abasic site is then excised by APE1, leaving behind a 5'-deoxiribose phosphate residue. Subsequently, this residue is removed by the AP-lyase activity of DNA polymerase β, and a correct nucleotide is inserted. Finally, DNA ligase DNA III seals the nick in the DNA strand to restore the integrity of DNA double strand. The XRCC1 gene encodes a protein that acts as a scaffolding protein by interacting with a complex of DNA repair proteins, including poly (ADP-ribose ADP) polymerase, DNA ligase III, and DNA polymerase β, and coordinates the gap-sealing process in the short-batch BER (Petermann et al., 2006; Maynard et al., 2009).

Polymorphic variants of OGG1, XRCC1, and APE1 genes have been reported to be responsible for functional changes at the protein level which may be related to cancer risk (Vodicka et al., 2007; Baute and Depicker, 2008), including nasopharyngeal carcinoma (Li et al., 2013), bladder cancer (Mittal et al., 2012), lung cancer (Osawa et al., 2010), esophageal cancer (Zhai et al., 2009), and cervical cancer (Li et al., 2012). Similarly, epidemiologic studies have examined the association between these inheritable BER pathway variations and the susceptibility to breast cancer; however, the results have been inconclusive. Although the genetic variants OGG1 Ser326Cys, APE1 Asp148Glu, and XRCC1 Arg399Gln in the BER pathway play critical roles in repairing base damage, few studies have investigated the association between breast cancer risk and these genetic polymorphisms in Chinese women. Furthermore, no studies have been conducted to examine the combined and synergistic effects of these 3 genes in Chinese women. To investigate potential differences in relative breast cancer risk by race, and the association between the genetic variants of these three BER genes and breast cancer risk, we conducted a hospital-based study of 194 patients with incident breast cancer and 245 cancer-free control subjects who were frequency-matched by age, age at menarche, age at menopause, menopausal status, pregnancy, body mass index, and family history of cancer.

Materials and Methods

Study Subjects

This case-controlled study included 194 new incident breast cancer patients and 245 cancer-free control subjects. All participants were of Chinese Han nationality. The cancer patients had been histologically diagnosed at the Daping Hospital, Third Military Medical University (Chongqing, China) during January 2007 to December 2010, and did not have a reported previous history of cancer. Control subjects were randomly selected among individuals receiving health examinations at the Health Examination Center of the same hospital during the same period; any of these subjects who had a history of cancer were excluded from the study. The control subjects were frequency-matched with the cancer patients based on age (mean age, age at menarche, age at menopause), family history of cancer, residential area, and nationality. The protocol for this study was approved by the ethics committee of Daping Hospital, and written informed consent was obtained from each participant. A structured questionnaire was administered by well-trained interviewers to collect information on demographic and anthropometric characteristics of the enrolled subjects. For purposes of the questionnaire, a family history of cancer was defined as any self-reported cancer in a first-degree relative, including parents, siblings and children.

Blood Sample Processing

Venous blood samples were collected from the antecubital vein of 194 cancer patients and 245 control subjects, and immediately centrifuged at 2000 rpm (10 min, 4 °C) to remove the serum. Genomic DNA was isolated from peripheral blood leukocytes using a standard phenol-chloroform extraction method (Saxena et al., 2006).

SNPs Selection and Genotyping

Based on the current literature, four common non-synonymous SNPs of BER genes: OGG1 (rs1052133; Ser326Cys; C/G; in exon 7), XRCC1 (rs25487; Arg399Gln; G/A; in exon 10), APE1 (rs1130409; Asp148Glu; T/G; in exon 5) and the promoter polymorphism of APE1: APE1 (rs1760944; -141T/G; in the promoter region) were chosen

Table 2. Distribution of Demographic Characteristics of Breast Cancer Cases and Control Participants

Characteristics	Cases	Controls	*P*-value
Age (%)			
Mean age, n (mean ± SD)	194 (51.4 ± 9.7)	245 (50.9 ± 16.0)	0.31
< 50	84 (43.3%)	118 (48.2%)	
≥ 50	110 (56.7%)	127 (51.8%)	
Age at menarche, n (mean ± SD)	194 (14.2± 1.4)	245 (14.0 ± 1.4)	0.529
Age at menopause, n (mean ± SD)	133 (47.9 ± 3.1)	146 (48.2 ± 2.8)	0.346
Pregnancy			0.824
No	4 (2.1%)	7 (2.8%)	
Yes	190 (97.9%)	238 (97.2%)	
Menopausal status			0.053
Premenopausa	61 (31.4%)	99 (40.4%)	
Postmenopausall	133 (68.6%)	146 (59.6%)	
Body mass index (kg/m^2)	23.33 ± 2.9	23.85 ± 2.2	0.385
< 24	111 (57.2%)	130 (53.1%)	
≥ 24	83 (42.8%)	115 (46.9%)	
Family history of cancer	0.22		
No	179 (92.2%)	233 (95.1%)	
Yes	15 (7.8%)	12 (4.9%)	
Histological type			
Infiltrating ductal arcinoma	186 (96.9%)		
Other carcinoma	8 (3.1%)		

for genotyping. Genetic polymorphisms were detected by PCR-CTPP (PCR with confronting two-pair primers) as previously described (Hamajima, 2001). Primer pairs and product lengths were designed for each allele and the correct allele was identified based on product length. PCR primers were designed based on GenBank reference sequences, and are shown in Table 1.

PCR amplification was performed in a 25-μL mixture volume in glass capillaries. The PCR mixtures contained 2 μL of genomic DNA, 0.5 U of TaqMan SNP Genotyping Assay Mix (40x) (Life Technologies, Grand Island, NY, USA), 12.5 pmol of each primer, 1.5 mM MgCl2, 5 μL of PCR buffer (5x), and 0.30 mM of dNTPs. Reaction conditions included an initial denaturation at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 1 min, annealing at 64°C (OGG1 Ser326Cys) or 66°C (XRCC1 Arg399Gln) or 58 (APE1_141T/G) or 60°C (APE1 Asp148Glu) for 1 min, and elongation at 72°C for 1 min. PCR products were analyzed by agarose gel electrophoresis. Genotype results were regularly confirmed by randomly selecting 5% of the samples for direct measurement of DNA sequencing. The results were reproducible and showed no discrepancies in genotyping.

Statistical Analysis

Breast cancer patients were compared with control subjects for basic demographic variables and genotypes. All statistical analyses were performed with SPSS software (v.16.0 for Windows). Differences in categorical variables among demographic variables (including pregnancy, menopausal status, body mass index (BMI), and family history of cancer) between cancer patients and control subjects were compared using Pearson's chi-squared (χ^2) test. Continuous variables, including age at menarche and age at menopausal were examined using the t-test. Unconditional logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) with adjustments for age, age at menarche, pregnancy, menopausal status, body mass index, and family history of cancer. Homozygous common alleles were used as a reference category. The statistical significance of interactions was determined using the likelihood ratio test, which compared logistic models with and without interaction terms. Statistical analyses of polymorphisms of OGG1, XRCC1, and APE1 genes as exposure variables and breast cancer as the dependent variable were performed using multivariate logistic regression analysis. A P value < 0.05 was used to indicate statistical significance in all studies. Deviations in the frequency of each SNP genotype in control subjects, as determined by the Hardy-Weinberg equilibrium (HWE) formulas, were assessed using the χ^2 test. The number of homozygous adverse genotypes per individual was calculated to evaluate the potential combined effects of OGG1 Ser326Cys, XRCC1 Arg399Gln, and APE1 Asp148Glu on breast cancer risk by unconditional logistic regression analysis.

Table 3. Observed and Expected Genotypic Frequencies of Each SNP in the Control Group

Genes	Genotype	Observed n (%)	Expected n (%)	*p* (HWE)
OGG1	Ser/Ser	45 (18.7%)	39.60 (16.2%)	0.151
Ser326Cys	Ser/Cys	107 (43.7%)	117.80 (48.1%)	
	Cys/Cys	93 (39.6%)	87.60 (35.7%)	
XRCC1	Arg/Arg	137 (55.9%)	135.94 (55.5%)	0.722
Arg399Gln	Arg/Gln	91 (37.1%)	93.12 (38.0%)	
	Gln/Gln	17 (7.0%)	15.94 (6.5%)	
APE1	TT	70 (28.6%)	73.29 (30.0%)	0.396
−141T/G	TG	128 (52.2%)	121.42 (49.6%)	
	GG	47 (19.2%)	50.29 (20.4%)	
APE1	Asp/Asp	80 (32.7%)	73.84 (30.1%)	0.112
Asp148Glu	Asp/Glu	109 (44.5%)	121.32 (49.5%)	
	Glu/Glu	56 (22.8%)	49.84 (20.4%)	

HWE, Hardy-Weinberg equilibrium

Results

Study Subjects

A total of 439 subjects (194 breast cancer patients and 245 cancer-free control subjects) were enrolled in this study. Characteristics of the study population are shown in Table 2. The cancer patients did not significantly differ from control subjects in terms of age, age at menarche, age at menopause, menopausal status, pregnancy, body mass index, and family history of cancer.

Genotype Distribution and Hardy-Weinberg Equilibrium

Table 3 shows the distributions of OGG1 (Ser326Cys), XRCC1 (Arg399Gln), and APE1 (Asp148Glu;-141T/G) genotypes and allele frequencies among control subjects. The frequency of each genotype in the control population was accordant with the Hardy-Weinberg equilibrium (P > 0.05).

Single Genotype Distribution and Breast Cancer Risk

The genotype distributions and allele frequencies for DNA repair gene polymorphisms in breast cancer patients and control subjects are shown in Table 4. We observed

Table 5. Associations Between XRCC1, APEX1, and OGG1 Polymorphisms and Breast Cancer Stratified by BMI and Menopausal Status

Genotype	Body mass index (kg/m^2)							Menopausal status						
	BMI < 24			BMI ≥ 24				Premenopausal women			Postmenopausal women			
	Cases/controls	OR[a] (95% CI)	*P*-value	Cases/controls	OR[a] (95% CI)	*P*-value	*P*-interaction	Cases/controls	OR[a] (95% CI)	*P*-value	Cases/controls	OR[a] (95%CI)	*P*-value	*P*-interaction
OGG1/Ser326Cys							0.117							0.467
Ser/Ser	29/17	1.00 (reference)		13/28	1.00 (reference)			17/17	1.00 (reference)		25/28	1.00 (reference)		
Ser/Cys	48/62	0.426 (0.200~0.905)	0.027*	39/45	1.562 (0.688~3.549)	0.286		23/42	0.587 (0.210~1.640)	0.309	64/65	1.010 (0.511~1.997)	0.976	
Cys/Cys	34/51	0.323 (0.146~0.715)	0.005*	31/42	1.300 (0.557~3.033)	0.543		21/40	0.412 (0.143~1.183)	0.213	44/53	0.834 (0.408~1.703)	0.618	
Ser/Cys+ Cys/Cys	82/113	0.379 (0.186~0.769)	0.007*	70/87	1.438 (0.670~3.087)	0.351		44/82	0.496 (0.194~1.271)	0.144	108/118	0.930 (0.492~1.760)	0.825	
XRCC1/Arg399Gln							0.839							0.852
Arg/Arg	54/71	1.00 (reference)		29/66	1.00 (reference)			27/53	1.00 (reference)		56/84	1.00 (reference)		
Arg/Gln	44/48	1.227 (0.6892.183)	0.487	46/43	2.336 (1.223~4.463)	0.010*		27/38	1.532 (0.667~3.518)	0.314	63/53	1.848 (1.084~3.153)	0.024*	
Gln/Gln	41956	1.293 (0.500~3.344)	0.596	41857	3.099 (0.918~10.461)	0.068		41828	0.771 (0.204~2.914)	0.702	41896	2.575 (0.961~6.896)	0.06	
Arg/Gln+ Gln/Gln	57/57	1.240 (0.721~2.132)	0.437	54/49	2.432 (1.304~4.538)	0.005*		34/46	1.332 (0.608~2.917)	0.473	77/62	1.948 (1.169~3.246)	0.011*	
APE1 −141T/G							0.059							0.118
TT	45/40	1.00 (reference)		19/30	1.00 (reference)			21/29	1.00 (reference)		43/56	1.00 (reference)		
TG	45/66	0.549 (0.300~1.008)	0.053	41/62	1.152 (0.554~2.398)	0.705		23/55	0.636 (0.260~1.557)	0.322	63/65	1.243 (0.694~2.227)	0.464	
GG	21/24	0.621 (0.284~1.355)	0.231	23/23	1.902 (0.792~4.569)	0.151		17/15	2.010 (0.670~6.030)	0.213	27/25	0.806 (0.376~1073)	0.58	
TG+GG	66/90	0.569 (0.322~1.004)	0.052	64/85	1.331 (0.661~2.677)	0.423		40/60	0.903 (0.394~2.069)	0.809	90/90	0.971 (0.558~1.690)	0.918	
APE1/Asp148Glu							0.334							0.317
Asp/Asp	36/44	1.00 (reference)		23/36	1.00 (reference)			19/24	1.00 (reference)		40/41	1.00 (reference)		
Asp/Glu	57/62	1.087 (0.593~1.991)	0.778	46/47	1.562 (0.778~3.135)	0.21		30/44	0.736 (0296~1.831)	0.736	73/73	1.710 (0.972~3.010)	0.063	
Glu/ Glu	18/24	0.947 (0.418~2.148)	0.897	14/32	0.723 (0.306~1.706)	0.459		42004	0.382 (0.130~1.120)	0.08	20/32	1.189 (0.556~2.539)	0.656	
Asp/Glu+Glu/Glu	75/86	1.389 (0.231~8.336)	0.864	60/79	1.228 (0.639~2.363)	0.538		42/75	0.586 (0.250~1.370)	0.217	93/105	1.561 (0.915~2.665)	0.103	

[a]OR adjusted for age, age at menarche, family history of breast cancer, pregnancy and menopausal status/body mass index in subgroup stratified by BMI/ menopausal status. All patients with complete information were included for unconditional logistic regression analysis; *$P < 0.05$

that subjects harboring either heterozygous or homozygous XRCC1 Gln allele showed a significantly increased risk of breast cancer [(OR = 1.529; 95% CI: 1.012~2.310; P = 0.044); (OR = 2.189; 95% CI: 1.063~4.507; P = 0.033), respectively], when compared to subjects harboring homozygous Arg allele, which was used as a reference. The data were adjusted for age, age at menarche, pregnancy, menopausal status, body mass index, and family history of cancer. A further analysis using the chi-square test demonstrated that when compared with the Arg allele, a significant susceptibility to breast cancer risk was associated with the XRCC1 Gln allele, (OR = 1.403; 95% CI: 1.044~1.886; P = 0.025). The OGG1 Cys/Cys genotype and APE1 Glu/Glu genotype both showed protective effects against developing breast cancer (OR = 0.693 and OR = 0.78, respectively), though the effects were not statistically significant. Also, there was no statistically significant difference in distribution of the -141T/G polymorphism of the APE1 gene between patients and control subjects.

A subgroup analysis stratified by body mass index (BMI) and menopausal status (Table 5) showed no statistically significant interaction between body mass index or menopausal status and the four BER gene loci polymorphisms. The OGG1 Ser/Cys and OGG1 Cys/Cys genotypes showed a significant protective effect against developing breast cancer for women with a low BMI (< 24 kg/m^2), (OR = 0.426, 95% CI 0.200~0.905, P = 0.027, and OR = 0.323, 95% CI 0.146~0.715, P = 0.005, respectively). Moreover, we found that among postmenopausal women, those with the XRCC1 Arg/Gln+ Gln/Gln allele were at a significantly increased risk of breast cancer (OR = 2.432; 95% IC,1.304~4.538;P = 0.005). The increased risk also was observed in women with a high BMI (≥ 24 kg/m^2) and allele XRCC1 Arg/Gln+ Gln/Gln (OR = 1.948; 95% IC 1.169~3.246; P = 0.011). The APE1 -141T/G polymorphism TG+GG showed a protective effect (OR = 0.569) in women with a low BMI (< 24 kg/m^2). However, the effect was not statistically significant. Similarly, no statistically significant differences were found in genotype or allele distributions of APE1 Asp148Glu between cancer patients and control subjects in a subgroup analysis.

When analyzing the incidence of different polymorphisms in the study population, we found more than one gene variant in a large number of individuals. Several studies have verified a higher susceptibility to cancer and

Table 4. Distribution of Genotypes and Odds Ratios (OR) Determined for All Breast Cancer Cases and Controls

Polymorphisms			Case n (%)	Control n (%)	Association OR[a] (95% CI)	*P*
OGG1	Genotype	Ser/Ser	42 (21.6%)	45 (18.7%)	1.00 (reference)	
Ser326Cys		Ser/Cys	87 (44.8%)	107 (43.7%)	0.823 (0.488~1.389)	0.465
		Cys/Cys	65 (33.6%)	93 (39.6%)	0.693 (0.402~1.195)	0.187
		Ser/Cys+ Cys/Cys	152 (78.4%)	200 (83.3%)	0.763 (0.469~1.240)	0.275
	Allele	Ser	171 (44.1%)	197 (40.2%)	1.00 (reference)	
		Cys	217 (55.9%)	293 (59.8%)	0.853 (0.651~1.117)	0.249
XRCC1	Genotype	Arg/Arg	83 (42.9%)	137 (55.9%)	1.00 (reference)	
Arg399Gln		Arg/Gln	90 (46.4%)	91 (37.1%)	1.529 (1.012~2.310)	0.044*
		Gln/Gln	21 (10.7%)	17 (7.0%)	2.189 (1.063~4.507)	0.033*
		Arg/Gln+ Gln/Gln	111 (57.1%)	108 (44.1%)	1.672 (1.129~2.477)	0.010*
	Allele	Arg	256 (66.0%)	365 (74.5%)	1.00 (reference)	
		Gln	132 (34.0%)	125 (25.5%)	1.506 (1.124~2.017)	0.006*
APE1	Genotype	TT	64 (33.0%)	70 (28.6%)	1.00 (reference)	
-141T/G		TG	86 (44.3%)	128 (52.2%)	0.729 (0.466~1.140)	0.166
		GG	44 (22.7%)	47 (19.2%)	1.066 (0.613~1.613)	0.822
		TG+GG	130 (67.0%)	175 (71.4%)	0.851 (0.536~1.241)	0.341
	Allele	T	214 (55.2%)	268 (54.7%)	1.00 (reference)	
		G	174 (44.8%)	222 (45.3%)	0.982 (0.751~1.283)	0.892
APE1	Genotype	Asp/Asp	59 (30.4%)	80 (32.7%)	1.00 (reference)	
Asp148Glu		Asp/Glu	103 (53.1%)	109 (44.5%)	1.274 (0.817~1.986)	0.285
		Glu/ Glu	32 (16.5%)	56 (22.8%)	0.781 (0.443~1.379)	0.394
		Asp/Glu+Glu/Glu	135 (69.6%)	165 (67.3%)	1.111 (0.730~1.690)	0.623
	Allele	Asp	221 (57.0%)	269 (54.9%)	1.00 (reference)	
		Glu	167 (43.0%)	221 (45.1%)	0.920 (0.703~1.203)	0.541

[a]OR adjusted for age, body mass index, age at menarche, family history of breast cancer, pregnancy and menopausal status. All patients with complete information were included for unconditional logistic regression analysis; *$P < 0.05$

Table 6. Breast Cancer Risk in Individuals Homozygous for More than One Risk Genotype: Joint Effects of Variants in Base Excision Repair (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser)

Total number of	Cases	Controls	Adjusted odds ratio[a]		
risk genotypes	n (%)	n (%)	OR	95% IC	*P**
0	99 (51.0%)	122 (49.8%)	1.000	(reference)	
1	69 (35.6%)	105 (42.9%)	0.767	0.505~1.165	0.214
2	25 (12.9%)	17 (6.9%)	2.206	1.091~4.468	0.028*
2 or 3	26 (13.4%)	18 (7.3%)	2.183	1.095~4.353	0.027*

Note: Accumulation of adverse alleles that indicated a trend for breast cancer risk when tested separately: XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser; [a]OR adjusted for age, body mass index, age at menarche, family history of breast cancer, pregnancy and menopausal status. All patients with complete information were included for unconditional logistic regression analysis; *$P < 0.05$

cancer recurrence with increasing numbers of putative risk alleles (Smith et al., 2003; Smith et al., 2008; Li et al., 2011). Therefore, we also considered whether gene–gene interactions may increase an individual's susceptibility to cancer. The incidence of the three genotypes in which a single polymorphism may increase the risk of cancer (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser) is shown in Table 6. Because individuals rarely contain all three of these risk genotypes, individuals with two or three risk alleles were combined into a single group. In our study, we found that breast cancer was associated with pair-wise combinations of these three alleles (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser), with an OR of 2.183 (95% CI = 1.095~4.353).

Discussion

Breast cancer is one of the most commonly diagnosed cancer and the leading cause of cancer death in females globally. Increasing evidence suggests that insufficient or faulty repair of DNA damage plays an important role in the carcinogenesis of breast cancer (Parshad et al., 1996; Jyothish et al., 1998). Several well established risk factors, including UV light, smoking, dietary factors, exposure to reactive oxygen species and endogenous or exogenous estrogens are thought to be associated with DNA damage. The BER pathway consists of a series of coordinated sequential reactions to recognize and dispose of damage resulting from reactive oxygen species, hydroxylation reactions, and other cellular processes (Krokan et al., 2000; Hung et al., 2005; Hoeijmakers, 2007). Accordingly, SNPs in key repair genes of the BER pathway may impair DNA repair capacity, which may have a subsequent impact on cancer susceptibility and occurrence. In this study we investigated the possible relation between sequence variants in four BER gene loci (OGG1 Ser326Cys, XRCC1 Arg399Gln, APE1 Asp148Glu, and APE1 -141T/G) and breast cancer risk. We found that Chinese women with the XRCC1 399Gln allele have an increased risk of breast cancer, while the OGG1 326Cys allele confers a significant protective effect against breast cancer in women with a low BMI (< 24 kg/m^2). When analyzing the effect of the gene–gene interactions on breast cancer susceptibility, we found that breast cancer susceptibility was associated with at least two risk genotypes (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser). To the best of our knowledge, this is the first case-controlled study to examine the association between the three BER SNPs and the risk of breast cancer in Chinese women.

The human 8-oxoguanine DNA glycosylase (OGG1) gene is located on chromosome 3p26, in a region that frequently shows a loss of heterozygosity in several human cancers. The product of this gene (8-oxoguanine DNA glycosylase) is a key enzyme involved in removal of 8-oxodeoxyguanosine, which is one of the most common substances produced by oxidative stress, and is highly mutagenic (Rossner et al., 2006; Tudek, 2007). A meta-analysis of 4,963 breast cancer cases and 4,776 control subjects on the role of OGG1 326Cys in breast cancer showed a significant protective effect against breast cancer in European women. However, no significant association between the OGG1 326Cys allele and breast cancer was found among Asian women (Yuan et al., 2010). Additionally, another meta-analysis of OGG1 Ser326Cys, which included data from 11 studies, also failed to observe an association between OGG1 Ser326Cys and breast cancer risk in the in European or Asian subjects or in an analysis stratified by ethnicity, source of control subjects, and menopausal status (Gu et al., 2010). We found that OGG1 Ser326Cys conferred a significant protective effect against breast cancer among women having a low BMI (< 24 kg/m^2) and the OGG1 Ser/Cys genotype (P = 0.027) or OGG1 Cys/Cys genotype (P = 0.005). The different findings in these studies may be related to different genetic backgrounds of the subjects and the heterogeneous nature of exposure to breast carcinogens within the study populations. Due to the relatively small sample size and limited demographic characteristics of our study population, the possibility that our results may be related to internal and external exposure to risk factors requires further verification.

The gene for X-ray repair cross-complementing group 1 (XRCC1) has 17 exons which span ~31.9 kb, and is located at chromosome 19q13.2. While the protein encoded by XRCC1 has no known enzymatic activity, it is thought to act as a scaffold protein which coordinates the activities of other proteins such as DNA polymerase β, DNA ligase III, and poly (ADP-ribose) polymerase (PARP), which function at the site of DNA damage (Campalans et al., 2005) by recognizing and binding to single-strand breaks (Dalhus et al., 2009). The XRCC1 Arg399Gln polymorphism is located within the XRCC1 BRCA1 carboxyl-terminal domain (BRCT I) and is suggested to affect protein structure and function. In the population of Chinese subjects, we found that individuals who were either the heterozygous or homozygous 399 codon of XRCC1Gln allele were at a significantly increased risk of breast cancer compared with those harboring the XRCC1-399 Arg allele (P = 0.044; P = 0.033). Furthermore, an analysis using the chi-square test showed a significant susceptibility to breast cancer was associated with the XRCC1 Gln allele, compared to the Arg allele (P = 0.025). A woman's breast cancer risk was also shown to be associated with postmenopausal status and high BMI (≥ 24 kg/m^2). In agreement with our findings, a meta-analysis of XRCC1 Arg399Gln conducted using 31 studies, which included 10,465 breast cancer cases and 10,888 control subjects, suggested an increased breast cancer risk with a recessive effect for the Arg399Gln variant in an Asian population (Gln/Gln vs. Arg/Arg+Arg/Gln: OR = 1.59) (Li et al., 2009). Similarly, another meta-analysis of XRCC1 polymorphisms and their association with breast cancer risk found that the 399Gln allele might act as a recessive allele in its association with breast cancer risk (Saadat and Ansari-Lari, 2009). In addition, this polymorphism has been associated with an increased risk of breast cancer in Caucasian (Roberts et al., 2011), Iranian (Saadat et al., 2008), and Portuguese (Silva et al., 2007) postmenopausal women.

The APE1 gene consists of five exons and four introns, has a 2.21-kb span, is located at chromosome 14q11.2-q12, and encodes a 317 amino acids protein. This protein is the rate-limiting enzyme in the BER pathway (Hoeijmakers, 2001). In addition to its role in DNA repair, APE1/Ref-1 also functions as a redox activator of numerous cellular transcription factors that are thought to be important in carcinogenesis, including AP-1, NF-kB, Myb, HIF-1a, HLF, PAX, and p53 (Ando et al., 2008). Polymorphisms in a promoter region can influence interactions between transcriptional factors and their ability to recognize DNA sequences in a promoter region, and thus affect gene expression. To our knowledge, no studies have yet been conducted to examine a possible link between the APE1-141T/G promoter polymorphism and the risk of breast cancer. In current study, we failed to find an association between the APE1-141T/G promoter polymorphism and breast cancer risk in Chinese Han women. However, other recent studies have investigated the role of this polymorphism in other cancers. Li et al (Li et al., 2011) reported that individuals homozygous for the variant APE-141GG were somewhat protected against lung cancer overall (OR = 0.62), and were particularly protected against lung adenocarcinoma (OR = 0.65). Additionally, a study in glioblastoma showed that individuals homozygous for the 141GG genotype exhibited a 46% reduced risk of glioblastoma compared to individuals who were 141TT homozygous (Zhou et al., 2011). APE1 Asp148Glu variants are the most common APE1 polymorphisms identified in the general population that result in a single amino acid substitution (Hung et al., 2005). Zhang et al. (2006) found no association between the APE1 Asp148Glu variant and breast cancer risk among non-Hispanic white Americans, whereas Suleeporn et al. (2008) reported a significant protective effect of the APE1 148Glu allele in Thai women. Subsequently, a meta-analysis of the APE1 Asp148Glu polymorphism , which included 5 studies with a total of 2,539 breast cancer patients and 2,572 control subjects, showed no obvious association between APE1 148Glu and breast cancer (Wu et al., 2012). Consistent with this result, we also found no significant association between APE1 Asp148Glu polymorphisms and breast cancer in our study.

When more than one genetic variant exist in an individual's BER related genes, it is possible that the total effect of these variants have a significant impact on DNA repair activity and breast cancer risk (Mohrenweiser et al., 2003; Smith et al., 2003). In our study, we analyzed the impact of gene–gene interactions on breast cancer susceptibility, and found that a high risk of breast cancer was associated with at least two risk genotypes (XRCC1 399Gln, APE1 148Asp, and OGG1 326Ser)

with OR values of 2.183 (95% CI = 1.095~4.353). This finding suggests that the interaction between these two genotypes in the BER pathway might contribute to a higher susceptibility to cancer. It should be noted that we currently lack a full understanding of how polymorphisms affect gene function, and a comprehensive analysis of polymorphisms in all known BER genes is needed to understand the various roles of the BER genes and their impact on breast cancer risk in more detail.

In conclusion, our study examined the possible correlation between breast cancer risk and polymorphisms of three DNA repair genes (OGG1, XRCC1, and APE1) involved in BER pathways. The results suggest that the XRCC1 399Gln allele is significantly associated with an increased risk for breast cancer in postmenopausal women and women with a high BMI (> 24 kg/m^2). Additionally, certain gene–gene interactions may also significantly increase the risk of breast cancer.

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References

Ando K, Hirao S, Kabe Y, et al (2008). A new APE1/Ref-1-dependent pathway leading to reduction of NF-kappaB and AP-1, and activation of their DNA-binding activity. *Nucleic Acids Res*, **36**, 4327-36.

Baute J, Depicker A (2008). Base excision repair and its role in maintaining genome stability. *Crit Rev Biochem Mol Biol*, **43**, 239-76.

Campalans A, Marsin S, Nakabeppu Y, et al (2005). XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. *DNA Repair (Amst)*., **4**, 826-35.

Dalhus B, Laerdahl JK, Backe PH, Bjoras M (2009). DNA base repair--recognition and initiation of catalysis. *FEMS Microbiol Rev*, **33**, 1044-78.

Dumitrescu RG, Cotarla I (2005). Understanding breast cancer risk- where do we stand in 2005? *J Cell Mol Med*, **9**, 208-21.

Goode EL, Ulrich CM, Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev*, **11**, 1513-30.

Gu D, Wang M, Zhang Z, Chen J (2010). Lack of association between the hOGG1 Ser326Cys polymorphism and breast cancer risk: evidence from 11 case-control studies. *Breast Cancer Res Treat*, **122**, 527-31.

Hamajima N (2001). PCR-CTPP: a new genotyping technique in the era of genetic epidemiology. Expert Rev. Mol. Diagn.

Hoeijmakers JH (2001). Genome maintenance mechanisms for preventing cancer. *Nature*, **411**, 366-74.

Hoeijmakers JHJ (2007). Genome maintenance mechanisms are critical for preventing cancer as well as other aging-associated diseases. *Mech Ageing Dev*, **128**, 460-2.

Hu JJ, Smith TR, Miller MS, et al (2002). Genetic regulation of ionizing radiation sensitivity and breast cancer risk. *Environ Mol Mutagen*, **39**, 208-15.

Hung RJ, Hall J, Brennan P, Boffetta P (2005). Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol*, **162**, 925-42.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Jyothish B, Ankathil R, Chandini R, et al (1998). DNA repair proficiency: a potential marker for identification of high risk members in breast cancer families. *Cancer Lett*, **124**, 9-13.

Krokan HE, Nilsen H, Skorpen F, et al (2000). Base excision repair of DNA in mammalian cells. *FEBS Lett*, **476**, 73-7.

Li Q, J.-M. Wang, Y. Peng, et al (2013). Association of DNA base-excision repair XRCC1, OGG1 and APE1 gene polymorphisms with nasopharyngeal carcinoma susceptibility in a Chinese population. *Asian Pac J Cancer Prev*, **14**, 5145-51.

Li H, Ha TC, Tai BC (2009). XRCC1 gene polymorphisms and breast cancer risk in different populations: a meta-analysis. *Breast*, **18**, 183-91.

Li Y, Liu F, Tan SQ, et al (2012). X-ray repair cross-complementing group 1 (XRCC1). genetic polymorphisms and cervical cancer risk: a huge systematic review and meta-analysis. *PLoS One*, **7**, e44441.

Li Z, Guan W, Li MX, et al (2011). Genetic polymorphism of DNA base-excision repair genes (APE1, OGG1 and XRCC1). and their correlation with risk of lung cancer in a Chinese population. *Arch Med Res*, **42**, 226-34.

Maynard S, Schurman SH, Harboe C, et al (2009). Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis*, **30**, 2-10.

Misra RR, Ratnasinghe D, Tangrea JA, et al (2003). Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer amongmale smokers in Finland. *Cancer Letters*, **191**, 171-8.

Mittal RD, Mandal RK, Gangwar R (2012). Base excision repair pathway genes polymorphism in prostate and bladder cancer risk in North Indian population. *Mech Ageing Dev*, **133**, 127-32.

Mohrenweiser HW, Wilson DM, Jones IM (2003). Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res-Fund Mol M*, **526**, 93-125.

Nock NL, Cicek MS, Li L, et al (2006). Polymorphisms in estrogen bioactivation, detoxification and oxidative DNA base excision repair genes and prostate cancer risk. *Carcinogenesis*, **27**, 1842-8.

Osawa K, Miyaishi A, Uchino K, et al (2010). APEX1 Asp148Glu gene polymorphism is a risk factor for lung cancer in relation to smoking in Japanese. *Asian Pac J Cancer Prev*, **11**, 1181-6.

Parshad R, Price FM, Bohr VA, et al (1996). Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br J Cancer*, **74**, 1-5.

Petermann E, Keil C, Oei SL (2006). Roles of DNA ligase III and XRCC1 in regulating the switch between short patch and long patch BER. *DNA Repair*, **5**, 544-55.

Roberts MR, Shields PG, Ambrosone CB, et al (2011). Single-nucleotide polymorphisms in DNA repair genes and association with breast cancer risk in the web study. *Carcinogenesis*, **32**, 1223-30.

Robertson AB, Klungland A, Rognes T, Leiros I (2009). DNA repair in mammalian cells: Base excision repair: the long and short of it. *Cell Mol Life Sci*, **66**, 981-93.

Rossner P, Jr., Terry MB, Gammon MD, et al (2006). OGG1 polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **15**, 811-5.

Saadat M, Ansari-Lari M (2009). Polymorphism of XRCC1 (at codon 399). and susceptibility to breast cancer, a meta-analysis of the literatures. *Breast Cancer Res Treat*, **115**, 137-44.

Saadat M, Kohan L, Omidvari S (2008). Genetic polymorphisms of XRCC1 (codon 399). and susceptibility to breast cancer in Iranian women, a case-control study. *Breast Cancer Res Treat*, **111**, 549-53.

Sangrajrang S, Schmezer P, Burkholder I, et al (2008). Polymorphisms in three base excision repair genes and breast cancer risk in Thai women. *Breast Cancer Res Treat*, **111**, 279-88.

Saxena S, Chakraborty A, Kaushal M, et al (2006). Contribution of germline BRCA1 and BRCA2 sequence alterations to breast cancer in Northern India. *BMC Med Genet*, **7**, 75.

Silva SN, Moita R, Azevedo AP, et al (2007). Menopausal age and XRCC1 gene polymorphisms: role in breast cancer risk. *Cancer Detect Prev*, **31**, 303-9.

Smith TR, Levine EA, Freimanis RI, et al (2008). Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. *Carcinogenesis*, **29**, 2132-8.

Smith TR, Levine EA, Perrier ND, et al (2003). DNA-repair genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **12**, 1200-4.

Smith TR, Miller MS, Lohman K, et al (2003). Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Letters*, **190**, 183-90.

Tudek B (2007). Base excision repair modulation as a risk factor for human cancers. *Mol Aspects Med*, **28**, 258-75.

Vodicka P, Stetina R, Polakova V, et al (2007). Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects. *Carcinogenesis*, **28**, 657-64.

Wood RD, Mitchell M, Lindahl T (2005). Human DNA repair genes, 2005. *Mutat Res*, **577**, 275-83.

Wu B, Liu HL, Zhang S, et al (2012). Lack of an Association between Two BER Gene Polymorphisms and Breast Cancer Risk: A Meta-Analysis. *PLOS ONE*, **7**.

Yuan W, Xu L, Feng Y, et al (2010). The hOGG1 Ser326Cys polymorphism and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat*, **122**, 835-42.

Zhai XD, Mo YN, Xue XQ, et al (2009). XRCC1 codon 280 and ERCC2 codon 751 polymorphisms and risk of esophageal squamous cell carcinoma in a Chinese population. *Bull Cancer*, **96**, E61-5.

Zhang Y, Newcomb PA, Egan KM, et al (2006). Genetic polymorphisms in base-excision repair pathway genes and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*, **15**, 353-8.

Zhou K, Hu D, Lu J, et al (2011). A genetic variant in the APE1/Ref-1 gene promoter -141T/G may modulate risk of glioblastoma in a Chinese Han population. *BMC Cancer*, **11**, 104.