Genetic Polymorphisms of Selected DNA Repair Genes, Estrogen and Progesterone Receptor Status, and Breast Cancer Risk

Kyoung-Mu Lee,[1,4] Ji-Yeob Choi,[1] Changwon Kang,[6] Changsoo Paul Kang,[6] Sue Kyung Park,[7] Hyunmi Cho,[6] Dae-Yeon Cho,[8] Keun-Young Yoo,[1] Dong-Young Noh,[2] Sei-Hyun Ahn,[5] Chung-Gyu Park,[3] Qingyi Wei,[9] and Daehee Kang[1]

Abstract

Purpose: Genetic polymorphisms of DNA repair genes seem to determine the DNA repair capacity, which in turn may affect the risk of breast cancer. To evaluate the role of genetic polymorphisms of DNA repair genes in breast cancer, we conducted a hospital-based case-control study of Korean women.

Experimental Design: We included 872 incident breast cancer cases and 671 controls recruited from several teaching hospitals in Seoul from 1995 to 2002. Twelve loci of selected DNA repair genes were genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (*XRCC2* $Arg^{188}His$, *XRCC4* 921G > T, *XRCC6* 1796G > T, *LIG4* 1977T/C, *RAD51* 135G > C, 172G > T, *RAD52* 2259C > T, *LIG1* 551A > C, *ERCC1* 8092A > C, 354C > T, *hMLH1* −93G > A, and $Ile^{219}Val$).

Results: We found that the *RAD52* 2259 CT or TT, *hMLH1* −93 GG, and *ERCC1* 8092 AA genotypes were associated with breast cancer risk after adjustment for known risk factors [odds ratio (OR), 1.33; 95% confidence interval (95% CI), 1.02-1.75; OR, 1.31; 95% CI, 0.99-1.74; and OR, 0.58; 95% CI, 0.38-0.89, respectively]. When Bonferroni's method was used to correct for multiple comparisons for nine polymorphisms with $P = 0.005$, all of these associations were not significant. However, the effects of *RAD52* 2259 CT or TT and *ERCC1* 354 CT or TT genotypes were more evident for the estrogen/progesterone receptor – negative cases (OR, 2.03; 95% CI, 1.24-3.34 and OR, 1.99; 95% CI, 1.35-2.94, respectively).

Conclusion: Our findings suggest that genetic polymorphisms of *RAD52, ERCC1*, and *hMLH1* may be associated with breast cancer risk in Korean women.

Exogenous carcinogens and endogenous oxygen species can induce DNA damage and genomic instability that may lead to carcinogenesis through activation of oncogenes and inactivation of tumor suppressor genes (1). Thus, DNA repair is expected to play a role in maintaining genomic stability (2). Because defects in DNA repair genes involved in double strand break repair (DSBR) such as *BRCA1* and *BRCA2* are implicated in familial breast cancer, overall DNA repair capacity may have an effect on the risk of sporadic breast cancer as well (3). Genetic polymorphisms of DNA repair genes seem to determine the DNA repair capacity (4), which in turn may affect the risk of breast cancer (5, 6).

A number of studies have evaluated the association between single nucleotide polymorphisms (SNP) of DNA repair genes and breast cancer risk, but only a few studies evaluated the association between genetic variants in DSBR genes and breast cancer risk (7–10). In those studies, SNPs of DSBR genes such as *XRCC2*, *XRCC4*, *XRCC6*, and *LIG4* have been reported to be associated with risk of breast cancer. Fu et al. (8) found that the combined genotypes of nonhomologous end-joining DSBR genes were associated with an elevated risk of breast cancer in Taiwanese women. Another study suggested that there is an interaction between polymorphisms of DNA repair genes and family history of breast cancer or plasma α-carotene level in the etiology of breast cancer (9).

Just as the DSBR genes may play an important role in the etiology of breast cancer, other DNA repair genes may also be involved in the development of breast cancer. Therefore, a large number of SNPs of these genes remain to be evaluated for their association with risk of breast cancer. To date, no study has reported the association between genetic polymorphisms of

Authors' Affiliations: Departments of [1]Preventive Medicine, [2]General Surgery, Cancer Research Institute, and [3]Microbiology and Immunology, Seoul National University College of Medicine; [4]Institute of Environmental Medicine, SNUMRC; [5]Department of Surgery, Ulsan University College of Medicine, Seoul, Korea; [6]Department of Biological Sciences, KAIST, Daejeon, Korea; [7]Department of Preventive Medicine, Konkuk University College of Medicine, Chuncheongbuk-Do, Korea; [8]Clinical Research Institute, Labgenomics Co., Ltd., Doosan Engineering Center, Kyungki-Do, Korea; and [9]Department of Epidemiology, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas

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Requests for reprints: Daehee Kang, Department of Preventive Medicine, Seoul National University College of Medicine, 28 Yongon-Dong Chongno-Gu, Seoul 110-799, Korea. Phone: 82-2-740-8326; Fax: 82-2-747-4830; E-mail: dhkang@snu.ac.kr.

LIG1, *ERCC1*, and *hMLH1* and breast cancer risk. Therefore, we extended our previous study on the association between genetic polymorphisms of *XRCC1* and *hOGG1* and breast cancer in Korean women (11, 12). In this study, we investigated the associations between additional 12 SNPs of six selected DSBR genes (i.e., *XRCC4*, *XRCC6*, *LIG4*, *XRCC2*, *RAD51*, and *RAD52*), one base excision repair gene (*LIG1*), one nucleotide excision repair gene (*ERCC1*), and one mismatch repair gene (*hMLH1*) and breast cancer risk in a hospital-based case-control study of a Korean population. A total 12 polymorphic sites in these nine genes were selected based on the consideration of their relatively high allele frequencies (i.e., at least 5% of the variant allele containing genotypes) and previous association studies conducted in Caucasian populations (6, 7, 10, 13–17).

Materials and Methods

Study subjects

Histologically confirmed incident breast cancer cases (n = 1,152) and controls (n = 1,057) were recruited from three teaching hospitals in Seoul between 1995 and 2002. Approximately 20% of cases and 14% of controls approached were excluded from the final study groups because of refusal to participate, failure to interview, and no blood collection. Selected characteristics (e.g., age and education) were not different between study participants and patients who refused to participate in this study. Those with present or previous history of cancer, amenorrhea, previous history of hysterectomy, oophorectomy, and hormone-related diseases such as thyroid problems were excluded from cases and controls. Benign breast tumor, other breast diseases (e.g.,mastitis, benign calcification, etc.), and other systemic problems like chronic liver diseases were also excluded from the controls.

After additional exclusion of the subjects without DNA samples, 872 cases and 671 controls were included in the final analysis. The main diseases of final control subjects were infection or stone of gall bladder/bile duct (42%), acute appendicitis (31%), hemorrhoid (11%), and the others (16%).

Informed consents were obtained at the time of blood drawing. The study design was approved by the Committee on Human Research of Seoul National University Hospital. Information on demographic characteristics; education; marital status; family history of breast cancer in first- and second-degree relatives; reproductive factors; menstruation; and lifestyles including alcohol consumption, smoking, and diet was collected using a questionnaire given by trained interviewers.

Genotyping

DNA was isolated by using the standard methods from blood drawn into 10-mL heparinized tubes and stored at −20°C until genotyping. Selected genetic polymorphisms of the DNA repair genes were genotyped by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (*XRCC2* $Arg^{188}His$, *XRCC4* 921G > T, *XRCC6* 1796G > T, *LIG4* 1977T/C, *RAD51* 135G > C, 172G > T, *RAD52* 2259C > T, *LIG1* 551A > C, *ERCC1* 8092A > C, 354C > T, *hMLH1* −93G > A, and $Ile^{219}Val$).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Multiplex PCR primers and optimized primer concentrations are listed in Appendix A. The PCR reactions were done in a volume of 5 μL containing 1× PCR buffer (TAKARA, Tokyo, Japan), 1.5 to 2.5 mmol/L $MgCl_2$, 0.2 mmol/L each deoxynucleotide triphosphate, 0.1 unit HotStar Taq Polymerase (Qiagen GmbH, Hilden, Germany), primers, and 1.0 to 4.0 ng of genomic DNA. The reaction profile consisted of denaturation at 95°C for 15 minutes followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 3 minutes.

Following the PCR, unincorporated deoxynucleotide triphosphates were removed by adding 0.3 unit of shrimp alkaline phosphatase and incubating for 20 minutes at 37°C followed by 5 minutes at 85°C for enzyme inactivation. The primer extension was started at 94°C for 2 minutes followed by 55 cycles of 94°C for 5 seconds, 52°C for 5 seconds, and 72°C for 5 seconds. The total volume of each reaction was 9 μL, and reaction mixture contained hME (homogeneous mass-extend) enzyme (Thermosequenase; Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), appropriate termination mix, and extension primers (see the Appendix A). After desalting of the reaction products with SpectroCLEAN (Sequenom, Inc., San Diego, CA), samples were dispensed on 384-well SpectroCHIP (Sequenom), using SpectroJET (Sequenom). The SpectroCHIPs were analyzed in the fully automated mode with the MALDI-TOF MassARRAY system (Bruker-Sequenom, San Diego, CA).

For quality control in genotyping, randomly selected 50 samples were genotyped and resequenced by oligonucleotide sequencing method. No discrepancy was found between MALDI-TOF genotyping and sequencing method.

Estrogen and progesterone receptor status

Immunohistochemical tests were done to determine expression levels of estrogen receptor (ER) and progesterone receptor (PR) status of the tumors from 544 cases. The immunohistochemical tests were done on paraffin blocks from tumor tissues fixed in 10% neutral buffered formalin. All immunohistochemical tests were carried out immediately after surgery for each case. All slides were reviewed by a pathologist without knowledge of demographics or treatment response. The primary antibodies for ER (DAKO, Glostrup, Denmark) and PR (DAKO) have been previously characterized (18). A cutoff value of 10% or more positively stained cells of the total cells per 10 high-power fields was used to determine ER and PR expression. The proportions of ER negative (ER^-) and PR negative (PR^-) were 38.6% and 54.0%, respectively.

Statistical analyses

The differences in mean age and body mass index were evaluated by Student's t test. The risk of breast cancer was estimated as odds ratios (OR) and 95% confidence intervals (95% CI) by unconditional logistic regression model.

Hardy-Weinberg equilibrium test was done for the genotype distribution in the controls for evaluating possible selection bias and genotyping errors. In multivariate analyses, the ORs for genotypes were adjusted for age, education, family history of breast cancer in first- or second-degree relatives, parity, and age at first full-term pregnancy, alcohol consumption, and smoking. To increase the statistical power based on findings of dominant or recessive model, three genotypes were grouped into two categories in subsequent analyses such as stratified analysis by menopausal status (i.e., premenopausal and postmenopausal) and the analysis with polychotomous model according to sex hormone receptor status (i.e., ER^+/PR^+, ER^-/PR^+ or $ER^+/ER^+/PR^-$, and ER^-/PR^-). The heterogeneity of the genotype distributions among the subgroups of cases according to ER and PR status was tested by the difference of polychotomous ORs. The possible interactions between genotypes and other risk factors (i.e., alcohol consumption, smoking, age at first full term pregnancy and parity, and family history of breast cancer in first- and second-degree relatives) in breast cancer development were evaluated by the likelihood ratio test. The difference of two −2LogL values of logistic models with and without interaction terms was referred to the tables of χ^2 using one degree of freedom.

Results

As shown in Table 1, elevated breast cancer risk was associated with education of at or over high school (OR, 1.28; 95% CI,

Table 1. Selected characteristics for 872 breast cancer cases and 671 control subjects

	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	Adjusted OR (95% CI)*
Age (mean ± SD)	46.7 (±9.86)	45.7 (±12.4)	0.09	
Body mass index (mean ± SD)	23.0 (±3.17)	22.8 (±2.85)	0.16	
Education				
Under high school	273 (31.4)	246 (37.3)	1.00 (reference)	1.00 (reference)
At and over high school	597 (68.6)	413 (62.7)	1.30 (1.05-1.61)	1.28 (0.98-1.67)
Age at FFTP†				
FFTP< 25	288 (36.0)	293 (48.8)	1.00 (reference)	1.00 (reference)
25≤ FFTP<30	423 (52.8)	260 (43.3)	1.66 (1.32-2.07)	1.78 (1.38-2.30)
30 ≤ FFTP	90 (11.2)	47 (7.8)	1.95 (1.32-2.87)	2.24 (1.46-3.43)
P_{trend}			<0.001	<0.001
Family history of breast cancer in first- and second-degree relatives				
No	816 (93.6)	655 (97.6)	1.00 (reference)	1.00 (reference)
Yes	56 (6.4)	16 (2.4)	2.81 (1.60-4.94)	2.82 (1.56-5.07)
Cigarette smoking				
<400 cigarettes/lifetime	798 (91.6)	631 (94.0)	1.00 (reference)	1.00 (reference)
≥400 cigarettes/lifetime	73 (8.4)	40 (6.0)	1.44 (0.97-2.15)	1.77 (1.15-2.73)

NOTE: Characteristics (age, education, body mass index, age at full-term pregnancy, etc.) of those excluded from the subjects because of no DNA samples were not significantly different from the final subjects.

*Adjusted for other covariates: age, body mass index, education, family history of breast cancer in first and second degree relatives, age at FTTP and parity, alcohol consumption, and smoking.

†Among parous women.

0.98-1.67) compared with under high school, older age at first full-term pregnancy (FFTP; 25 ≤ FFTP < 30: OR, 1.78; 95% CI, 1.38-2.30; 30 ≤ FFTP: OR, 2.24; 95% CI, 1.46-3.43) compared with FFTP < 25, family history of breast cancer in first- and second-degree relatives (OR, 2.82; 95% CI, 1.56-5.07) compared with those without such family history, and the consumption of at least 400 cigarettes during lifetime (OR, 1.77; 95% CI, 1.15-2.73) compared with <400 cigarettes (Table 1).

Three SNPs (i.e., the *XRCC2* 31479G > A, *XRCC6* 1796 G > T, and *LIG4* 1997T > C) of the 12 tested were not polymorphic in this study population. Those monomorphic sites were confirmed by oligonucleotide sequencing of 30 samples. The genotype distributions of other nine SNPs did not diverted significantly from Hardy-Weinberg equilibrium ($P > 0.05$) and the frequencies of minor alleles were 0.28 for *XRCC4* 921T, 0.13 for *RAD51* 135C, 0.05 for *RAD51* 172T, 0.47 for *RAD52* 2259C, 0.34 for *LIG1* 551C, 0.28 for *ERCC1* 8092A, 0.24 for *ERCC1* 354T, 0.44 for *hMLH1* −93G, and 0.03 for *hMLH1* ^{219}Val. When the two loci in the same gene were evaluated for linkage disequilibrium, two loci in *RAD51* gene (*135G>C* and *171G>T*) were in negative linkage disequilibrium ($D' = 0.88$), whereas two loci in *ERCC1* or *hMLH1* gene did not show strong linkage disequilibrium ($D' = 0.38$ and 0.49, respectively).

Increased breast cancer risk was associated with the *RAD52* 2259 CT or TT genotypes (OR, 1.33; 95% CI, 1.02-1.75 compared with the CC genotype) and the *hMLH1* −93 GG genotype (OR, 1.31; 95% CI, 0.99-1.74 compared with the AA or AG genotypes), whereas decreased risk was associated with the *ERCC1* 8092 AA genotype (OR, 0.58; 95% CI, 0.38-0.89 compared with the CC or CA genotype; Table 2).

Stratification by menopausal status revealed that the risk of breast cancer was significantly elevated for the *RAD52* CT or TT and *ERCC1* 8092 AA carriers among premenopausal women (OR, 1.47; 95% CI, 1.06-2.05 and OR, 0.52; 95% CI, 0.31-0.86, respectively), and the risk increased in a dose response manner as the number of *hMLH1* −93 G allele increased in postmenopausal women (OR, 1.33; 95% CI, 0.81-2.19; OR, 2.24; 95% CI, 1.21-4.17, respectively; $P_{trend} = 0.01$). However, when Bonferroni's method was used to correct for multiple comparisons for nine polymorphisms with P of 0.006 (≅0.05/9), all of these associations were no longer significant.

When the cases were divided into subgroups by ER/PR status, the effects of *RAD52* 2259C > T and *ERCC1* 354C > T genotypes were more evident for the ER^-/PR^- cases (Table 3). However, the effects of other genotypes were not different by ER/PR status. The *RAD52 2259* CT or TT genotypes and the *ERCC1* 354 CT or TT genotypes were associated with 2-fold increased risk of breast cancer for ER^-/PR^- cases (OR, 2.03; 95% CI, 1.24-3.34 and OR, 1.99; 95% CI, 1.35-2.94). In the analysis of combined genotypes, as the number of risk alleles (*RAD52* 2259T and *ERCC1* 354T) increased, the breast cancer risk also increased among the ER^-/PR^- cases; that is, those women with one or two of those alleles were at the 2.9-fold (95% CI, 1.25-6.90) and 5.3-fold (95% CI, 2.23-12.6) increased breast cancer risk, respectively, compared with those without those alleles ($P_{trend} < 0.01$; data not shown).

When possible interaction between these genotypes and known risk factors for breast cancer was evaluated, only *RAD52* CT or TT genotype showed a moderate interaction with family history; that is, those women with the CT + TT

Table 2. The distributions of genetic polymorphisms of selected DNA repair genes and breast cancer risks by menopausal status

	All women			Premenopausal women			Postmenopausal women		
	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*
XRCC4 c. 921G > T									
GG	430 (53.6)	343 (52.8)	1.00 (reference)	310 (53.6)	226 (51.6)	1.00 (reference)	118 (53.2)	116 (55.5)	1.00 (reference)
GT	327 (40.7)	257 (39.5)	1.10 (0.87-1.40)	238 (41.2)	178 (40.6)	0.98 (0.73-1.31)	88 (39.6)	77 (36.8)	1.30 (0.83-2.05)
TT	46 (5.7)	50 (7.7)	0.74 (0.47-1.16)	30 (5.2)	34 (7.8)	0.65 (0.37-1.15)	16 (7.2)	16 (7.7)	0.99 (0.43-2.28)
GT + TT	373 (46.5)	307 (47.2)	1.04 (0.82-1.30)	268 (46.4)	212 (48.4)	0.92 (0.70-1.22)	104 (46.9)	93 (44.5)	1.24 (0.81-1.92)
RAD51 nt 135G > C									
GG	611 (78.1)	450 (76.7)	1.00 (reference)	443 (79.3)	301 (76.4)	1.00 (reference)	167 (75.6)	147 (77.4)	1.00 (reference)
GC	143 (18.3)	123 (21.0)	0.83 (0.62-1.12)	98 (17.5)	83 (21.1)	0.77 (0.54-1.11)	44 (19.9)	39 (20.5)	1.02 (0.59-1.76)
CC	28 (3.6)	14 (2.4)	1.40 (0.68-2.88)	18 (3.2)	10 (2.5)	1.32 (0.54-3.21)	10 (4.5)	4 (2.1)	1.05 (0.29-3.88)
GC + CC	171 (21.9)	137 (23.3)	0.89 (0.67-1.17)	116 (20.8)	93 (23.6)	0.83 (0.59-1.16)	54 (24.4)	43 (22.6)	1.02 (0.61-1.72)
RAD51 nt 172G > T									
GG	721 (92.0)	533 (90.2)	1.00 (reference)	514 (91.5)	361 (91.2)	1.00 (reference)	205 (93.2)	169 (88.0)	
GT	54 (6.9)	54 (9.1)	0.77 (0.50-1.18)	42 (7.5)	32 (8.1)	1.05 (0.62-1.80)	12 (5.5)	22 (11.5)	0.51 (0.23-1.12)
TT	9 (1.2)	4 (0.7)	0.69 (0.51-5.59)	6 (1.1)	3 (0.8)	1.34 (0.33-5.51)	3 (1.4)	1 (0.5)	4.27 (0.43-42.9)
GT + TT	63 (8.0)	58 (9.8)	0.84 (0.56-1.26)	48 (8.5)	35 (8.8)	1.08 (0.65-1.80)	15 (6.8)	23 (12.0)	0.65 (0.31-1.36)
RAD52 nt 2259C > T									
CC	155 (18.7)	151 (23.0)	1.00 (reference)	108 (18.1)	108 (24.4)	1.00 (reference)	47 (20.5)	42 (19.7)	1.00 (reference)
CT	432 (52.2)	318 (48.3)	1.42 (1.06-1.90)	318 (53.4)	209 (47.3)	1.61 (1.13-2.29)	112 (48.9)	107 (50.2)	0.79 (0.45-1.36)
TT	241 (29.1)	189 (28.7)	1.20 (0.87-1.65)	170 (28.5)	125 (28.3)	1.27 (0.86-1.87)	70 (30.6)	64 (30.1)	0.81 (0.45-1.47)
CT + TT	637 (81.3)	507 (77.1)	1.33 (1.02-1.75)	488 (81.9)	334 (75.6)	1.47 (1.06-2.05)	182 (79.5)	171 (80.3)	0.80 (0.47-1.33)
LIG1 exon 6 nt 551A > C									
AA	351 (46.0)	258 (42.5)	1.00 (reference)	257 (47.5)	181 (44.6)	1.00 (reference)	93 (42.5)	77 (38.9)	1.00 (reference)
AC	317 (41.6)	282 (46.5)	0.76 (0.60-0.98)	218 (40.3)	186 (45.8)	0.70 (0.52-0.96)	97 (44.3)	94 (47.5)	0.87 (0.54-1.39)
CC	95 (12.5)	67 (11.0)	1.14 (0.78-1.66)	66 (12.2)	39 (9.6)	1.22 (0.75-2.01)	29 (13.2)	27 (13.6)	1.02 (0.52-2.01)
AC + CC	412 (54.1)	349 (57.5)	0.83 (0.66-1.05)	284 (52.5)	225 (55.4)	0.79 (0.59-1.05)	126 (57.5)	121 (61.1)	0.90 (0.58-1.41)
ERCC1 3′UTR c. 8092C > A									
CC	417 (53.6)	319 (54.0)	1.00 (reference)	299 (53.5)	212 (53.8)	1.00 (reference)	117 (54.2)	104 (53.6)	1.00 (reference)
CA	310 (39.9)	216 (36.6)	1.03 (0.80-1.32)	223 (39.9)	141 (35.8)	0.99 (0.73-1.35)	85 (39.4)	75 (38.7)	1.15 (0.73-1.82)
AA	51 (6.6)	56 (9.5)	0.59 (0.38-0.91)	37 (6.6)	41 (10.4)	0.52 (0.31-0.87)	14 (6.5)	15 (7.7)	0.85 (0.35-2.06)
CC + CA	727 (93.4)	535 (90.5)	1.00 (reference)	522 (93.4)	353 (89.6)	1.00 (reference)	202 (93.5)	178 (91.8)	1.00 (reference)
AA	51 (6.6)	56 (9.5)	0.58 (0.38-0.89)	37 (6.6)	41 (10.4)	0.52 (0.31-0.86)	16 (8.3)	14 (6.5)	0.73 (0.31-1.70)
ERCC1 c. 354C > T									
CC	411 (58.3)	323 (58.7)	1.00 (reference)	296 (57.9)	223 (61.1)	1.00 (reference)	114 (59.7)	98 (53.9)	1.00 (reference)
CT	257 (36.5)	187 (34.0)	1.11 (0.85-1.43)	192 (37.6)	115 (31.5)	1.41 (1.02-1.95)	63 (33.0)	72 (39.6)	0.71 (0.43-1.16)
TT	37 (5.3)	40 (7.3)	0.94 (0.55-1.62)	23 (4.5)	27 (7.4)	0.72 (0.35-1.47)	14 (7.3)	12 (6.6)	1.20 (0.50-2.87)
CT + TT	294 (41.8)	227 (41.3)	1.08 (0.84-1.39)	215 (42.1)	142 (39.3)	1.30 (0.96-1.77)	77 (40.3)	84 (46.2)	0.78 (0.49-1.24)
hMLH1 5′ region c. −93G > A									
AA	234 (29.9)	185 (31.1)	1.00 (reference)	164 (29.4)	113 (28.1)	1.00 (reference)	69 (31.1)	71 (37.6)	1.00 (reference)
AG	348 (44.4)	292 (49.2)	1.02 (0.78-1.34)	250 (44.8)	204 (50.8)	0.85 (0.61-1.20)	97 (43.7)	87 (46.0)	1.33 (0.81-2.19)
GG	201 (25.7)	117 (19.7)	1.33 (0.96-1.84)	144 (25.8)	85 (21.1)	1.00 (0.67-1.49)	56 (25.2)	31 (16.4)	2.24 (1.21-4.17)
AA + AG	582 (74.3)	477 (80.3)	1.00 (reference)	414 (74.2)	317 (78.9)	1.00 (reference)	166 (74.8)	158 (83.6)	1.00 (reference)
GG	201 (25.7)	117 (19.7)	1.31 (0.99-1.74)	144 (25.8)	85 (21.1)	1.10 (0.78-1.55)	56 (25.2)	31 (16.4)	1.91 (1.10-3.30)
hMLH1 5′ exon8 Ile^{219}Val (A > G)									
AA	602 (95.4)	506 (94.8)		425 (95.3)	336 (95.2)		174 (95.6)	167 (93.8)	
AG	29 (4.6)	28 (5.2)	0.88 (0.49-1.58)	21 (4.7)	17 (4.8)	0.87 (0.41-1.83)	8 (4.4)	11 (6.2)	1.01 (0.37-2.81)
GG	—	—	—	—	—	—	—	—	—

Abbreviation: nt, nucleotide.

*Adjusted for age, body mass index, education (under high school versus at and over high school), family history of breast cancer in first- and second-degree relatives (yes versus no), age at FTTP and parity, alcohol consumption (≥1/month versus <1/month), and smoking (≥400 versus <400 cigarettes/lifetime).

Table 3. Breast cancer risks among subgroups of cases by ER and PR status

	Controls, *n* (%)	Cases*					
		ER+/PR+		ER−/PR+ or ER+/PR−		ER−/PR−	
		n (%)	OR† (95% CI)	*n* (%)	OR† (95% CI)	*n* (%)	OR† (95% CI)
RAD52 nt 2259C > T							
CC	151 (23.0)	39 (17.1)		26 (22.6)		24 (13.1)	
CT + TT	507 (77.1)	189 (82.9)	1.51 (0.99-2.30)	89 (77.4)	1.12 (0.66-1.91)	159 (86.9)	2.03 (1.24-3.34)
ERCC1 c. 354C > T							
CC	323 (58.7)	114 (59.7)		70 (70.7)		69 (44.2)	
CT + TT	227 (41.3)	77 (40.3)	1.02 (0.71-1.47)	29 (29.3)	0.74 (0.45-1.24)	87 (55.8)	1.99 (1.35-2.94)

Abbreviation: nt, nucleotide.
*$P_{heterogeneity}$ for polychotomous ORs was 0.07 and <0.01, respectively.
†Adjusted for age, body mass index, education (under high school versus at and over high school), family history of breast cancer in first- and second-degree relatives (yes versus no), age at FTTP and parity, alcohol consumption (≥1/month versus <1/month), and smoking (≥400 versus <400 cigarettes/lifetime).

genotype and family history of breast cancer had 4.8-fold risk (95% CI, 2.15-10.7) compared with those with CC genotype and no family history of breast cancer ($P_{interaction}$ = 0.07; Table 4). No significant interaction was observed with other genotypes.

Discussion

The results of this study suggest that genetic polymorphisms *RAD52* 2259C > T, *ERCC1* 8092C > A and 354C > T, and *hMLH1* −93G > A are associated with risk of breast cancer in Korean women. Particularly, the effects of *RAD52* 2259C > T and *ERCC1* 354C > T genotypes were evident for the ER$^-$/PR$^-$ cases.

Whereas we found an association between the *RAD52* 2259C > T polymorphism and breast cancer risk, a previous study conducted by Kushel et al. (7) did not, in which only crude ORs were estimated. In the present study, initial crude OR for the *RAD52* 2259 CT or TT genotype was not significant, either (crude OR, 1.22; 95% CI, 0.95-1.58). Therefore, the discrepancy between these two studies might be partly attributed to the adjustment for other risk factors of breast cancer, in addition to the differences of populations (Caucasian versus Korean) and variant allele frequencies (0.44 versus 0.53).

Table 4. Interactive effects between *RAD52* nt 2259C > T and family history of breast cancer on breast cancer risk in Korean women

	RAD52 nt 2259C > T	
	CC	CT + TT
Family history of breast cancer*		
No	1.00 (reference) [148/146]	1.27 (0.97-1.68)† [627/496]
Yes	1.96 (0.28-3.32)† [7/5]	4.80 (2.15-10.7)† [46/11]
$P_{interaction}$‡		0.07

Abbreviation: nt, nucleotide.
*Among the first and second relatives.
†Adjusted for age, body mass index, education (under high school versus at and over high school), age at FFTP and parity, alcohol consumption (≥1/month versus <1/month), and smoking (≥400 versus <400 cigarettes/lifetime).
‡Assessed by likelihood ratio test with the difference of two −2LogL values between the model with interaction term and the model without the interaction term.

There may be biological plausibility for our finding of an association between the *hMLH1* polymorphism in the 5′ region and breast cancer risk, because this polymorphism may have a potential influence on the expression of this mismatch repair protein. A number of studies found that mutations on mismatch repair genes cause colon cancer. Growing evidences, however, support the hMLH1 expression is also involved in breast cancer development. Frequent loss of heterozygosity at the *hMLH1* locus was found in breast cancer (46% of 22 sporadic cases; ref. 14), and lower normal expression of hMLH1 was observed for 12 cases of 14 sporadic breast cancer cases with microsatellite instability (19).

The finding of an association between the *ERCC1* 8092 CC or CA genotype and increased breast cancer risk is comparable with that of previous epidemiologic study, in which the *ERCC1* 8029C > A was found to be associated with risk of adult-onset glioma (15). The potential biological significance of this *ERCC1* polymorphism located in 3′-untranslated region can be inferred from the fact that ERCC1 is involved in nucleotide excision repair pathway as the XPF-ERCC1 complex making the 5′ incision of bulky DNA adducts (5) and that previous phenotype study reported that the expression level of ERCC1 was slightly lower in lung cancer cases than in controls (P = 0.091; ref. 20).

It is known that patients who have ER$^-$ or PR$^-$ receptors tend to have a poor prognosis than patients with these receptors and the hormone receptor status has a profound effect on therapeutic decisions. A number of studies suggested the different relationship between risk factors such as parity, age, body mass index, family history of breast cancer and smoking, and breast cancer by ER and PR status (21–27). Colditz et al. (27) have concluded that the incidence rates and risk factors for breast cancer differ according to ER and PR status and that breast cancer risk should be estimated

according to the ER and PR status. However, other studies (28, 29) did not find any significant differences in the profile of risk factors by breast cancer subtypes. Although the underlying biological mechanisms still remain to be investigated, examining potentially modifiable breast cancer risk factors by tumor ER and PR status may provide us greater

Appendix A: Oligonucleotide sequences of PCR primer for each polymorphisms

Genotyping method	SNP	PCR primers	Concentration (nmol/L)	hME extention primers	Concentration (μmol/L)
MALDI-TOF	*XRCC2* 31479G > A	(F) 5′-*ACGTTGGATG*CCCA TCTCTCTGCCTTTTGA	56	5′-TTGTCGTTGCAA AAAGAACCAGG	0.6
		(R) 5′-*ACGTTGGATG*GATG AGCTCGAGGCTTTCTG			
	LIG4 1977T > C	(F) 5′-*ACGTTGGATG*AAGC AGCAGAGATCGTACCC	56	5′-CAGCAGAGATC GTACCCAGTGA	1.3
		(R) 5′-*ACGTTGGATG*GCCT TCCCCCTAAGTTGTTC			
	RAD52 2259C > T	(F) 5′-*ACGTTGGATG*CTGG AGTTCAGTGGTGCAAA	88	5′-TCTCTCCACAA CCTCTTGGGC	0.9
		(R) 5′-*ACGTTGGATG*TGTA AGGCAGAGGTGGGAGT			
	XRCC4 921G > T	(F) 5′-*ACGTTGGATG*GGCC TGATTCTTCACTACCTG	120	5′-CTGATTCTTCACT ACCTGAGACGTC	0.6
		(R) 5′-*ACGTTGGATG*CTTC TGGGCTGCTGTTTCTC			
	XRCC6 1796G > T	(F) 5′-*ACGTTGGATG*AAGG CCCAAGGTGGAGTATT	80	5′-GCTTACGGGC TGAAGAGTGG	0.6
		(R) 5′-*ACGTTGGATG*AGTC CTGGAAGTGCTTGGTG			
	RAD51 135G > C	(F) 5′-*ACGTTGGATG*AGCT GGGAACTGCAACTCAT	200	5′-GAGAAGTGGA GCGTAAGCCA	0.6
		(R) 5′-*ACGTTGGATG*CGCC TCACACACTCACCTC			
	RAD51 172G > T	(F) 5′-*ACGTTGGATG*AGCT GGGAACTGCAACTCAT	200	5′-GGTCGGGA GCGTGCCAC	0.6
		(R) 5′-*ACGTTGGATG*CGC CTCACACACTCACCTC			
	LIG1 exon 6 583A > C	(F) 5′-*ACGTTGGATG*GCCA TCTGACCGTTCTGTCT	200	5′-CCTCACAGAGG CTGAAGTGGC	0.6
		(R) 5′-*ACGTTGGATG*TCTG ACCCCAAAATCAGGAG			
	ERCC1 3′ untranslated region 8092A > C	(F) 5′-*ACGTTGGATG*CAGA GACAGTGCCCCAAGAG	67	5′-CAGGCTGCT GCTGCTGCT	0.6
		(R) 5′-*ACGTTGGATG*CAGA CTACACAGGCTGCTGCT			
	ERCC1 354C > T	(F) 5′-*ACGTTGGATG*TCCC TATTGATGGCTTCTGC	67	5′-TTTGCCAAATT CCCAGGGCAC	0.6
		(R) 5′-*ACGTTGGATG*TCCA GAACACTGGGACATGA			
	hMLH1 5′ region −93G > A	(F) 5′-*ACGTTGGATG*AATC AATAGCTGCCGCTGAA	67	5′-CTGGATGGCGT AAGCTACAGCT	0.6
		(R) 5′-*ACGTTGGATG*TTCA GCCAATCACCTCAGTG			
	hMLH1 exon8: Ile^{219}Val (A > G)	(F) 5′-*ACGTTGGATG*TGGG GGATGGTTTTGTTTTA	200	5′-ACCGTGGACA ATATTCGCTCC	0.6
		(R) 5′-*ACGTTGGATG*CCGA CTAACAGCATTTCCAA			

NOTE: 5′-ACGTTGGATG is 10mer tag. This sequence which is contained in M13 genome is not coincide with human genome.
Abbreviations: F, forward PCR primer; R, reverse PCR primer.

insight into breast cancer etiology and the mechanisms underlying the risk of associations (22). Recently, Cotterchio et al. (26) hypothesized that some hormonal factors may increase the risk of ER^+/PR^+ breast cancer, as opposed to ER^-/PR^- breast cancer, and that certain nonhormonal factors may be more strongly associated with ER^-/PR^- than ER^+/PR^+ breast cancer risk.

We evaluated the heterogeneity of the association observed between genotypes and breast cancer risk by ER and PR status. We found that the association between *RAD52* 2259 CT or TT and *ERCC1* 354 CT or TT genotype and breast cancer risk was stronger for the ER^-/PR^- cases. Biological mechanisms underlying the stronger effect of *RAD52* and *ERCC1* genotype observed in this study can only be speculated. *In vitro* assay (30) reported that ER^- breast tumor cell line (MDA-231) showed higher expressions of DNA repair genes (i.e., *BRCA1* and *BRCA2*) thus showed improved DNA repair capacity compared with ER^+ cell line (MCF-7). Therefore, the effect of genetic polymorphisms of DNA repair genes on the DNA repair capacity could be possibly larger among subgroup of ER^-/PR^- cases as observed in this study. However, this hypothesis remains to be investigated.

We found a suggestive interactive effect for family history in first- and second-degree relatives and the *RAD52* 2259G > T polymorphism ($P_{interaction}$ = 0.07). Another association study conducted by Han et al. (9) has also suggested the interaction between *LIG4* polymorphism (1977C) and the first-degree family history of breast cancer, but we were not able to evaluate the interaction between this locus and family history because it was not polymorphic in our study population. The interactive effect between *RAD52* and family history of breast cancer might be supported by the previous study which found that repair of radiation-induced DNA damage was reduced in breast cancer cases and their female relatives compared with healthy women without a family history of breast cancer (31).

There are several limitations in this study that include moderate sample size for evaluating gene-environment and gene-gene interactions, limited evidence of functional effect of SNPs selected in the study because selection of polymorphisms was based on the allele frequencies and previous association studies, and a hospital-based case-control design that may have uncontrolled biases. However, we used the comprehensive, candidate gene approach for studying the association between genetic polymorphisms of DNA repair genes and risk of breast cancer in a Korean population. To the best of our knowledge, this is the first study that investigated the association between genetic polymorphisms of *ERCC1* and *hMLH1* and breast cancer risk.

In conclusion, the results of this study suggest that genetic polymorphisms of *RAD52*, *ERCC1*, and *hMLH1* may be associated with risk of breast cancer in Korean women. Further larger studies, however, are needed to address the genotype-phenotype relationship and gene-environment and gene-gene interaction in breast cancer development.

References

1. Barnes DE. DNA damage: air-breaks? Curr Biol 2002; 12:R262–4.
2. Dixon K, Kopras E. Genetic alterations and DNA repair in human carcinogenesis. Semin Cancer Biol 2004;14:441–8.
3. Shi Q, Wang LE, Bondy ML, Brewster A, Singletary SE, Wei Q. Reduced DNA repair of benzo(a)pyrene diol epoxide-induced adducts and common XPD polymorphisms in breast cancer patients. Carcinogenesis 2004;25:1695–700.
4. Qiao Y, Spitz MR, Guo Z, et al. Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. Mutat Res 2002;509:165–74.
5. Yu Z, Chen J, Ford BN, Brackley ME, Glickman BW. Human DNA repair systems: an overview. Environ Mol Mutagen 1999;33:3–20.
6. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1513–30.
7. Kuschel B, Auranen A, McBride S, et al. Variants in DNA double-stranded break repair genes and breast cancer susceptibility. Hum Mol Genet 2002;11:1399–407.
8. Fu YP, Yu JC, Cheng TC, et al. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res 2003;63:2440–6.
9. Han J, Hankinson SE, Rannu H, De Vivo I, Hunter DJ. Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the Nurses' Health Study. Carcinogenesis 2004;25:189–95.
10. Rafii S, O'Regan P, Xinarianos G, et al. A potential role for the XRCC2 R186H polymorphic site in DNA-damage repair and breast cancer. Hum Mol Genet 2002;11:1433–8.
11. Kim SU, Park SK, Yoo KY, et al. *XRCC1* genetic polymorphism and breast cancer risk. Pharmacogenetics 2002;12:335–8.
12. Choi JY, Hamajima N, Tajima K, et al. *hOGG1 Ser^{326}Cys* polymorphism and breast cancer risk among Asian women. Breast Cancer Res Treat 2003; 79:59–62.
13. Ford BN, Ruttan CC, Kyle VL, Brackley ME, Glickman BW. Identification of single nucleotide polymorphisms in human DNA repair genes. Carcinogenesis 2000; 22:1977–81.
14. Shen H, Spitz MR, Qiao Y, Zheng Y, Hong WK, Wei Q. Polymorphism of DNA ligase I and risk of lung cancer: a case-control analysis. Lung Cancer 2002;36:243–7.
15. Chen P, Wiencke J, Aldape K, et al. Association of an *ERCC1* polymorphism with adult-onset glioma. Cancer Epidemiol Biomarkers Prev 2000;9:843–7.
16. Benachenhou N, Guira S, Gorska-Flipot IG, Labuda D, Sinnentt D. High resolution deletion mapping reveal frequent allelic losses at the DNA mismatch repair loci *hMLH1* and *hMSH3* in non-small lung cancer. Int J Cancer 1998;77:173–80.
17. Ito E, Yanagisawa Y, Iwahashi Y, et al. A core promoter and a frequent single-nucleotide polymorphism of the mismatch repair gene *hMLH1*. Biochem Biophys Res Commu 1999;256:488–94.
18. Kang HJ, Kim SW, Kim HJ, et al. Polymorphisms in the estrogen receptor-α gene and breast cancer risk. Cancer Lett 2002;178:175–80.
19. Murata H, Khattar NH, Kang Y, Gu L, Li GM. Genetic and epigenetic modification of mismatch repair genes hMSH2 and hMLH1 in sporadic breast cancer with microsatellite instability. Oncogene 2002; 21:5696–703.
20. Cheng L, Spitz MR, Hong WK, Wei Q. Reduced expression of nucleotide excision repair genes in lung cancer: a case-control analysis. Carcinogenesis 2000; 21:1527–30.
21. Potter JD, Cerhan JR, Sellers TA, et al. Progesteron and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? Cancer Epidemiol Biomarkers Prev 1995;4:319–26.
22. Enger SM, Ross RK, Paganini-Hill A, Carpenter CL, Bernstein L. Body size, physical activity, and breast cancer hormone receptor status: results from two case-control studies. Cancer Epidemiol Biomarkers Prev 2000;9:681–7.
23. Huang WY, Newman B, Millikan RC, Schell MJ, Hulka BS, Moorman PG. Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status. Am J Epidemiol 2000;151:703–14.
24. Manjer J, Malina J, Berglund G, Bondeson L, Garne JP, Janzon L. Smoking associated with hormone receptor negative breast cancer. Int J Cancer 2001;91:580–4.
25. Britton JA, Gammon MD, Schoenberg JB, et al. Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20-44 years of age. Am J Epidemiol 2002;156:507–16.
26. Cotterchio M, Kreiger N, Theis B, Sloan M, Bahl S. Hormonal factors and the risk of breast cancer according to estrogen- and progesterone-receptor subgroup. Cancer Epidemiol Biomarkers Prev 2003;12:1053–60.
27. Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst 2004;96:218–28.
28. Zue K, Beiler J, Hunter S, et al. The relationship between menstrual factors and breast cancer according to estrogen receptor status of tumor: a case-control study in African-American women. Ethn Dis 2002; 12:S3-23-9.
29. McCredie MR, Dite GS, Southey MC, Venter DJ, Giles GG, Hopper JL. Risk factors for breast cancer in young women by oestrogen receptor and progesterone receptor status. Br J Cancer 2003;89:1661–3.
30. Skog S, He Q, Khoshnoud R, Fornander T, Rutqvist L. Genes related to growth regulation, DNA repair and apoptosis in an oestrogen receptor-negative (MDA-231) versus an oestogen receptor-positive (MCF-7) breast tumour cell line. Tumour Biol 2004;25:1–2.
31. Helzlsouer KJ, Harris EL, Parshad R, Perry HR, Price FM, Sanford KK. DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl Cancer Inst 1996;88:754–5.