

Synergistic Effects of Polymorphisms in DNA Repair Genes and Endogenous Estrogen Exposure on Female Breast Cancer Risk

Ming-Shiean Hsu, MD¹, Jyh-Cherng Yu, MD, PhD², Hsiao-Wei Wang, MSc³, Shou-Tung Chen, MD, PhD⁴, Chia-Ni Hsiung, MSc³, Shian-ling Ding, PhD⁵, Pei-Ei Wu, MSc^{3,6}, Chen-Yang Shen, PhD^{3,6,7}, and Chun-Wen Cheng, PhD^{3,8,9}

¹Division of General Surgery, Yuanli Lee's General Hospital, Lee's Medical Corporation, Miaoli, Taiwan; ²Department of Surgery, Tri-Service General Hospital, Taipei, Taiwan; ³Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; ⁴Department of Surgery, Changhua Christian Hospital, Changhua, Taiwan; ⁵Department of Nursing, Kang-Ning Junior College of Medical Care and Management, Taipei, Taiwan; ⁶Taiwan Biobank, Taipei, Taiwan; ⁷Graduate Institute of Environmental Science, China Medical University, Taichung, Taiwan; ⁸Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan; ⁹Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan

ABSTRACT

Background. Endogenous estrogen is suggested to initiate cell proliferation and cause oxidative DNA damage during breast tumorigenesis. Cells eliminate DNA damage by means of repair enzymes. Genotypic variants of DNA damage repair genes, participating in base excision repair (BER) and nucleotide excision repair (NER) pathways, may act as modifiers that affect the association between estrogen exposure and breast cancer.

Methods. In a hospital-based case-control study of female breast cancer, DNA samples were obtained from 401 cases and 533 enrolled healthy controls, all of whom were Chinese women in Taiwan. Genotyping of polymorphisms of *XRCC1* (*Arg194Trp* and *Arg399Gln*), *OGG1* (*Ser326Cys* and *Arg229Gln*), *ERCC2 Lys751Gln*, *ERCC4 Ser662Pro*, and *ERCC5 His1104Asp* was performed and used to evaluate breast cancer susceptibility.

Results. Of the nonsynonymous polymorphisms, the *ERCC5 1104Asp* variant was significantly associated with breast cancer (odds ratio = 1.42; 95% confidence

interval = 1.08–1.97), and this association was more pronounced in women with lengthy estrogen exposure. A trend toward an increased risk of developing breast cancer was observed in women who carried greater numbers of combined high-risk genotypes of BER and NER genes ($P_{\text{trend}} = .038$). The synergistic effect of multiple genes on the increase of risk was significant in women with a longer period of estrogen exposure (>26 years), greater age at first full-term pregnancy (>26 years), a longer menarche-to-first full-term pregnancy interval (>11 years), and higher body mass index (>22) (all $P < .05$).

Conclusions. This study demonstrates that genotype polymorphisms related to DNA damage repair confer greater susceptibility to endogenous estrogen in the development of breast cancer in women.

Compared to most Western populations, there is a low incidence rate of breast cancer in women in the Taiwanese population. However, it remains the second leading cause of cancer death in women after cervical cancer; more importantly, the prevalence of breast cancer has increased in recent years. Similar to that of Western women, breast cancer in women in the Taiwanese population is also highly correlated with estrogen-mediated risk factors, including early age at menarche, late age at menopause, nulliparity, and late age at first full-term pregnancy (FFTP).^{1,2} Findings from cohort studies revealed that a high circulating level of estrogen is associated with an

increased risk of breast cancer in women.^{3,4} The well-known effect of estrogen on breast cancer risk is because estrogen is a strong mitogen for human mammary epithelial cells, causing proliferation of these cells. Estrogen has recently been considered a genotoxic carcinogen because it has been reported that estrogen-induced potentiation for replication errors and DNA damage in genes that are responsible for normal growth, death, and differentiation are required for tumor cell initiation.⁵⁻⁷ In this initiating mechanism, estrogen can be converted to catechol estrogen metabolites, and quinone intermediates are formed by the oxidation that follows. These oxidized quinone metabolites can bind to DNA and react with the purine bases of DNA to form depurinating adducts, leading to mutagenic apurinic sites in cells. In addition, reactive oxidative species (ROS) are produced during the conversion of catechol estrogen-quinone metabolites. Human cells treated with increasing concentrations of catechol estrogen-derived metabolites were found to have increasingly higher levels of ROS. The cumulative oxidative stress is capable of inducing DNA damage that may contribute to tumor initiation of cells. This evidence strongly indicated that estrogen was important in the initiation of breast tumorigenesis.⁸⁻¹⁰ As a result, the enzymes involved in correcting ROS-damaged DNA bases, which are derived from endogenous estrogen exposure, are required to maintain genomic stability.

Repair of ROS-damaged bases occurs mainly via the base excision repair (BER) and nucleotide excision repair (NER) pathways.^{11,12} It has been reported that the presence of functional polymorphisms is closely correlated with cancer risk. Rather than certain high-penetrance genes that were thought to be the high-risk factors for hereditary diseases, polymorphisms in these DNA repair genes would predispose carriers to a relatively higher risk of developing cancer as a result of the poorer ability to repair DNA damage than those who carried wild-type genotypes, enhancing the carriers' susceptibility to estrogen exposure. Breast cancer is a common, polygenic, and heterogeneous disease. However, the extent to which genetic variations in DNA repair genes are involved in breast cancer development is unknown. In addition, it remains unclear what indices of reproductive risk factors linked to which kind of DNA repair gene polymorphism actually contribute to breast cancer risk.

We hypothesized that interindividual vulnerability due to differences in DNA repair enzymes in the BER and NER pathways may modify breast cancer risk as a result of the burdens of genetic mutations in the context of estrogen exposure. To test this hypothesis, the present study was performed as a hospital-based case-control study to evaluate the association between breast cancer risk and seven nonsynonymous single nucleotide polymorphisms in genes involved in the BER (*XRCC1 Arg194Trp* and *Arg399Gln*;

OGG1 Ser326Cys and *Arg299Gln*) and NER (*ERCC2 Lys751Gln*, *ERCC4 Ser662Pro*, and *ERCC5 His1104Asp*) pathways. Therefore, carriers of polymorphisms of enzymes in the BER and NER pathways may have an increased risk of breast cancer in relation to the estrogen-related risk factors.

MATERIALS AND METHODS

Study Population

This hospital-based case-control study is part of an ongoing cooperative study, initiated in 1991, aimed at understanding the causes of breast cancer in Taiwan. The relevant information had previously been published, and breast cancer in the Taiwanese population is characterized by low incidence, early tumor onset, hormone dependency, and novel genomic alterations.^{2,13-15} The low incidence of breast cancer in this population shows an overall lower effect of common risk factors. Additionally, because of the homogeneous genetic background, investigation of breast cancer in women in the Taiwanese population has certain advantages for studying the effects of genetic variations, such as genotypic polymorphisms.¹⁶ The present study included data from 401 female patients with breast cancer and 533 healthy female controls, collected between August 2001 and October 2004. This study was approved by the ethics committee of the Institutional Review Board of Biomedical Sciences, Academia Sinica, and all subjects provided informed consent. All breast cancer patients had pathologically confirmed primary infiltrating ductal carcinoma of the breast, and approximately 9% had a family history of breast cancer. Their ages ranged from 24 to 82 years. The clinics taking part in this study are two breast cancer clinics located in northern Taiwan. Other patients were excluded because of a lack of suitable blood specimens. No statistically significant differences were found in breast cancer risk factors between the recruited and non-recruited women.

Control subjects were cancer-free individuals randomly selected from women attending the health examination clinic at the same hospital during the same study period. Those with a history of benign breast diseases were excluded. Their ages ranged from 24 to 87 years and were very similar to those of cases. These women underwent a one-day comprehensive health examination, including routine breast screening by X-ray mammography and ultrasonic examination of the breast. Individuals with any evidence of breast cancer, suspicious precancerous lesions of the breast, or other cancers were excluded from the control group. The control subjects consisted of approximately 20% of all the women who attended the clinics, and

no statistically significant differences were observed in socioeconomic status between those included and those not included. All blood specimens were collected from the control subjects after they agreed to complete the questionnaire.

Questionnaire

Experienced research nurses were assigned to administer a structured questionnaire to both the cases and controls. The validity of this questionnaire was addressed and confirmed in our previous studies.^{13,17} The information collected included demographic characteristics (ethnic background, residence area, family income, and educational level), medical history (age at diagnosis of breast cancer, family history of breast cancer in first-degree female relatives, breast biopsy history, and breast screening history), reproductive risk factors (age at menarche and/or menopause, parity, age at FFTP, number of pregnancies, history of breast feeding), and usage of hormones (use of oral contraceptives, dietary habits, and hormone replacement therapy). In addition, body mass index (BMI) was calculated from measurements taken when women were diagnosed with breast cancer at the participating clinics or were attending health examination clinic during the study period. Diet, history of cigarette smoking and alcohol consumption, and radiation exposure were also recorded. Women younger than 55 years who had undergone a hysterectomy, but not a bilateral oophorectomy, were classified as unknown with respect to menopausal status.

Determination of Genotypic Polymorphisms

A 10-ml sample of peripheral blood, collected in acetate-citrate dextrose, was obtained from each control subject and breast cancer patient before operation. Genomic DNA was immediately extracted by a QIAamp DNA blood mini kit (Qiagen) following the manufacturer's protocol and then stored at -20°C for subsequent genotype analysis.

The nonsynonymous variants of interest were chosen for genotyping on the basis of the following criteria: (1) the single nucleotide polymorphism (SNP) resulted in an amino acid substitution; (2) the frequency of the variant allele was greater than five percent in the general population. Genotype determination of the polymorphisms was performed by real-time polymerase chain reaction (PCR) with the TaqMan assay system. The SNP information was obtained from the public SNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>, National Institutes of Health). The sequences for probes and primer sets in genotyping assays of the polymorphisms *Arg194Trp* (a C-to-T transition, rs1799782) and *Arg399Gln* (a G-to-A transition, rs25487) in

XRCC1, *Ser326Cys* (a C-to-G transversion, rs1052133) and *Arg299Gln* (a G-to-A transition, rs1805373) in *OGG1*, *Lys751Gln* (a A-to-C transversion, rs13181) in *ERCC2*, *Ser662Pro* (a T-to-C transition, rs2020955) in *ERCC4*, and *His1104Asp* (a C-to-G transversion, rs17655) in *ERCC5* were determined by TaqMan(R) Assays-On-Demand software (Applied Biosystems, ABI; Foster City, CA). The probes were labeled fluorescently with either FAM or VIC on the 5' end and a nonfluorescent minor groove binder quencher on the 3' end. Allelic discrimination of the TaqMan assay was used for genotyping these seven polymorphisms in a 96-well format on an ABI Prism 7900HT sequence detection system (Applied Biosystems), each with a 20- μl reaction containing 200 nM of each probe, 900 nM of each primer set, 1X TaqMan Universal PCR Master Mix (Applied Biosystems), and 50 ng DNA template. To ensure that the observed polymorphisms were specific and not the result of experimental variation, 10% of the samples were randomly selected and run in duplicate with complete verification. Independent control samples, which were template free, were included and analyzed on 96-well plates by batch.

Statistical Analysis

The following statistical analyses were sequentially performed. (i) Univariate and multivariate analyses were used to assess risk factors and to establish background risk profiles for breast cancer. On the basis of our previous studies, important reproductive risk factors were considered as important indices to estimate the estrogen exposure level or susceptibility to estrogen exposure in the subsequent analysis.^{13,17-19} (ii) The allelic and genotypic frequencies of each polymorphism of the individual BER- and NER-related genes were compared between cases and controls by χ^2 and Fisher's exact tests when appropriate. Multivariate logistic regression models were used to consider the known risk factors for breast cancer, and the adjusted odds ratio (aOR) and the corresponding 95% confidence interval (95% CI) for the association were estimated. In the present study, increased exposure to estrogen was hypothesized to contribute to an increased risk of breast cancer. The presence of putative high-risk alleles, such as the *XRCC1 Trp194* and *Gln399* and *OGG1 Cys326* for BER-related genes, as well as *ERCC2 Gln751* and *ERCC5 Asp1104* for NER-related genes, mimicked a functional deficiency in cells with lower efficacy in response to DNA damage repair, and thus these were considered to be higher risk genotypes for breast cancer. (iii) Additionally, because we considered that long-term cumulative estrogen is suggested to be associated with breast cancer development compared to risk factors reflecting short-term exposure to estrogen, we adopted four

biological indices that are the most critical criteria to estimate estrogen exposure and its related factors.^{13,14} As shown in Table 1, the susceptibility of breast cancer in women was principally related to estrogenic risk factors, including (a) the number of years of estrogen exposure, which was calculated by subtracting the age at menarche from the age at interview for premenopausal women or the age at menopause for postmenopausal women; (b) age at FFTP; (c) the number of years between menarche and FFTP; and (d) BMI. The relationship between BER- and NER-related genes and breast cancer risk in women with different levels of estrogen exposure was examined by using joint and stratified methods.²⁰ We assumed that these genotypic polymorphisms contributed equally to an increase in breast cancer risk and that individuals who carried the same number of high-risk genotypes would have the same increased risk of breast cancer development. In the joint method, we assessed the association between increased breast cancer risk and the combination of the putative high-risk genotypes. (v) Moreover, in the stratified model, possible modification of the increased risk associated with these DNA repair gene polymorphisms in response to estrogen exposure was evaluated by calculating the risk susceptibility of breast cancer according to the number of high-risk genotypes within different categories of estrogen-related risk factors. All data were analyzed by SAS version 9.1 (SAS Institute, Cary, NC), and statistical tests were based on a two-tailed probability.

RESULTS

Risk Profile of Breast Cancer

The distributions of selected demographic characteristics and major risk factors for breast cancer were similar to those reported in our previous studies.^{13,17–19} The average age was similar for cases and controls (cases, 51.5 ± 11.2 years vs.

controls, 49.8 ± 9.9 years). Of the various reproductive risk factors, those reflecting prolonged exposure to estrogen were consistently found to be statistically significantly associated with increased risk. By means of multivariate logistic regression analysis, an increased risk was found for a family history of breast cancer in first-degree female relatives (aOR = 1.22; 95% CI = 0.80–1.87). Compared to the controls, the cases were younger at menarche (<14 years vs. ≥ 14 years, aOR = 1.13; 95% CI = 0.81–1.41), older at FFTP (>26 years or nulliparity vs. ≤ 26 years, aOR = 1.16; 95% CI = 0.87–1.54), and had a history of smoking (aOR = 3.31; 95% CI = 1.60–7.16). Although not all the differences were statistically significant, reproductive risk factors for breast cancer occurred more frequently among the cases than among the controls. Protection conferred by pregnancy against the development of breast cancer was observed (number of pregnancies = 1–3 vs. nulliparity, aOR = .90; 95% CI = 0.57–1.41), possibly because pregnancy causes permanent differentiation of vulnerable breast stem cells, thus reducing susceptibility to estrogen exposure, as suggested in a previous study.²¹ No association was found between breast cancer risk and radiation exposure, hormone replacement therapy, or dietary intake of specific kinds of foods or vegetables. Additionally, women with a BMI of $>22 \text{ kg/m}^2$ had a higher risk of breast cancer, but this did not reach statistical significance (aOR = 1.08; 95% CI = 0.85–1.42).

Polymorphisms in DNA Repair Genes and Breast Cancer Risk

Genotype distributions of the seven nonsynonymous polymorphisms in the *XRCC1*, *OGG1*, *ERCC2*, *ERCC4*, and *ERCC5* genes in the controls did not deviate from expectation on the basis of the Hardy–Weinberg equilibrium ($P > .05$). Genotype frequencies for these polymorphisms were compared between cases and controls, and the effects of

TABLE 1 Representation of different levels of estrogen exposure or different degrees of estrogen-induced susceptibility to breast cancer

Estrogen-related risk factors	Stratification (years)	Description
Total years of estrogen exposure	≤ 26 vs. > 26	For premenopausal women, this index = age when attending our breast cancer or health examination clinic – age at menarche For postmenopausal women, this index = age at menopause – age at menarche
Age at menarche	≥ 14 vs. < 14	The age at onset of menarche
Age at FFTP	≤ 26 vs. > 26	The age at onset of the FFTP
Menarche-to-FFTP interval	≤ 11 vs. > 11	For premenopausal nulliparous women, this index = age when attending our breast cancer or health examination clinic – age at menarche For postmenopausal nulliparous women, this index = age at menopause – age at menarche For other women, this index = age at FFTP – age at menarche
BMI	≤ 22 vs. > 22	BMI was calculated for individuals who attended our breast cancer or health examination clinic

FFTP first full-term pregnancy, BMI body mass index

breast cancer risk factors were adjusted in the multiple logistic regression model. Because of the small percentage of subjects homozygous for variants and on the basis of inferred phenotypic manifestations, women harboring the heterozygous and homozygous variant genotypes of these genes were grouped together and compared to subjects who carried the homozygous wild-type genotype.^{22–27} This grouping resulted in increased statistical power in detecting the association between repair gene polymorphisms and breast cancer risk. In this study, the heterozygous and homozygous variants of *XRCC1 Trp194* and *Gln399*, *OGG1 Cys326*, *ERCC2 Gln751*, and *ERCC5 Asp1104* were considered high-risk genotypes; this grouping strategy has also been widely used in previous molecular epidemiological studies.^{26–28} As shown in Table 2, when considering subjects who carried the homozygous *ERCC5 His/His* genotype as a reference, we found ORs of 1.34 (95% CI = 0.96–1.97) for *His/Asp*, 1.43 (95% CI = 1.00–2.08) for *Asp/Asp*, and 1.42 (95% CI = 1.08–1.97) for the combined *His/Asp* and *Asp/Asp* genotypes, comparing between cases and controls. The homozygous variants of *Arg194Trp* or *Arg399Gln* in the *XRCC1* gene resulted in higher risk of breast cancer; however, the combined heterozygous and homozygous variants showed nonsignificantly increased risk when compared to the respective wild-type genotypes. Genotype frequencies of the *OGG1 Ser326Cys* and *ERCC2 Lys751Gln* polymorphisms were higher in cases than in the control subjects, but none of the variant genotypes of either gene were significantly associated with breast cancer. On the other hand, the heterozygous and homozygous of the *OGG1 Gln229* variants and *ERCC4 Ser662* variants were not seen in almost all of our case and control subjects and thus seem to have no correlation with breast cancer. Moreover, the frequencies of the variant alleles of both polymorphisms were similar to those shown on the NCBI Web site for the Chinese population, suggesting that there was no selection bias for the subjects enrolled in terms of these two genotypes. To focus our consideration on the assessment of the contribution of genotypes pertinent to breast cancer development, we therefore excluded these two polymorphisms in the subsequent analyses.

Association Between Genetic Polymorphisms in DNA Repair Genes and Breast Cancer Risk Modified by Estrogen Exposure

Interactions between the variant alleles and estrogen-related risk factors were examined by determining the frequency of an individual genotype among the cases and controls. The results are summarized in Table 3. As expected, a greater proportion of the *ERCC5 Asp1104* variant genotypes (*His/Asp* and *Asp/Asp*) was seen in cases than in controls, conferring a 2.46-fold increased risk in the

subset that had more years of estrogen exposure (>26 years) (95% CI = 1.13–5.32). Similarly, genotype frequencies of either the *XRCC1 Arg399Gln* or *ERCC2 Lys751Gln* polymorphism were found to be higher in cases in which there had been prolonged estrogen exposure (>26 years), leading to higher risk in carriers with the *XRCC1 Gln399* (*Arg/Gln* and *Gln/Gln*) (aOR = 1.98; 95% CI = 1.21–2.96) or *ERCC2 Gln751* (*Lys/Gln* and *Gln/Gln*) variant (aOR = 1.64; 95% CI = 1.01–2.82). A significant association between *ERCC5 Asp1104* variant genotypes and breast cancer risk was found in women with early age at onset of menarche (<14 years) (aOR = 1.54; 95% CI = 1.00–2.38). Although individuals carrying either *XRCC1 Trp194* or *OGG1 Cys326* showed an increased cancer risk among subjects within the same category of estrogen exposure, most of these associations lacked statistical significance ($P > .05$) (Table 3).

Effect of Gene-to-Gene Interaction on the Risk of Breast Cancer

Given that an individual DNA repair gene plays an important role in eliminating damaged DNA for the maintenance of genome stability, there may be an increased risk of cancer as a result of the combined effects of genetic variations in multiple DNA repair pathways. Additionally, it has been suggested that a higher susceptibility to cancer development is in line with an increasing number of high-risk alleles,^{19,28,29} we thus explored the joint effect of these DNA repair genes by determining the association between gene-to-gene interactions and the higher breast cancer risk. By means of a dummy variable coding scheme and the β estimate from the regression model,^{30,31} the effect of a multigenic model incorporating the high-risk genotypes of DNA repair genes on breast cancer is summarized in Table 4. By means of the homozygous genotypes *XRCC1* (*Arg194* and *Arg399*) and *OGG1* (*Ser326*) as a reference, there was an increasing breast cancer risk in women carrying an increasing number of high-risk genotypes for *XRCC1* (*Trp194* and *Gln399*) and *OGG1* (*Cys326*) (aOR = 1.15; 95% CI = 0.95–1.36; $P_{\text{trend}} = .169$). In the NER pathway, subjects carrying the *ERCC2 Lys751Gln* and *ERCC5 His1104Asp* variants displayed a marginally significant association with breast cancer risk compared to those carrying the *ERCC2 Lys/Lys* and *ERCC5 His/His* genotypes (aOR = 1.48; 95% CI = 0.94–2.41). This joint effect on breast cancer risk is greater in women having one additional high-risk genotypes for *ERCC2* and *ERCC5* (aOR = 1.23; 95% CI = 0.98–1.57; $P_{\text{trend}} = .079$) than in women with the homozygous genotypes *ERCC2 Lys751* and *ERCC5 His1104* (Table 4).

TABLE 2 Genotype frequencies of polymorphisms of DNA repair genes among cases and controls and aOR in relation to breast cancer risk

Gene and SNP ^a	Case, <i>n</i> (%) ^b	Control, <i>n</i> (%) ^b	cOR (95% CI)	aOR (95% CI) ^c	aORs (95% CIs) ^d
BER pathway					
<i>XRCC1 R194W</i> (rs1799782; C > T)					
<i>Arg/Arg</i>	187 (46.6)	270 (50.7)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Arg/Trp</i>	170 (42.4)	217 (40.7)	1.09 (0.83–1.44)	1.12 (0.84–1.47)	1.20 (0.87–1.62)
<i>Trp/Trp</i>	44 (11.0)	46 (8.6)	1.36 (0.88–2.14)	1.37 (0.87–2.17)	
<i>XRCC1 R399Q</i> (rs25487; G > A)					
<i>Arg/Arg</i>	198 (50.1)	276 (52.0)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Arg/Gln</i>	149 (37.7)	202 (38.0)	1.03 (0.78–1.36)	1.05 (0.81–1.38)	1.12 (0.87–1.42)
<i>Gln/Gln</i>	48 (12.2)	53 (10.0)	1.26 (0.82–1.93)	1.31 (0.83–1.96)	
<i>OGG1 S326C</i> (rs1052133 C > G)					
<i>Ser/Ser</i>	64 (16.0)	87 (16.3)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Ser/Cys</i>	165 (41.1)	231 (43.3)	0.99 (0.67–1.47)	0.97 (0.66–1.42)	1.04 (0.80–1.46)
<i>Cys/Cys</i>	172 (42.9)	215 (40.4)	1.09 (0.74–1.63)	1.09 (0.74–1.59)	
<i>OGG1 R229Q</i> (rs1805373; G > A)					
<i>Arg/Arg</i>	401 (100.0)	533 (100.0)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Arg/Gln</i>	0 (0.0)	0 (0.0)			
<i>Gln/Gln</i>	0 (0.0)	0 (0.0)			
NER pathway					
<i>ERCC2 K751Q</i> (rs13181; A > C)					
<i>Lys/Lys</i>	334 (83.2)	450 (84.4)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Lys/Gln</i>	60 (15.0)	77 (14.4)	1.05 (0.73–1.51)	1.05 (0.72–1.53)	1.09 (0.73–1.57)
<i>Gln/Gln</i>	7 (1.8)	6 (1.2)	1.57 (0.52–4.72)	1.51 (0.51–4.56)	
<i>ERCC4 S662P</i> (rs2020955; T > C)					
<i>Ser/Ser</i>	401 (100.0)	533 (100.0)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>Ser/Pro</i>	0 (0.0)	0 (0.0)			
<i>Pro/Pro</i>	0 (0.0)	0 (0.0)			
<i>ERCC5 H1104D</i> (rs17655; C > G)					
<i>His/His</i>	76 (19.0)	129 (24.3)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<i>His/Asp</i>	191 (47.6)	243 (45.8)	1.31 (0.93–1.85)	1.34 (0.96–1.97)	1.42 (1.08–1.97)*
<i>Asp/Asp</i>	134 (33.4)	159 (29.9)	1.39 (0.97–2.03)	1.43 (1.00–2.08)*	

SNP single nucleotide polymorphism, cOR crude odd ratio, 95% CI 95% confidence interval, aOR adjusted odds ratio, BER base excision repair, NER nucleotide excision repair, Ref reference group

* $P < .05$

^a The rs number shown is the National Center for Biotechnology Information dbSNP cluster ID for each SNP

^b The minor difference of sample size in individual comparisons was due to a lack of DNA specimens in study subjects

^c aORs were calculated by unconditional logistic regression and adjusted for age, age at first full-term pregnancy, cigarette smoking, body mass index, and family history of breast cancer

^d In these regression models, heterozygous and homozygous variants were grouped together and compared to homozygous wild-type

Considering these five polymorphisms simultaneously, there was a statistically significant increase in breast cancer risk in those subjects with a greater number of high-risk genotypes, as determined by using the same logistic regression model. For the analysis that used the joint method, we classified our women into two groups, those with no more than one high-risk genotypes (0–1) and those with at least two high-risk genotypes (2–5) in the genes involved in either of the two DNA damage repair pathways, because such a definition would provide sufficient

statistical power to address the question. When compared to women with zero or one high-risk genotype, cases that carried a greater number of high-risk genotypes were at a significantly higher cancer risk of 2.72-fold (95% CI = 1.57–5.18) in the subset with two risk genotypes, and of 3.19-fold (95% CI = 1.26–8.13) in the subset with five risk genotypes, respectively. The trend test for one additional high-risk genotype in association with breast cancer was significant in women with at least two high-risk genotypes, compared to those with 0–1 high-risk genotype

TABLE 3 Interaction between individual genotypic polymorphism and estrogen-related risk factors in breast cancer

Genotype	Total years of estrogen exposure				Age at menarche (years)				Age at FFTP (years)				Menarche-to-FFTP interval				BMI (kg/m ²)	
	Exposure (years)	Ca/Co	aOR (95% CI) ^a	Age (years)	Age (years)	Ca/Co	aOR (95% CI)	Age (years)	Age (years)	Ca/Co	aOR (95% CI)	Age (years)	Age (years)	Ca/Co	aOR (95% CI)	BMI	Ca/Co	aOR (95% CI)
<i>XRCC1 R194W</i>																		
CC	≤26	27/50	1.00 (Ref.) ^b	≥14	115/165	1.00 (Ref.)	≤26	106/140	1.00 (Ref.)	≤11	88/113	1.00 (Ref.)	≤22	71/101	1.00 (Ref.)			
CC	>26	157/220	1.35 (0.81–2.28)	<14	68/104	0.95 (0.63–1.37)	>26	78/128	0.81 (0.57–1.26)	>11	93/154	0.78 (0.57–1.21)	>22	115/169	1.01 (0.67–1.47)			
CT/TT	≤26	35/70	0.94 (0.49–1.71)	≥14	121/171	1.02 (0.73–1.42)	≤26	111/133	1.12 (0.80–1.57)	≤11	88/114	1.03 (0.67–1.46)	≤22	76/101	1.07 (0.70–1.63)			
CT/TT	>26	171/192	1.65 (0.98–2.78)	<14	85/91	1.32 (0.91–1.95)	>26	102/128	1.05 (0.73–1.51)	>11	118/146	1.07 (0.71–1.53)	>22	138/162	1.22 (0.83–1.77)			
<i>XRCC1 R399Q</i>																		
GG	≤26	28/65	1.00 (Ref.)	≥14	116/180	1.00 (Ref.)	≤26	107/137	1.00 (Ref.)	≤11	88/115	1.00 (Ref.)	≤22	73/110	1.00 (Ref.)			
GG	>26	165/210	1.84 (1.19–3.17)*	<14	78/95	1.26 (0.86–1.85)	>26	88/136	0.82 (0.57–1.20)	>11	103/157	0.88 (0.60–1.25)	>22	124/165	1.13 (0.82–1.65)			
GA/AA	≤26	34/53	1.71 (0.88–2.81)	≥14	116/156	1.15 (0.82–1.67)	≤26	106/136	1.02 (0.74–1.49)	≤11	86/112	1.16 (0.77–1.76)	≤22	71/90	1.18 (0.87–1.82)			
GA/AA	>26	157/202	1.98 (1.21–2.96)**	<14	73/98	1.18 (0.78–1.69)	>26	90/118	0.99 (0.67–1.42)	>11	104/141	1.08 (0.74–1.57)	>22	125/165	1.23 (0.85–1.76)			
<i>OGG1 S326C</i>																		
CC	≤26	9/18	1.00 (Ref.)	≥14	42/66	1.00 (Ref.)	≤26	38/41	1.00 (Ref.)	≤11	28/34	1.00 (Ref.)	≤22	20/23	1.00 (Ref.)			
CC	>26	54/69	1.52 (0.64–3.67)	<14	22/21	1.65 (0.81–3.36)	>26	30/46	0.85 (0.46–1.56)	>11	35/53	0.83 (0.41–1.67)	>22	43/64	0.83 (0.40–1.57)			
CG/GG	≤26	53/102	1.04 (0.43–2.41)	≥14	194/270	1.18 (0.73–1.75)	≤26	184/232	1.03 (0.63–1.63)	≤11	148/193	0.98 (0.58–1.64)	≤22	127/179	0.86 (0.44–1.54)			
CG/GG	>26	274/343	1.60 (0.69–3.92)	<14	131/174	1.23 (0.76–1.94)	>26	150/210	0.92 (0.55–1.51)	>11	176/247	0.92 (0.52–1.53)	>22	209/266	0.96 (0.50–1.71)			
<i>ERCC2 K751Q</i>																		
AA	≤26	50/97	1.00 (Ref.)	≥14	195/281	1.00 (Ref.)	≤26	184/226	1.00 (Ref.)	≤11	149/188	1.00 (Ref.)	≤22	125/168	1.00 (Ref.)			
AA	>26	277/352	1.53 (1.05–2.23)*	<14	130/167	1.12 (0.85–1.52)	>26	149/221	0.83 (0.59–1.07)	>11	176/257	0.86 (0.64–1.16)	>22	208/281	1.02 (0.75–1.37)			
AC/CC	≤26	12/23	1.01 (0.47–2.21)	≥14	41/55	1.07 (0.67–1.71)	≤26	33/47	0.89 (0.55–1.39)	≤11	27/39	0.91 (0.51–1.49)	≤22	22/34	0.91 (0.47–1.53)			
AC/CC	>26	51/60	1.64 (1.01–2.82)*	<14	23/28	1.21 (0.69–2.20)	>26	31/35	1.21 (0.69–2.21)	>11	35/43	1.04 (0.62–1.73)	>22	44/49	1.22 (0.76–2.01)			
<i>ERCC5 H1104D</i>																		
CC	≤26	9/26	1.00 (Ref.)	≥14	48/89	1.00 (Ref.)	≤26	45/59	1.00 (Ref.)	≤11	39/55	1.00 (Ref.)	≤22	29/48	1.00 (Ref.)			
CC	>26	64/100	1.79 (0.79–4.08)	<14	25/39	1.19 (0.69–2.28)	>26	29/71	0.62 (0.43–1.17)	>11	32/73	0.71 (0.43–1.15)	>22	47/81	0.97 (0.56–1.71)			
CG/GG	≤26	53/94	1.61 (0.69–3.58)	≥14	188/247	1.42 (0.96–2.11)	≤26	172/214	1.04 (0.79–1.61)	≤11	137/171	1.13 (0.71–1.82)	≤22	118/154	1.25 (0.78–2.12)			
CG/GG	>26	264/310	2.46 (1.13–5.32)*	<14	128/154	1.54 (1.00–2.38)*	>26	151/184	1.09 (0.75–1.81)	>11	179/226	1.18 (0.73–1.81)	>22	205/247	1.39 (0.84–2.34)			

aOR adjusted odds ratio, Ca case, Co control, FFTP first full-term pregnancy, BMI body mass index, Ref. reference group

* $P < .05$ ** $P < .01$ ^a aOR was calculated in a logistic regression model with adjustment for age, cigarette smoking, and family history of breast cancer^b Ref indicates CC in *Arg194* and GG in *Arg399* in the *XRCC1* gene, CC in *OGG1 Ser326*, AA in *ERCC2 Lys751*, and CC in *ERCC5 His1104* genotypes

TABLE 4 Multivariate logistic regression analysis of gene-to-gene interactions of the genes involved in the BER and NER pathways in association with an increased risk of breast cancer in women

Genotype ^a	OR (95% CI) ^b
BER pathway	
<i>XRCC1 R194W/R399Q</i> and <i>OGG1 S326C</i>	
0	1.00 (Ref.)
1	1.14 (0.56–2.32)
2	1.43 (0.74–2.81)
3	1.41 (0.68–2.89)
One additional risk genotype	1.15 (0.95–1.36)
	$P_{\text{trend}} = .169$
NER pathway	
<i>ERCC2 L751Q</i> and <i>ERCC5 H1104D</i>	
0	1.00 (Ref.)
1	1.36 (0.96–1.94)
2	1.48 (0.94–2.41)
One additional risk genotype	1.23 (0.98–1.57)
	$P_{\text{trend}} = .079$
Combination of high-risk genotypes of the BER and NER pathways	
0–1	1.00 (Ref.) ^c
2	2.72 (1.57–5.18)**
3	2.38 (1.29–4.26)*
4	2.93 (1.54–5.21)**
5	3.19 (1.26–8.13)*
As a continuous variable	1.29 (1.03–1.36)*
	$P_{\text{trend}} = .038$

BER base excision repair, NER nucleotide excision repair, OR odds ratio, 95% CI 95% confidence interval

* $P < .05$

** $P < .01$

^a The high-risk genotypes are identified as combined genotypes of heterozygous and homozygous variants of the polymorphisms of DNA repair genes

^b A multivariate logistic regression model after adjusting for age, body mass index, cigarette smoking, and family history of breast cancer

^c The risks were estimated by using the women harboring no more than one high-risk genotype as the reference

after adjusting for age, BMI, cigarette smoking, and family history of breast cancer (aOR = 1.29; 95% CI = 1.03–1.36; $P_{\text{trend}} = .038$) (Table 4).

Higher Breast Cancer Risk and Prolonged Exposure to Estrogen in Women With a Greater Number of High-Risk Polymorphisms in DNA Repair Genes

We used both the joint method and the stratified method to investigate the relationship between cancer risk and increased time of estrogen exposure, modified by an increasing number of high-risk genotypes. We stratified the

data according to different statuses of estrogen exposure, together with high-risk genotypes in a combination of BER and NER genes described above, to evaluate the contribution to breast cancer tumorigenesis. As presented in Table 5, a consistent significant association between an increased cancer risk and harboring at least two high-risk genotypes was seen in the subset who had more years of estrogen exposure (>26 years) (aOR = 2.71; 95% CI = 1.52–4.94), an older age at menarche (≥ 14 years) (aOR = 2.47; 95% CI = 1.34–5.46), an older age at FFTP (>26 years) (aOR = 3.91; 95% CI = 1.59–9.59), a longer menarche-to-FFTP interval (>11 years) (aOR = 3.82; 95% CI = 1.56–9.36), and in those women with a higher BMI (>22) (aOR = 3.17; 95% CI = 1.54–6.51) (all $P < .05$).

DISCUSSION

On the basis of a multigenic model, the present study addresses the issue of genotypic polymorphisms in DNA repair genes in relation to breast cancer risk in a female population characterized by features of estrogen exposure. This study used the candidate pathway approach that was based on SNPs located in genes of the BER and NER pathways, to evaluate the risk association with breast cancer. Because these SNPs occur in exonic sequences and affect amino acid coding, resulting in a functional change, women with multiple high-risk genotypes may have relatively low DNA repair enzyme activity, and the breast cancer risk of these women was increased when compared to those carrying wild-type genotypes. Our results also provide an insight into the modified effects of combined DNA repair genes on breast tumorigenesis that was initiated by endogenous estrogen exposure. The statistically significant associations can be interpreted as the presence of an individual impairment in ability to repair damaged DNA causally linked to estrogen-related effects on breast cancer in women.

The *XRCC1* gene has been reported to play a pivotal role in the multistep BER pathway, demonstrating the influence of the *XRCC1* enzyme on the ability to remove DNA adducts and oxidative DNA damage from single-stranded DNA. As compared with molecular epidemiological studies, the frequencies of the *Trp194* and *Gln399* polymorphisms in the *XRCC1* gene found in this study were similar to those reported among the Taiwanese population as well as in the Han Chinese population tested through GenBank.^{32,33} Recent studies have found that the low enzymatic activity caused by the *Gln399* polymorphism resulted in a statistically significantly increased breast cancer risk.^{34,35} However, our results suggest that the *Gln399* variant plays a recessive role in breast cancer risk. In previous studies, a null or protective effect against breast cancer was reported in female carriers of the *Gln399*

TABLE 5 Increased risk of breast cancer associated with the combination of high-risk genotypes in the DNA repair genes stratified by the estrogen-related risk factors

Risk factor	No. of high-risk genotype	Case (%)	Control (%)	aOR (95% CI) ^a	P value
Total years of estrogen exposure					
≤26	0–1	3 (4.8)	10 (8.5)	1.00 (Ref.)	
≤26	≥2	59 (95.2)	108 (91.5)	1.82 (0.48–6.87)	.377
>26	0–1	15 (4.7)	48 (11.7)	1.00 (Ref.)	
>26	≥2	307 (95.3)	362 (88.3)	2.71 (1.52–4.94)	.003
Age at menarche (years)					
≥14	0–1	13 (5.6)	46 (13.7)	1.00 (Ref.)	
≥14	≥2	219 (94.4)	290 (86.3)	2.47 (1.34–5.46)	.005
<14	0–1	5 (3.3)	12 (6.3)	1.00 (Ref.)	
<14	≥2	146 (96.7)	179 (93.7)	1.89 (0.65–5.51)	.242
Age at FFTP (years)					
≤26	0–1	11 (5.2)	27 (9.9)	1.00 (Ref.)	
≤26	≥2	202 (94.8)	245 (90.1)	1.96 (0.97–4.05)	.074
>26	0–1	6 (3.4)	31 (12.3)	1.00 (Ref.)	
>26	≥2	172 (96.6)	222 (87.7)	3.91 (1.59–9.59)	.004
Menarche-to-FFTP interval (years)					
≤11	0–1	11 (6.3)	27 (11.9)	1.00 (Ref.)	
≤11	≥2	163 (93.7)	199 (88.1)	1.94 (0.92–4.07)	.080
>11	0–1	6 (2.9)	31 (10.4)	1.00 (Ref.)	
>11	≥2	201 (99.1)	266 (89.6)	3.82 (1.56–9.36)	.003
BMI (kg/m ²)					
≤22	0–1	7 (4.9)	18 (9.0)	1.00 (Ref.)	
≤22	≥2	137 (95.1)	182 (91.0)	1.83 (0.75–4.68)	.187
>22	0–1	11 (4.4)	40 (12.2)	1.00 (Ref.)	
>22	≥2	238 (95.6)	288 (87.8)	3.17 (1.54–6.51)	.002

aOR adjusted odds ratio, 95% CI 95% confidence interval, FFTP first full-term pregnancy, BER base excision repair, NER nucleotide excision repair
^a The aOR for female breast cancer development associated with combination of high-risk genotypes in the BER and NER pathways and reproductive risk factors was calculated in a multivariate logistic regression model considering age, cigarette smoking, and family history of breast cancer. The risks were estimated by using individuals with a low number (0–1) of high-risk genotypes as the reference

polymorphism.^{28,36,37} In addition to the relative difference in allelic frequency that is ethnicity specific, these conflicting findings may also be attributed to the reliability of the different backgrounds of the populations being studied, including familial history, smoking habits, fruit and vegetable consumption, antioxidant intake, and gene-to-gene interactions in the different pathways.^{29,36,38–40} Therefore, an investigation of genetic susceptibility with a larger sample size is the best solution to resolve the current discrepancies regarding the role of *XRCC1 Gln399* in breast cancer.

8-Oxoguanine (8-oxoG) is a major DNA lesion that is attacked by ROS in the genome. *OGGI* mutant mice exhibit a relatively higher level of 8-oxoG and an increase in mutation frequency during the cell cycle.⁴¹ Our present data did not show a statistically significant association between the *Cys326* variant and breast cancer risk, and this lack of an association with breast cancer risk is similar to the results of studies in different populations.^{26,42,43} On the other hand, an association between the *Cys326* polymorphism and increased risk of developing breast cancer was demonstrated in postmenopausal Thai women.³⁹ The

power calculations in breast cancer risk of this polymorphism are inconsistent; the major limitation in most case-control studies is ascribed to differential frequencies among genetically susceptible individuals in different ethnic backgrounds. In addition, numerous epidemiological findings indicate that high levels of 8-oxoG linked *OGGI* polymorphism on cancer risk modified by environmental chemicals differ between ethnicities.^{39,42–45} As a result, it is important to note that the growing list of nonsynonymous polymorphisms in the *OGGI* gene may provide candidates for further investigations of breast cancer.

It has been demonstrated that the homozygous genotype for *ERCC5 1104Asp* is associated with reduced risk in breast and lung cancer in African Americans.^{28,46} However, no statistically significant difference in DNA repair kinetics has been found between *1104His/His* and *1104Asp/Asp* genotypes.⁴⁷ In this study, we found that carriers with the *ERCC5 1104Asp* and *ERCC2 751Gln* genotypes have a joint effect of increased susceptibility of breast cancer (Table 3). In addition, we focused on understanding the effect of polymorphisms in the *ERCC2* and *ERCC5* genes on modifying the association between

estrogen and breast cancer. Our data hereby suggest that rather than being high-penetrance alleles, the subtle effect of the genotypic polymorphism of an individual gene would predispose carriers to a higher risk of developing cancer. Furthermore, the enhanced cancer risk may be due to interactions between an increasing number of genetic variants after adjusting for environmental risk factors, i.e., the effects of interactions between polymorphisms in the *ERCC2* and *ERCC5* genes and estrogen exposure on breast cancer susceptibility. Therefore, a large sample size is required to reinforce the role of the *ERCC5* polymorphism in individuals vulnerable to damaged DNA caused by oxidative stress due to environmental hormones, and there is a need for extensive information about lifestyle factors that may correlate with susceptibility to cancer disease.

On the basis of our understanding that reproductive risk factors may initiate breast tumorigenesis by causing various forms of oxidative DNA damage, the effects of estrogen on breast cancer are subject to individual variations in the ability of DNA repair enzymes to counteract the effects of ROS generated by estrogen metabolism.^{17,48–50} The increased but nonsignificant risk associations between DNA repair gene polymorphisms and breast cancer shown in previous studies may be the result of a lack of consideration of estrogen-related risk factors.^{44,51–53} Those associations may lack an adequate spectrum of reproductive risk factors because such categorizations exclude the inclusive significances of an early induction period, long duration of exposure, or prevention from the high latency of estrogen-related ROS, which may correlate with breast cancer risk. In the stratified method, on the other hand, we were aware of whether breast cancer risk associated with at least one putative high-risk genotype was modified by estrogen-related risk factors. The present study used a case–control design to figure out the associations between polymorphisms in the DNA repair genes and breast cancer risk, which, in theory, might be subjected to differential recall bias in the estrogen-related variables due to inappropriate information provided by both case and control groups. However, considerable efforts, including the application of a standardized interview to ensure the validity of information collected by questionnaire, were made to avoid such biases. Our observation that the risk profiles of women with different estrogen exposure statuses defined in this series of patients were similar to those reported in other breast cancer studies and were consistent with the current understanding of breast tumorigenesis provides solid justification of our study results.^{13,17,19,54}

Because the molecular mechanism underlying tumorigenesis extends from single-gene mechanisms to multigenic or etiological pathway-wide networks, the consideration of whether there is a link between a putative

cancer-associated gene and tumor development might be extended to entire tumorigenic networks.^{13,17,28,55,56}

Recently, an increased risk of breast cancer has been reported to be associated with increased frequency of polymorphisms in genes participating in estrogen metabolism and DNA double-strand break repair, leading to the carcinogenic effect of estrogen metabolite-induced ROS on breast epithelial cells.^{17,19,54} Therefore, we adopted the concept that epistatic analysis can improve statistical power for detection of the influence of genetic effects on breast cancer risk. To the best of our knowledge, the present study is the first to shed light on individual susceptibility genotypes with suboptimal DNA repair capacities in a combination of BER- and NER-related genes that permits sufficient accumulation of damaged DNA derived from prolonged exposure to estrogen, which then allows breast cells to display growth advantages. Going beyond the current study of the tumorigenic effects of estrogen on breast cancer due to generation of DNA damage, given the etiologic role of other environmental risk factors, such as cigarette smoking, polyaromatic hydrocarbon metabolites, and ion irradiation, in stimulating cell proliferation and DNA adduct formation, which in turn leads to neoplasia in cancers, it would be very informative to be able to identify additional genetic factors. The present breast cancer study shows that polymorphisms in BER- and NER genes may potentially help with risk prediction.

Our study design for genetic investigation integrates both gene-to-gene and gene-to-environment interactions to determine the molecular mechanism underlying breast cancer complexity. In conclusion, susceptibility to breast cell tumorigenesis is clearly influenced by genetic variants of DNA repair genes in association with estrogen-related risk factors. These multiple interactions are potentially involved in female breast oncology and therefore may be applied in identifying novel functional genotypic polymorphisms in susceptible populations.

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