Polymorphisms in the DNA Repair Gene *XRCC1* and Breast Cancer[1]

Eric J. Duell,[2] Robert C. Millikan,[3] Gary S. Pittman, Scott Winkel, Ruth M. Lunn, Chiu-Kit J. Tse, Allison Eaton, Harvey W. Mohrenweiser, Beth Newman, and Douglas A. Bell

Department of Epidemiology, School of Public Health, Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599 [E. J. D., R. C. M., S. W., C-K. J. T., A. E.]; Laboratory of Computational Biology and Risk Assessment, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 [G. S. P., R. M. L., D. A. B.]; Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94550 [H. W. M.]; and School of Public Health, Queensland University of Technology, Kelvin Grove, QLD 4059, Australia [B. N.]

Abstract

X-ray repair cross complementing group 1 (*XRCC1*) encodes a protein involved in base excision repair. We examined the association of polymorphisms in *XRCC1* (codon 194 Arg→Trp and codon 399 Arg→Gln) and breast cancer in the Carolina Breast Cancer Study, a population-based case-control study in North Carolina. No association was observed between *XRCC1* codon 194 genotype and breast cancer, and odds ratios (ORs) were not modified by smoking or radiation exposure. A positive association for *XRCC1* codon 399 Arg/Gln or Gln/Gln genotypes compared with Arg/Arg was found among African Americans (253 cases, 266 controls; OR = 1.7, 95% confidence interval, 1.1–2.4) but not whites (386 cases, 381 controls; OR =1.0, 95% confidence interval, 0.8–1.4). Among African-American women, ORs for the duration of smoking were elevated among women with *XRCC1* codon 399 Arg/Arg genotype (trend test; $P < 0.001$) but not Arg/Gln or Gln/Gln ($P = 0.23$). There was no difference in OR for smoking according to *XRCC1* codon 399 genotype in white women. ORs for occupational exposure to ionizing radiation were stronger for African-American and white women with codon 399 Arg/Arg genotype. High-dose radiation to the chest was more strongly associated with breast cancer among white women with *XRCC1* codon 399 Arg/Arg genotype. Our results suggest that *XRRC1* codon 399 genotype may influence breast cancer risk, perhaps by modifying the effects of environmental exposures. However, interpretation of our results is limited by incomplete knowledge regarding the biological function of *XRCC1* alleles.

Introduction

Epidemiological studies using functional measurements of DNA repair suggest that DNA repair capability is variable within human populations (1–3). Because inactivating mutations in DNA repair genes are rare (4), it has been hypothesized that variation in DNA repair capability in the general population is a product of combinations of multiple alleles that show subtle variations in biological function (3). In support of this hypothesis, investigators at Lawrence Livermore National Laboratory recently discovered common variants in a large number of DNA repair genes (5), and it is proposed that those variants may act in combination with environmental factors to increase susceptibility to human cancer (3). A possible role for DNA repair deficiencies in cancer development has been the subject of increasing interest. In particular, previous studies suggested that breast cancer patients might be deficient in the repair of radiation-induced DNA damage (6–10). Several of these studies reported reduced DNA repair capacity in family members of breast cancer cases, suggesting a potential genetic contribution to radiation sensitivity.

One of the DNA repair genes exhibiting polymorphic variation is *XRCC1*, which is located on chromosome 19q13.2 and encodes a M_r 70,000 protein (11). XRCC1 has no known catalytic activity, but appears to play a pivotal role in BER[4] by bringing together DNA polymerase β, DNA ligase III, and PARP at the site of DNA damage (12–16). BER targets endogenous DNA damage induced through hydrolysis, oxidative stress, and alkylation, as well as adducts and fragmented bases caused by exogenous agents such as ionizing radiation and alkylating or oxidative agents (4, 17–19). Thus, XRCC1 may participate in the removal of "non-bulky" DNA adducts, the repair of oxidative DNA damage, and the repair of DNA damage attributable to ionizing radiation (14, 20, 21). Shen *et al.* (5) identified nonconservative amino acid substitutions in conserved regions of *XRCC1*, including an arginine to tryptophan change at codon 194 (C→T) in exon 6 and an arginine to glutamine change at codon 399 (G→A) in exon 10. The functional characteristics of these alleles are unknown, but the codon 399 variant lies within the BRCT-1 domain of *XRCC1* (codons 314–402; Ref. 22). The BRCT-1 domain is a region with extensive homology to *BRCA1* (22, 23) and includes a binding site for PARP (codons 301–402; Ref. 16). Chinese hamster ovary cell lines with nonconservative amino acid substitutions in the BRCT-1 domain of *XRCC1* show a reduced

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[2] Present address: Department of Cancer Cell Biology, Division of Radiobiology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.
[3] To whom requests for reprints should be addressed, at Department of Epidemiology, CB# 7400, School of Public Health, University of North Carolina, Chapel Hill, NC 27599-7400. Phone: (919) 966-7437; Fax: (919) 966-2089; E-mail: bob_millikan@unc.edu.

[4] The abbreviations used are: BER, base excision repair; CBCS, Carolina Breast Cancer Study; CI, confidence interval; nt, nucleotide; OR, odds ratio; q, allele frequency; PARP, poly(ADP-ribose) polymerase; XRCC1, X-ray repair cross complementing group 1.

ability to repair single-strand breaks and a hypersensitivity to ionizing radiation (24). Hu *et al.* (25) examined the association of a polymorphism in a *PARP* pseudogene and breast cancer in a case-control study. The authors did not observe a significant association of *PARP* genotype and disease, but PARP activity was reduced among breast cancer cases compared with controls. Sturgis *et al.* (26) recently evaluated two variants in *XRCC1* and observed increased ORs for the codon 399 Gln allele and cancers of the head and neck among current smokers.

We examined the role of *XRCC1* as a candidate susceptibility gene for breast cancer using DNA samples and exposure information collected from participants in the CBCS, a population-based case-control study of African-American and white women in North Carolina. We estimated the main effects for *XRCC1* codon 194 and codon 399 genotypes, as well as modification of ORs for two environmental factors, ionizing radiation and cigarette smoking. Single strand breaks and base damage induced by ionizing radiation and oxidative stress are repaired through BER-dependent pathways (4, 18, 19). High-dose exposure to ionizing radiation is an established cause of breast cancer (27, 28), but the effects of low-dose exposure through occupational practices or diagnostic procedures are controversial (28, 29). Modification of ORs for smoking and breast cancer were examined because exposure to cigarette smoke can lead to oxidative DNA damage (30), and the role of smoking in breast carcinogenesis is unclear (31). We hypothesized that less common (variant) alleles in the BER gene *XRCC1* could be associated with an increased risk of smoking- or radiation-induced breast cancer.

Materials and Methods

Study Design. The present study used participants from the CBCS (1993–1996), a population-based, case-control study of breast cancer conducted in 24 counties of central and eastern North Carolina (32). Incident cases of invasive breast cancer were identified using a Rapid Case Ascertainment System in cooperation with the North Carolina Central Cancer Registry. Controls were identified from Division of Motor Vehicle and Medicare lists. Randomized recruitment (a form of probability sampling) was used to frequency-match controls to cases on the basis of 5-year age intervals and race and to oversample African-American participants and women under the age of 50 (33). A total of 862 cases and 790 controls were enrolled. Response rates were 74% for cases and 53% for controls (34). Over 98% of cases and controls agreed to provide a blood sample. Interviews were completed in participants' homes.

Laboratory Methods. DNA was extracted from peripheral blood lymphocytes using standard methods (35). For codon 194 of *XRCC1*, genotyping was completed on 412 cases (161 African-American and 251 white) and 400 controls (166 African-American and 234 white) in order of enrollment in the CBCS. Variants in codon 194 were detected using a multiplex PCR-RFLP assay that included codons 194 and 399, as described by Lunn *et al.* (36). Two reviewers independently scored all genotypes. Genotypes were repeated on a 10% random sample of participants, and in all cases, these results were found to agree with the initial analysis. For codon 399 of *XRCC1*, we completed genotyping on 639 cases (253 African American and 286 white) and 647 controls (266 African American and 381 white). The multiplex PCR-RFLP assay (36) described previously was used for the first 412 cases and 400 controls. The remaining samples were genotyped for codon 399 using a 5′-exoncuclease (Taqman) assay and the ABI Prism SDS 7700 system (PE Applied Biosystems). The two assay methods yielded complete agreement on a 10% repeat sample.

For the Taqman assay, PCR primers and probes were designed using Primer Express software (PE Applied Biosystems). Assay design and conditions were based on the allelic discrimination protocol from PE Applied Biosystems. The nt 28152 G (Arg) allele probe was labeled on the 5′ end with the VIC (PE Applied Biosystems) reporter dye and contained the following nt sequence: 5′-CTGCCCTCCCGGAGGTAAGGC-3′. The melting temperature was 66.3°C. The nt 28152 A (Gln) allele probe was labeled on the 5′ end with the 6-carboxyfluorescein reporter dye and contained the following nt sequence: 5′-CTGCCCTCCCAGAGGTAAGGCC-3′. Both probes contained the quencher dye 6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine on the 3′ end. Forward and reverse primers were used to amplify the region surrounding the nt 21852 polymorphism. The nt sequence for the forward primer was 5′-GAGTGGGTGCTGGACTGTCA-3′. The melting temperature was 58.2°C, with a 60% G:C content and 20 bp in length. The nt sequence for the reverse primer was listed 3′-CTTATCCTGTGCTGGGCAA-5′. The melting temperature was 58.3°C with a 58% G:C content and 19 bp in length. PCR reactions were performed in a 50-ml reaction volume using the hot-start format. The reaction components were as follows: 1× Taqman Universal PCR Master Mix, 250 nM of each primer, 200 nM wild-type probe, 200 nM mutant probe, and 25 ng of genomic DNA. The PCR was run on a Perkin-Elmer GenAmp 9700 thermocycler under the following conditions: 50°C for 2 min (AmpErase UNG Activation), 95°C for 10 min (AmpliTaq Gold Activation), and then 40 cycles of 95°C for 15 s (denature) and 62°C for 1 min (anneal/extend). Samples that could not be scored were repeated. Unreadable results on the second run were scored as missing ($n = 41$). In addition to comparing Taqman results with the PCR-RFLP assay, we repeated the Taqman assay on a 10% sample and results were identical to the initial analysis.

Statistical Methods. qs were calculated as the number of alleles divided by the number of chromosomes. Genotype frequencies were calculated as the number of participants with a particular genotype divided by the total number of participants. Case-control differences were assessed using χ^2 tests. Departures from Hardy-Weinberg equilibrium were assessed by comparing expected genotype frequencies (based on observed qs) to observed genotype frequencies. Statistical significance was evaluated using χ^2 tests.

ORs and 95% CIs were calculated using unconditional logistic regression. PROC GENMOD in SAS (SAS Institute, Cary, NC) was used to adjust for age (as an 11-level ordinal variable reflecting 5-year age categories), race (African-American, white), and to incorporate offset terms derived from sampling fractions used to identify eligible participants. Race was classified according to self-report. Less than 2% of study participants listed their race as Native American, Hispanic, Asian-American, or "multi-racial," and these women were classified as "white" in the analysis. Results were unchanged when we adjusted for age as a continuous variable. ORs were not altered significantly after adjustment for age at first birth, parity, age at menarche, family history of breast cancer, history of breast biopsy, alcohol consumption, history of breast-feeding, smoking (for radiation effects), or radiation exposure (for smoking effects). Therefore, ORs are presented adjusted for age only.

Menopausal status was assigned as follows. (*a*) Women were classified as postmenopausal if they had undergone natural menopause, bilateral oophorectomy, or irradiation to the ovaries; and (*b*) women 50 and over were classified as postmenopausal if they had ceased menstruation or if they were

Table 1 *XRCC1* codon 194 (exon 6) allele frequencies, genotype frequencies, and ORs for breast cancer

	Cases	Controls
African Americans	*n* = 161	*n* = 166
Allele		
Arg	0.95	0.94
Trp	0.05	0.06
χ^2:[a] *P* = 0.4		
Genotype		
Arg/Arg	141 (91.0%)	140 (87.5%)
Arg/Trp	13 (8.4%)	20 (12.5%)
Trp/Trp	1 (0.6%)	0 (0%)
Missing	6	6
χ^2:[a] *P* = 0.3		
OR[b] (Arg/Trp or Trp/Trp *vs.* Arg/Arg) = 0.7 (95% CI 0.3–1.5)		
Whites	*n* = 251	*n* = 234
Allele		
Arg	0.95	0.93
Trp	0.05	0.07
χ^2:[a] *P* = 0.2		
Genotype		
Arg/Arg	209 (89.7%)	190 (86.0%)
Arg/Trp	24 (10.3%)	29 (13.1%)
Trp/Trp	0 (0%)	2 (0.9%)
Missing	18	13
χ^2:[a] *P* = 0.2		
OR[b] (Arg/Trp or Trp/Trp *vs.* Arg/Arg) = 0.7 (95% CI 0.4–1.3)		

[a] Comparing cases and controls.
[b] Adjusted for age.

Table 2 *XRCC1* codon 399 (exon 10) allele frequencies, genotype frequencies, and ORs for breast cancer

	Cases	Controls
African Americans	*n* = 253	*n* = 266
Allele		
Arg	0.81	0.86
Gln	0.19	0.14
χ^2:[a] *P* = 0.02		
Genotype		
Arg/Arg	164 (65%)	198 (74%)
Arg/Gln	82 (32%)	64 (24%)
Gln/Gln	7 (3%)	4 (2%)
Missing	6	5
χ^2:[a] *P* = 0.05		
OR[b] (Arg/Gln or Gln/Gln *vs.* Arg/Arg) = 1.7 (1.1–2.4)		
Whites	*n* = 386	*n* = 381
Allele		
Arg	0.65	0.64
Gln	0.35	0.36
χ^2:[a] *P* = 0.73		
Genotype		
Arg/Arg	162 (42%)	164 (43%)
Arg/Gln	175 (45%)	158 (41%)
Gln/Gln	49 (13%)	59 (16%)
Missing	18	12
χ^2:[a] *P* = 0.41		
OR[b] (Arg/Gln or Gln/Gln *vs.* Arg/Arg) = 1.0 (0.8–1.4)		

[a] Comparing cases and controls.
[b] Adjusted for age.

taking hormone replacement therapy, regardless of menstrual status. Participants who smoked <100 cigarettes over their lifetime were classified as never smokers. Women who smoked on the reference date (date of diagnosis in cases or selection in controls) were designated current smokers, whereas women who no longer smoked on the reference date were classified as former smokers. Duration of smoking was calculated by asking the participant to sum the total number of years that they smoked regularly.

Occupational exposure to ionizing radiation was based on participants' reports of the two jobs held longest since age 18 (37). These were classified according to potential exposure to radiation using the most recent International Commission of Radiological Protection classification (38). In the CBCS, jobs with potential exposure to ionizing radiation included nurse, medical doctor, and X-ray technician (37). Participants were also asked about exposure to ionizing radiation through medical procedures. High-dose radiation to the chest included coronary catheterization, coronary angioplasty, and treatment of the upper body with radiation (excluding treatment or diagnosis for breast cancer).

Here, we report only the results of modification of ORs for breast cancer and smoking or exposure to ionizing radiation by *XRCC1* genotypes. Effect measure modification was assessed on a multiplicative scale by calculating ORs for environmental factors after stratifying on *XRCC1* genotypes. Effect measure modification was also assessed on an additive scale by calculating ORs for the combined effect of genotype and environmental exposures (data not shown). In the subset of data where both genotypes were available (412 cases and 400 controls), we examined the combined effect of codon 194 and codon 399 alleles on an additive scale by calculating ORs for combinations of alleles, using codon 194 Arg/Arg and codon 399 Arg/Arg homozygotes as a common referent group.

T tests were used to compare age at onset in cases across *XRCC1* genotypes. Tests for trend were conducted by calculating the *P* for the β coefficient of smoking duration coded as an ordinal variable.

Results

Characteristics of cases and controls have been presented previously (39). The distribution of traditional risk factors for breast cancer in the present dataset did not differ from the CBCS as a whole (data not shown). Mean age was 50.5 years for cases and 51.6 for controls. Cases were 50% premenopausal and 50% postmenopausal, and controls were 46% premenopausal and 54% postmenopausal.

qs, genotype frequencies, and ORs for breast cancer and the *XRCC1* codon 194 variant are presented in Table 1. Allele and genotype frequencies were similar in African Americans and whites, and there were no statistically significant case-control differences in either group. No significant departures from Hardy-Weinberg equilibrium were observed among cases or controls of either racial group (χ^2; $P \geq 0.6$ for each group). ORs for breast cancer and one or more copies of the *194Trp* allele were similar among African Americans and whites. Combining both racial groups, the age and race-adjusted OR for 194 Trp/Trp and Trp/Arg genotypes *versus* 194 Arg/Arg was 0.7 (95% CI, 0.5–1.1).

Results for the *XRCC1* codon 399 variant are presented in Table 2. The codon Gln allele was more common among white controls (q = 0.36) than African-American controls (q = 0.14). Among African Americans, the codon 399 Gln allele was more common among cases than controls ($P = 0.02$; Table 2). There were no significant departures from Hardy-Weinberg equilibrium among cases or controls of either racial group ($P \geq 0.35$ for each group). A positive association between codon 399 Arg/Gln or Gln/Gln genotype and breast cancer was observed in African Americans but not in whites (Table 2). Among African Ameri-

Table 3 ORs for breast cancer and smoking and breast cancer and radiation exposure stratified by *XRCC1* codon 399 genotype among African-American participants

	XRCC1 codon 399 genotype			
	Arg/Gln or *Gln/Gln*		*Arg/Arg*	
	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[a]
Never smoker	52/35	Referent	86/126	Referent
Current smoker	13/21	0.4 (0.2–0.9)	30/35	1.4 (0.8–2.4)
Former smoker	24/12	1.4 (0.6–3.2)	48/37	2.2 (1.3–3.6)
Duration of smoking (yr)				
Never	52/35	Referent	86/126	Referent
≤10	14/7	1.4 (0.5–3.9)	19/24	1.2 (0.6–2.4)
11–20	6/8	0.5 (0.2–1.6)	19/24	1.3 (0.7–2.6)
>20	17/18	0.7 (0.3–1.5)	39/23	2.9 (1.6–5.2)
P for trend test		0.23		<0.001
High-dose radiation to chest				
No	83/62	Referent	152/183	Referent
Yes	5/6	0.7 (0.2–2.3)	12/15	1.0 (0.4–2.2)
Occupational exposure to ionizing radiation				
No	82/63	Referent	153/190	Referent
Yes	7/5	1.1 (0.4–3.8)	11/8	1.9 (0.7–4.8)

[a] Adjusted for age.

Table 4 ORs for breast cancer and smoking and breast cancer and radiation exposure stratified by *XRCC1* codon 399 genotype among white participants

	XRCC1 codon 399 genotype			
	Arg/Gln or *Gln/Gln*		*Arg/Arg*	
	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[a]
Never smoker	105/102	Referent	82/83	Referent
Current smoker	42/47	0.9 (0.5–1.4)	29/33	0.9 (0.5–1.6)
Former smoker	77/68	1.0 (0.7–1.6)	51/48	1.1 (0.7–1.9)
Duration of smoking (yr)				
Never	105/102	Referent	82/83	Referent
≤10	41/33	1.0 (0.6–1.8)	16/21	0.6 (0.3–1.3)
11–20	21/33	0.6 (0.3–1.1)	23/22	1.0 (0.5–2.0)
>20	57/49	1.2 (0.8–2.0)	41/38	1.3 (0.7–2.2)
P for trend test		0.85		0.44
High-dose radiation to chest				
No	207/203	Referent	148/155	Referent
Yes	17/14	1.2 (0.6–2.5)	14/9	1.9 (0.8–4.7)
Occupational exposure to ionizing radiation				
No	211/211	Referent	147/159	Referent
Yes	13/6	2.3 (0.8–6.2)	15/5	3.3 (1.2–9.4)

[a] Adjusted for age.

cans, ORs were 2.2 (95% CI, 0.6–7.8) for Gln/Gln and 1.6 (95% CI, 1.1–2.4) for Arg/Gln genotypes compared with Arg/Arg. The corresponding ORs in whites were 0.8 (95% CI, 0.5–1.3) and 1.1 (95% CI, 0.8–1.5). Combining all participants, the overall age and race-adjusted OR comparing 399 Gln/Gln and Arg/Gln genotypes with 399 Arg/Arg was 1.2 (95% CI, 1.0–1.5).

ORs for *XRCC1* codon 194 and codon 399 genotypes did not differ according to menopausal status (data not shown). There was no evidence for a combined effect of codon 194 and codon 399 alleles. Using 194 Arg/Arg and 399 Arg/Arg compound homozygotes as a common referent group, age- and race-adjusted ORs were 1.1 (95% CI, 0.8–1.5) for 194 Arg/Arg and 399 Arg/Gln or Gln/Gln; 0.7 (95% CI, 0.4–1.2) for 194 Arg/Trp or Trp/Trp and 399 Arg/Arg; and 0.9 (95% CI, 0.4–2.1) for 194 Arg/Trp or Trp/Trp and 399 Arg/Gln or Gln/Gln.

No differences in ORs for environmental factors were observed, according to *XRCC1* codon 194 genotype (data not shown). ORs for breast cancer and smoking and breast cancer and radiation exposure stratified by *XRCC1* codon 399 genotype are presented for African Americans in Table 3 and for whites in Table 4. Among African-American women, there was a modest positive association for smoking in the past, and a statistically significant association with duration of smoking, for participants with codon 399 Arg/Arg genotype. No association was observed for high-dose radiation to the chest in either genotype group, whereas the OR for occupational exposure to ionizing radiation was stronger among women with the Arg/Arg genotype (Table 3). Among white women, there was a weak positive association with longer duration of smoking in each genotype group. ORs for high-dose radiation to the chest and occupational exposure to ionizing radiation were higher among white women with Arg/Arg compared with Arg/Gln or Gln/Gln genotypes (Table 4). Results were unchanged when we stratified on menopausal status or age (data not shown).

Age at onset of disease did not differ among breast cancer cases according to *XRCC1* codon 194 or 399 genotype. For

codon 194, mean age was 51 (SD = 12.4) for women with Arg/Arg genotype and 50 (11.2) for Arg/Trp or Trp/Trp (P = 0.48). For codon 399, mean age was 51 (12.4) for Arg/Arg genotype and 51 (12.2) for Arg/Gln or Gln/Gln (P = 0.86).

Discussion

We examined *XRCC1* as a candidate susceptibility gene for breast cancer in a population-based case-control study of African-American and white women in North Carolina. We found no association between *XRCC1* codon 194 genotype and breast cancer. A positive association was observed for *XRCC1* codon 399 Arg/Gln or Gln/Gln genotypes compared with Arg/Arg among African Americans (OR = 1.7; 95% CI ,1.1–2.4) but not whites (OR = 1.0; 95% CI, 0.8–1.4). We found no evidence for a combined effect of the *194Trp* and *399Gln* alleles and breast cancer. Surprisingly, among African-American women, *XRCC1* codon 399 Arg/Arg homozygotes showed a stronger positive association for smoking than women with Arg/Gln or Gln/Gln genotypes. Among African-American and white women, ORs for radiation exposure were higher among women with Arg/Arg genotype. We observed no modification of ORs for smoking and radiation exposure, according to *XRCC1* codon 194 genotype. These findings suggest that *XRCC1* codon 399 genotype may be related to breast cancer risk. However the direction and magnitude of associations observed in this study are difficult to interpret on the basis of current knowledge of the functional status of *XRCC1* alleles.

Functional studies of *XRCC1* suggest that the codon 399 Gln allele may be associated with multiple DNA damage phenotypes in human cells and tissues. Lunn *et al.* (36) reported that the *399Gln* allele was associated with an increased aflatoxin DNA adduct burden in placental tissue and an elevated glycophorin A mutant frequency in erythrocytes. Duell *et al.* (40) reported a positive association between the same allele and detection of polyphenol DNA adducts from blood mononuclear cells, as well as a positive association between the variant *399Gln* allele and baseline sister chromatid exchange frequencies in lymphocytes from smokers. Together, these studies suggest a role for *XRCC1* in the repair of multiple DNA damage end points in human cells and tissues, and imply that the *399Gln* allele of *XRCC1* has an important, potentially harmful phenotype. Consistent with this hypothesis, two recent epidemiological studies have shown a positive association between the *XRCC1* Gln allele and cancer. Sturgis *et al.* (26) reported an OR of 1.6 (95% CI, 1.0–2.6) for the *XRCC1* codon 399 Gln/Gln genotype in a case-control study of head and neck cancer, and Divine *et al.* (41) observed an odds ratio of 2.8 (95% CI, 1.2–7.9) for *XRCC1* codon 399 Gln/Gln genotype in a case-control study of lung cancer. However, three other epidemiological studies reported contrary findings. Stern *et al.* (42) observed an inverse association between *XRCC1* codon 399 Gln/Gln genotype and bladder cancer; ORs for smoking were stronger among carriers of the codon 399 Arg/Arg genotype compared with Gln-containing genotypes. Similarly, Watson *et al.* (43) reported an inverse association between *XRCC1* codon 399 Gln/Gln genotype and cancer of the head and neck; ORs for smoking were stronger among carriers of the codon 399 Arg/Arg genotype. Nelson *et al.* (44) found an inverse association between *XRCC1* codon 399 Gln-containing genotypes and risk of non-melanoma skin cancer.

Our finding of a positive association between the *XRCC1* codon 399 Gln/Gln genotype and breast cancer is consistent with two previous epidemiological studies (26, 41) as well as with published functional studies (36, 40). However, the fact that a positive association was observed only among African Americans suggests that unmeasured genetic factors (*e.g.*, other alleles in linkage disequilibrium with the codon 399 Gln allele), as well as unmeasured environmental factors, could be responsible for the observed effect. Our observation of stronger associations between smoking and breast cancer and radiation exposure and breast cancer among carriers of the *XRCC1* Arg/Arg genotype was contrary to our *a priori* hypothesis. On the basis of functional studies (36, 40), we expected that ORs for smoking and radiation exposure would be elevated among *XRCC1* codon 399 Gln carriers.

There are several potential explanations for our findings. We cannot rule out chance or random associations. To address this possibility, we conducted genotyping for *XRCC1* codon 399 on additional DNA samples from the CBCS, and the results did not change. Selection bias could have played a role, because the response rate was lower among controls than cases. Previous analyses (39, 45) have shown that ORs for traditional breast cancer risk factors in the CBCS are similar to those reported in the literature, and comparisons of our controls with surveys of the North Carolina population do not reveal significant differences in prevalence of smoking and other risk factors (34, 39). It is unlikely that the *XRCC1* genotype was related to participation in the study, because the genotypes are in Hardy-Weinberg equilibrium, and genotype frequencies among controls are similar to previous studies of both African Americans and whites (26, 36, 40–44).

It is biologically plausible that the *XRCC1* Arg allele, rather than the Gln allele, could increase breast cancer risk in the presence of specific environmental exposures. To explore further the functional role of *XRCC1* alleles, we examined the association between the *XRCC1* genotype and the occurrence of somatic mutations in the tumor suppressor gene, *P53*.[5] Breast cancer cases from the CBCS with the codon 399 Arg/Arg genotype who were exposed to occupational radiation had a higher prevalence of *P53* deletions in breast tumors when compared with exposed cases with Gln-containing genotypes or unexposed cases of either genotype. Similarly, cases with codon 399 Arg/Arg genotype who smoked had a higher prevalence of transversion mutations in *P53* compared with Gln carriers who smoked, as well as unexposed cases with either genotype. The *P53* results are compatible with the patterns observed when we compared ORs for smoking and radiation across categories of *XRCC1* codon 399 genotype, but are not consistent with the aforementioned functional assays (36, 40). The discrepancy could result if assays using peripheral blood cells or placental cells do not duplicate metabolic conditions within breast tissue. DNA repair systems have overlapping substrate specificity and contain sufficient functional redundancy such that deficiencies in one pathway are compensated for by other pathways, depending upon the tissue or organ being studied (4). Our results for smoking and *XRCC1* are consistent with some epidemiological studies (42–44), but not with others (26, 41). Differences in results across epidemiological studies could occur if the biological effects of *XRCC1* codon 399 alleles depend upon context. As recently pointed out by Weiss and Terwilliger (46), the effects of any given genetic variant will depend upon other genetic as well as environmental factors that interact with that variant. Thus, the effects of *XRCC1* alleles potentially depend upon competing biochemical pathways operating in the tissue being analyzed, as well as on differing distributions of genetic and environmental factors in the study population.

We conclude that *XRCC1* genotype may be related to breast cancer risk, but this relationship is complex. Additional information is needed, including functional studies correlating

[5] R. Millikan, S. Edmiston, and K. Conway. *XRCC1* genotype and occurrence of somatic P53 mutations in breast tumors from the CBCS, manuscript in preparation.

genotype and phenotype for specific *XRCC1* alleles within breast tissue. Our results and those of other recent epidemiological studies of *XRCC1* suggest that BER represents an important biochemical pathway for future epidemiological studies of cancer. Polymorphisms in several genes within the BER pathway have been discovered (5). These polymorphisms could help to clarify the contribution of smoking, ionizing radiation, and other environmental exposures to cancer risk. However, functional studies both *in vitro* and *in vivo* will also be required for the remaining genes within the BER pathway.

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