Polymorphisms in XRCC1 Modify the Association between Polycyclic Aromatic Hydrocarbon-DNA Adducts, Cigarette Smoking, Dietary Antioxidants, and Breast Cancer Risk

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Abstract

The variability in DNA repair capacity of the general population may depend in part upon common variants in DNA repair genes. X-ray repair cross complementing group 1 (XRCC1) is an important DNA base excision repair gene and exhibits polymorphic variation. Using the Long Island Breast Cancer Study Project, a population-based case-control study, we evaluated the hypothesis that two common single nucleotide polymorphisms of XRCC1 (codon 194 Arg→Trp and 399 Arg→Gln) influence breast cancer susceptibility and interact with polycyclic aromatic hydrocarbon (PAH)-DNA adducts, cigarette smoking, and intake of fruits and vegetables and antioxidants. The available sample for genotyping included 1,067 cases and 1,110 controls. Genotyping was done by a high-throughput single-nucleotide extension assay with fluorescence polarization detection of the incorporated nucleotide. We observed no significant increases in risk among all subjects who were carriers of XRCC1 194Trp or 399Gln alleles. Among never smokers, we observed an increased risk of breast cancer in 399Gln carriers [odds ratio (OR), 1.3; 95% confidence interval (CI), 1.0-1.7]. Further analysis indicated a suggestive weak additive interaction between the 399Gln allele and detectable PAH-DNA adducts (OR for exposure with mutant genotype, 1.9; 95% CI, 1.2-3.1). The estimated age-adjusted interaction contrast ratio (ICR) and 95% CI (ICR, 0.38; 95% CI, –0.32 to 1.10) indicated that the departure from additivity was not statistically significant, but that there was some suggestion of a relative excess risk due to the interaction. In subjects with at least one copy of XRCC1 194Trp allele, there was a moderate interaction with high intake of fruits and vegetables (≥35 half-cup servings per week of any fruits, fruit juices, and vegetables, OR, 0.58; 95% CI, 0.38-0.89; ICR, –0.49; 95% CI, –0.03 to –0.95), and dietary plus supplement antioxidant intake with 33% to 42% decreases in breast cancer risk compared with those with the Arg194Arg genotype and low-intake individuals. These results do not show that the two genetic polymorphisms of XRCC1 independently influence breast cancer risk. However, there is evidence for interactions between the two XRCC1 single nucleotide polymorphisms and PAH-DNA adducts or fruit and vegetable and antioxidant intake on breast cancer risk. Further understanding of the biological function of XRCC1 variants and their interactions with PAH-DNA adducts, antioxidants, and other genes in the pathway are needed.

Introduction

Whether the risk of breast cancer is increased in relation to environmental exposures such as polycyclic aromatic hydrocarbons (PAH) or by decreased intake of dietary and/or supplemental antioxidants is unclear. The Long Island Breast Cancer Study Project (LIBCSP) reported a modest increase in risk among women with the highest level of mononuclear cell PAH-DNA adducts, but there was no dose response (1). Little or no elevation in risk was noted for cigarette smoking (2). Numerous nutritional epidemiologic studies have noted inverse associations between dietary antioxidants and breast cancer risk, including results from the LIBCSP (3), although some data are conflicting (4, 5). To help identify women who may be particularly susceptible to these environmental factors, we examined a low penetrance susceptibility DNA repair gene.

DNA-repair pathways are critical for maintaining the integrity of DNA and protecting against mutations due to exposure-induced damage or replication errors (6, 7). X-ray repair cross complementing group 1 (XRCC1) protein is involved in the base-excision repair pathway and plays a critical role in repairing DNA base damage and DNA single-strand breaks (8-10). Shen et al. (11) found two common single nucleotide polymorphisms: Arg194Trp and Arg399Gln, which are nonconservative amino acid changes and occur in evolutionarily conserved regions. That is, the wild-type and variant residues have dissimilar physical and/or chemical properties, suggesting that these substitutions may affect protein structure and potentially have functional relevance (6, 11).

In phenotypic studies, the codon 399Gln allele was associated with higher levels of genotoxic damage (12, 13), increased level of sister chromatid exchanges, chromosome deletions, polyphenol- and aflatoxin B1-DNA adducts, and chromosomal breaks (12, 14-16). However, the codon 194Trp allele seemed to be protective against DNA damage, although no significant difference was observed (12, 13). Others showed positive or no association with altered levels of DNA damage biomarkers (16, 17). Most molecular epidemiologic studies on XRCC1 Arg194Trp also reported that the 194Trp allele was associated with reduced risk of bladder, breast, lung, and stomach cancer and squamous cell carcinoma of the head and neck. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of antioxidants. Several reports have suggested that poly-
genome integrity from oxidative damage caused by low levels
repair system also plays a pivotal role in protecting the
inactivation of reactive oxygen species (22, 23). The DNA
supplements can prevent oxidative DNA damage through the
strand breaks (21). Intake of antioxidants via the diet or as
polymorphisms in XRCC1 and XRCC3 may influence repair of
bulky DNA adducts and oxidative DNA damage caused by
tobacco smoking, other PAH sources, or decreased intake of
antioxidants (15, 19, 24, 25).

In the present study, we hypothesized that polymorphisms of
XRCC1 in codon 194 and 399 were predictors of breast
cancer risk because of their associations with DNA repair
phenotypes (16). Because this gene is involved in the repair of
bulky DNA adducts and oxidative DNA damage caused by
PAH exposure, we also hypothesized that detectable blood
PAH-DNA adducts, as intermediate markers of both PAH
exposure and DNA damage caused by PAHs, might interact
with the two XRCC1 single nucleotide polymorphisms and
increase individual susceptibility to breast cancer. We also
examined whether high intake of antioxidants from diet and/or
supplemental sources, which may reduce the risk of breast
cancer, is modulated by the effect of functional polymorphisms of
XRCC1.

Materials and Methods

Study Design. The study methods of the LIBCSP, a
population-based case-control study have been described in
detail previously (1, 26). In brief, potentially eligible cases
were identified through frequent contact with the pathology
departments of 31 institutions in the Long Island-New York
City area. Verification of the diagnosis and consent for
recruitment was obtained from the diagnosing physician for
90.5% of potentially eligible case women. Case eligibility
included adult female residents of Nassau and Suffolk
counties on Long Island, NY, who were of any age or race,
who spoke English, and were newly diagnosed with in situ or
invasive breast cancer between August 1, 1996, and July 31,
1997. Potentially eligible controls were frequency matched to
the age distribution of the cases and identified through
random digit dialing for women ages $\geq 65$ years, and through
Health Care Finance Rosters for women ages $\geq 65$ years.
Eligible controls were defined as women who spoke English
and resided in the same Long Island counties as the cases but
with no personal history of breast cancer. This study was
conducted with approval from participating institutional
review boards, and in accordance with an assurance filed
with and approved by the U.S. Department of Health and
Human Services.

In-person interviews were completed for 82.1% of cases
($n = 1,508$) and 62.8% of controls ($n = 1,556$). The main
questionnaire assessed information on known and suspected
risk factors for breast cancer, including cigarette smoking,
alcohol use, menstrual and reproductive histories, hormone
use, dietary habits, prior disease history, and family history of
breast cancer (http://epi.grants.cancer.gov/LIBCSP/projects/
Questionnaire.html). Of those who completed an interview,
73.1% of cases (1,102) and 73.3% of controls (1,141) donated a
blood sample.

Laboratory Methods. Genomic DNA was extracted by
standard RNase-proteinase K and phenol-chloroform treat-
ment and genotyped by a fluorescence polarization method
using a commercial AcycloPrime fluorescence polarization
single nucleotide polymorphism detection kit (PerkinElmer
Life Sciences, Boston, MA). This technique distinguishes the
polymorphic base of a single nucleotide polymorphism by the
template-directed incorporation of a dye-labeled deoxyxynu-
cleotide onto an oligonucleotide primer that anneals just 5’
to the polymorphic base (27). Briefly, the first stage involves PCR
amplification of genomic DNA containing the single nucleo-
tide polymorphisms. The forward and reverse primers were
designed according to the human XRCC1 gene sequence
(GenBank accession no. L34079), which were, respectively, 5’-
ATGAGAGGCGCACTTCTCG-3’, 5’-CATCCTCTTCTCTT-
CACACC-3’ for XRCC1 Arg194Trp and 5’-CCCAAGTAT-
CAGCCAGGTTC-3’, 5’-ATTGCCACGAGGATAGG-3’ for
XRCC1 Arg399Gln. Stage 2 cleaned the PCR products by
digesting the excess primers and deoxynucleotides. The final
stage was primer extension and single nucleotide polymor-
phism analysis. The sequences of forward TDI probes were
designed using Primer 3 software (http://frodo.wi.mit.edu/
cgi-bin/primer3/primer3_www.cgi) as 5’-CGGGGCTCT-
TCTTCTCAGC-3’ for codon 194 and 5’-GTCCGGCGCT-
GCCCCTCCC-3’ for codon 399. Full technical details are
available on request. An automatically created genotype
cluster chart and output worksheet was in dimensionless units
(nM) as previously described (27). The assay was validated by
sequencing subjects with all three genotypes and these known
samples were used as positive controls on each plate. The
PAH-DNA adducts levels, determined by competitive ELISA,
was available from the parent study (1, 28).

The laboratory staff was blind to the identity of the subjects.
Duplicate quality control samples were randomly included in
genotyping, and 1.62% (4/246) was discordant. Genotyping
data were available for 1,067 (96.8%) cases and 1,110 (97.3%)
controls who donated blood, which represent 70.8% (1,067/
1,508) of eligible cases and 71.3% (1,110,1,556) of eligible
controls. PAH-DNA adducts were conducted on 873 (79.2%)
breast cancer cases and 941 (82.5%) controls who donated
blood, which were, respectively, 57.9% (873/1,508) and 60.5%
(941/1,556) of the eligible cases and controls (28). A total of 866
cases and 938 controls had both genotyping and PAH-DNA
adduct data.

Statistical Methods. Hardy-Weinberg Equilibrium was
tested to compare the observed and expected genotype
frequencies among cases and controls, respectively. $\chi^2$
tests for categorical variables were used to assess case-control
differences in frequencies of XRCC1 genotypes (29). Uncondi-
tional logistic regression with SAS version 9.0 was used to
estimate odds ratios (OR) and corresponding 95% confidence
intervals (CI), adjusting for matching by age at reference (date
of diagnosis for cases and identification for controls; ref. 30).
Covariates considered as potential confounders included age
at menarche, parity, lactation, months of lactation, age at first
birth, number of miscarriages, history of fertility problems,
body mass index [weight (kg)/height (m$^2$)] at reference, body
mass index at age 20, first-degree family history of breast
cancer, history of benign breast disease, menopausal status,
oral contraceptive use, hormone replacement use, race,
education, religion, and marital status (as previously defined;
refs. 1, 26, 28). The potential confounding variables were
assessed by examining the percent changes (>10%) in the ORs
of the main effects of genotyping.

Effect modification was assessed on a multiplicative scale
by calculating ORs for XRCC1 genotypes stratified by
indicators of race, stage of disease, menopausal status, family
history of breast cancer, cigarette smoking, alcohol drinking,
body mass index, and intake of fruits, vegetables, and
antioxidants levels as previously defined (1, 3, 26, 28).
running separate models for each exposure category and by including multiplicative interaction terms (use cross-product terms) in the logistic regression model. We further evaluated interactions on additive and multiplicative scales between XRCC1 genotypes and exposures (PAH-DNA adducts, cigarette smoking, and intake of dietary and/or supplemental antioxidants) by using indicator terms for those with the genotypes only, exposures only, and those with both the genotypes and exposures of interest. The magnitude of an additive interaction effect was determined by estimating the age-adjusted interaction contrast ratio (ICR) and 95% CI departures from additivity of effect with the following formula (31) and PROC LOGISTIC program in SAS (32): ICR = OR_{eg} - OR_e - OR_g + 1, where OR_{eg} is the OR for exposure with mutant genotype, OR_e is the OR for exposure with a wild-type genotype, and OR_g is the OR for mutant genotype among nonexposed. ICR >0 implies a greater than additive interaction effect (positive interaction), whereas ICR = 0 implies no interaction, and ICR <0 implies a less than additive effect (negative interaction).

All statistical tests were two-sided. Cigarette smoking was defined as never or ever smoking. PAH-DNA adducts data was assessed by a single binary variable detectable (>15% inhibition in the ELISA) versus nondetectable adducts. To assess diet for the 12 months before the reference date, 98% of participants completed a self-administered modified National Cancer Institute-Block food frequency questionnaire. Fruit and vegetable intake was calculated, and intake of carotenoids, ascorbic acid, and alpha-tocopherol were computed from food composition data as previously described (3). Assuming that the variant frequencies of both XRCC1 polymorphisms are >10%, we have 87% statistical power to see an OR of 1.5 with an alpha of 0.05 for gene-environment interaction effects.

Results

**XRCC1** and Breast Cancer Risk. Genotype distributions at each locus were consistent with the Hardy-Weinberg equilibrium among controls. Allele frequencies of XRCC1 codon 194Trp and 399Gln in the control group were 6.72% and 35.86%, respectively. Only 12 subjects (four cases, eight controls) were of the homozygous Trp/Trp genotype for codon 194. Therefore, for all subsequent analyses, we combined the Arg/Trp and Trp/Trp genotypes as one group. For codon 399, we considered G as the higher risk allele and combined Arg/Gln and Gln/Gln genotypes as 399Gln carriers when conducting subgroup analyses. ORs were not altered significantly (changes were <6%) when the potential confounding variables were considered individually or in multivariate models (data not shown). Thus, only age-adjusted models are shown. The ORs for breast cancer were not substantially elevated in relation to genotypes for either XRCC1 codon 194 or 399 (Table 1). These associations of 194Trp and 399Gln alleles with breast cancer did not vary greatly with menopausal status, although 399Gln carriers showed a higher risk of breast cancer in premenopausal compared with postmenopausal women. Analyses stratified by stage of disease (in situ or invasive), ethnicity (White or non-White), first-degree family history of breast cancer (yes or no), alcohol drinking status (never, ever), and body mass index at reference (<25 or ≥25) also did not seem to modify the risk of breast cancer among codon 194Trp or 399Gln carriers (data not shown).

**XRCC1 Interaction with PAH-DNA Adducts and Cigarette Smoking on Breast Cancer Risk.** We assessed the potential multiplicative interactions between the two XRCC1 polymorphisms and risk exposures (PAH-DNA adducts and cigarette smoking) by running separate logistic regression models including cross-product terms. No apparent evidence of interaction was observed between codon 194Trp and PAH-DNA adducts or cigarette smoking (Table 2). For codon 399, we found a modest increased risk of breast cancer among subjects with both detectable PAH-DNA adducts and carrying the 399Gln allele (OR, 1.33, as compared with those who had neither, 95% CI, 0.98-1.84), although the multiplicative interaction term was not statistically significant (P = 0.71). We also observed a significantly increased risk of breast cancer in 399Gln carriers who also were never smokers (OR, 1.31; 95% CI, 1.01-1.69) as compared with those with the Arg/Arg genotype; the multiplicative interaction term was statistically significant (P = 0.03).

Further analysis was undertaken among never smokers (Table 3). Substantially elevated risk was associated with a 399Gln allele and detectable adducts, as compared with women with neither (adjusted OR_{eg}, 1.92; 95% CI, 1.21-3.07). Although there was a suggestion of additive interaction, there was no statistically significant departure from additivity (ICR, 0.38; 95% CI, −0.32, 1.10) indicating only a weak interaction between the 399Gln allele and PAH-DNA adducts. There was no evidence of interaction between the 194Trp allele and PAH-DNA adducts among never smokers. Among ever smokers, no consistent pattern emerged when interactions between XRCC1 genotypes and PAH-DNA adducts were evaluated (Table 3).

**Combined Effect of XRCC1 Genotypes and Intake of Fruits and Vegetables or Dietary and Supplemental Antioxidants.** Table 4 shows the joint effects of XRCC1 genotypes and fruit and vegetable consumption and dietary or dietary plus supplemental intake of antioxidants. Codon 194Trp carriers with high intake seemed to have significantly decrease breast cancer risk compared with those with the Arg194Arg genotype and low intake of these factors. The multiplicative interaction terms were statistically significant

| Table 1. XRCC1 codon 194 and 399 genotype frequencies and OR for breast cancer risk among pre- and postmenopausal women, Long Island Breast Cancer Study Project, 1996-1997 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **XRCC1 Genotype** | **Total cases/controls (1,067/1,110)** | **OR* (95% CI)** | **Premenopausal cases/controls (341/376)** | **OR* (95% CI)** | **Postmenopausal cases/controls (701/688)** | **OR* (95% CI)** |
| Codon 194 | | | | | | |
| Arg/Arg | 938/967 | Reference | 300/321 | Reference | 616/605 | Reference |
| Arg/Trp or Trp/Trp | 128/141 | 0.93 (0.72-1.21) | 41/55 | 0.78 (0.50-1.20) | 84/81 | 1.00 (0.72-1.38) |
| Codon 399 | | | | | | |
| Arg/Arg | 412/444 | Reference | 131/162 | Reference | 274/267 | Reference |
| Arg/Gln or GLN/Gln | 655/666 | 1.06 (0.89-1.26) | 210/214 | 1.23 (0.91-1.66) | 427/421 | 0.99 (0.80-1.24) |
| Arg/Gln | 539/536 | 1.08 (0.90-1.29) | 174/169 | 1.28 (0.93-1.75) | 347/342 | 0.99 (0.79-1.24) |
| Gln/Gln | 116/130 | 0.97 (0.73-1.29) | 36/45 | 1.03 (0.62-1.70) | 80/79 | 1.01 (0.71-1.44) |

*Adjusted for age at reference (continuous).
in those with higher intake (any fruits, fruit juices, and vegetables, dietary and supplemental vitamin C) and 194Trp carriers ($P < 0.04$). The OR was 0.58 (95% CI, 0.38-0.89) for $\geq 35$ half-cup servings per week of any fruits, fruit juices, and vegetables compared with 0 to 34 half-cup servings per week (ICR, $-0.49$; 95% CI, $-0.35$; 0.11). These ORs remained essentially unchanged after controlling for other sources of antioxidants one at a time or all simultaneously (data not shown). For $\beta$-carotene and $\alpha$-carotene, the combined effects with the codon 194Trp allele were also significant, although the multiplicative interaction terms were not statistically significant. However, after adjustment for intake of any fruits, fruit juices, and vegetables, the ORs did not change materially but were no longer significant (data not shown). There was no evidence that there was a significant interaction effect between the 194Trp allele and high intake of fruits and vegetables. The OR was 0.63 (95% CI, 0.43-0.93) for $\geq 131.1$ mg/d dietary and supplemental vitamin C compared with 0 to 131.0 mg/d (ICR, $-0.35$; 95% CI, $-0.81$, 0.11). These ORs remained essentially unchanged after controlling for other sources of antioxidants one at a time or all simultaneously (data not shown). For $\beta$-carotene and $\alpha$-carotene, the combined effects with the codon 194Trp allele were also significant, although the multiplicative interaction terms were not statistically significant. However, after adjustment for intake of any fruits, fruit juices, and vegetables, the ORs did not change materially but were no longer significant (data not shown).

### Table 2. Association between XRCC1 polymorphisms and breast cancer risk by PAH-DNA adducts and cigarette smoking status, Long Island Breast Cancer Study Project, 1996-1997

<table>
<thead>
<tr>
<th>XRCC1 Genotype</th>
<th>Non-detectable PAH-DNA adducts</th>
<th>Detectable PAH-DNA adducts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Codon 194 Arg/Arg</td>
<td>194</td>
<td>249</td>
</tr>
<tr>
<td>Arg/Trp or Trp/Trp</td>
<td>31</td>
<td>42</td>
</tr>
<tr>
<td>Arg/Arg Never smoking</td>
<td>431</td>
<td>433</td>
</tr>
<tr>
<td>Arg/Trp or Trp/Trp</td>
<td>59</td>
<td>69</td>
</tr>
</tbody>
</table>

| Codon 399 Arg/Arg | 83    | 107    | 1.00 (reference) | 249   | 264 | 1.23 (0.88-1.73) |
| Arg/Gln or Gln/Gln | 142  | 185    | 1.00 (0.69-1.43) | 392   | 382 | 1.33 (0.97-1.84) |
| Arg/Arg Never smoking | 192  | 232 | 1.00 (reference) | 220   | 213 | 1.26 (0.96-1.64) |
| Arg/Gln or Gln/Gln | 298  | 272    | 1.31 (1.01-1.69) | 357   | 393 | 1.11 (0.88-1.42) |

*Adjusted for age at reference (continuous).

### Discussion

In the present study, the allele frequencies of XRCC1 194Trp and 399Gln in the control group were 6.72% and 35.86%, respectively, which were very similar to those reported previously from American and European Caucasians (194Trp, 5-7%; 399Gln, 34-36%; refs. 19, 20, 33) and Asian (399Gln, 27-31%; refs. 34, 35), but quite different from African American women for 399Gln (14%) and Asian women for 194Trp (34%; refs. 20, 35).

We observed no significant relationships between either XRCC1 codon 194 or 399 rare alleles and breast cancer risk, nor a variation with menopausal status, stage of disease, ethnicity, first-degree family history of breast cancer, alcohol drinking, and body mass index at reference. The possible relationships between the two XRCC1 single nucleotide polymorphisms and cancer risk have been investigated in >60 studies with nearly 10 in breast cancer, but the results are inconsistent, especially concerning their interactions with environmental carcinogens (9). Most molecular epidemiologic studies on XRCC1 codon 194 reported a decreased risk of breast cancer (20) or cancer at other sites associated with the 194Trp allele (9). This association was also observed in one relatively large study of breast cancer, but significant differences occurred only in the highest plasma carotene subgroup (19). In several other studies, the 194Trp allele showed an increased risk for breast
allele and detectable PAH-DNA adducts on breast cancer risk. We observed a weak additive interaction between the 399Gln polymorphisms and PAH-DNA adducts in breast cancer risk.

Any fruits, fruit juices, and vegetables

Dietary + supplemental vitamin C (mg/d)

Dietary + supplemental vitamin E (a-tet/d)

Dietary α-carotene (μg/d)

<table>
<thead>
<tr>
<th>Antioxidant intake*</th>
<th>XRCC1 codon 194</th>
<th></th>
<th>XRCC1 codon 399</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arg/Arg</td>
<td>Arg/Trp or Trp/Trp</td>
<td>Arg/Arg</td>
<td>Arg/Gln or Gln/Gln</td>
</tr>
<tr>
<td>Any fruits, fruit juices, and vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-34</td>
<td>572/580</td>
<td>1.0 (ref)</td>
<td>92/83</td>
<td>1.13 (0.83-1.55)</td>
</tr>
<tr>
<td>≥35</td>
<td>351/371</td>
<td>0.94 (0.79-1.11)</td>
<td>34/58</td>
<td>0.58 (0.38-0.89)</td>
</tr>
<tr>
<td>Dietary + supplemental β-carotene (μg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0-3817.2</td>
<td>562/548</td>
<td>1.0 (ref)</td>
<td>85/77</td>
<td>1.10 (0.80-1.51)</td>
</tr>
<tr>
<td>≥3817.3</td>
<td>352/391</td>
<td>0.89 (0.75-1.06)</td>
<td>40/63</td>
<td>0.64 (0.42-0.97)</td>
</tr>
<tr>
<td>Dietary + supplemental vitamin C (mg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-131.0</td>
<td>539/558</td>
<td>1.0 (ref)</td>
<td>80/67</td>
<td>1.23 (0.88-1.73)</td>
</tr>
<tr>
<td>≥131.1</td>
<td>375/381</td>
<td>1.01 (0.86-1.20)</td>
<td>45/73</td>
<td>0.63 (0.43-0.93)</td>
</tr>
<tr>
<td>Dietary + supplemental vitamin E (α-tet/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-29.0</td>
<td>520/563</td>
<td>1.0 (ref)</td>
<td>83/71</td>
<td>1.22 (0.88-1.70)</td>
</tr>
<tr>
<td>≥29.1</td>
<td>394/376</td>
<td>1.10 (0.93-1.30)</td>
<td>42/69</td>
<td>0.65 (0.44-0.96)</td>
</tr>
<tr>
<td>Dietary α-carotene (μg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-267.7</td>
<td>574/564</td>
<td>1.0 (ref)</td>
<td>81/75</td>
<td>1.09 (0.79-1.51)</td>
</tr>
<tr>
<td>≥267.8</td>
<td>340/375</td>
<td>0.91 (0.77-1.08)</td>
<td>44/65</td>
<td>0.67 (0.45-0.99)</td>
</tr>
</tbody>
</table>

*Variables were derived from a two-step process. Quintiles of the control distribution were used to form fifths. ORs of the last two fifths and the first three fifths were similar and therefore collapsed to form these binary variables.

**Adjusted for age at reference (continuous).**

In half-cup servings per week.

cancer although none of these estimated effects were statistically significant (33, 35-37). Most studies on the XRCC1 399Gln allele suggested that breast cancer risk was elevated, but more pronounced only in specific subgroup analyses (7, 19, 20, 34). We found no independent influences for 194Trp or 399Gln on breast cancer risk. This is in agreement with the findings of two relatively large breast cancer studies with sample sizes of both cases and controls >1,000 (the Nurses’ Health Study and Shanghai Breast Cancer Study; refs. 19, 34). Although our results were consistent with studies of cancers at other sites (9, 18), they were inconsistent with other studies of breast cancer (20, 35). One of these studies found a significant positive association for XRCC1 399Gln carriers and breast cancer risk compared with the Arg/Arg genotype among African Americans (20). Kim et al. (35) observed that an increased breast cancer risk was associated with the number of 399Gln alleles carried in Korean women (homozygous variant allele increasing breast cancer risk by 2.4-fold). This difference might be attributed to the different ethnic groups studied.

To the best of our knowledge, this study is the first to evaluate the interaction between the XRCC1 single nucleotide polymorphisms and PAH-DNA adducts in breast cancer risk. We observed a weak additive interaction between the 399Gln allele and detectable PAH-DNA adducts on breast cancer risk among never-smoking women (ORadj = 1.92; 95% CI, 1.21-3.07), although it did not show a statistically significant departure from additivity (ICR, 0.38; 95% CI, −0.32 to 1.10). No similar effects were noted in relation to cigarette smoking. This gene-adduct interaction among never smokers is consistent with previous observations among never or light smokers in other cancer sites (lung, bladder, and nonmelanoma skin cancer; refs. 24, 38-40). There was also no evidence that the 399Gln allele alone could significantly elevate breast cancer risk in never smokers, consistent with our finding in all subjects. Although the biological mechanisms are still unclear, a potential explanation is that cigarette smoking may stimulate DNA repair in response to the DNA damage caused by tobacco carcinogens. Smoking or heavy tobacco exposure may lead to more proficient repair in lymphocytes compared with little or no smoke exposure. This might overwhelm any effect of the XRCC1 codon 399 variant in smokers (38, 40) and may explain the more pronounced interaction of 399Gln with PAH-DNA adducts (formed from perhaps other PAH sources) among never smokers (15, 24). Thus, the interaction observed in never smokers may not mean that there is no interaction effect between PAH-DNA adducts and XRCC1 codon 399 variant in smokers, only that it may be hard to detect. It would be prudent to pursue this observation through biological investigations as well as epidemiologic studies.

Our observation is also biologically plausible based on prior genotype-phenotype and PAH-DNA adduct data (41-43). It is generally assumed that bulky DNA adducts, such as those induced by PAHs, are repaired by the nucleotide excision repair pathway (44). PAHs can be also metabolized via radical cation intermediates to electrophiles that bind to DNA and destabilize the N-glycosyl bond, inducing rapid depurination or depyrimidation of adducted bases (25, 44). In addition, when DNA is extensively damaged by PAH compounds, single-strand breaks or oxidative damage, nucleotide excision repair–independent excision repair mechanisms (e.g., base excision repair pathway) have been detected (25, 44-46).

The XRCC1 protein participates in the base excision repair pathway (47), acting as a scaffold protein by binding DNA ligase III at its COOH terminus, DNA polymerase β at its NH2 terminus, and to poly(ADP-ribose) polymerase that binds at the site of DNA damage (20). It is possible that the efficiency of the repair process might be influenced by amino acid substitutions at key positions in functional domains of the XRCC1 protein. The Arg399Gln variant (at nucleotide 28152) is located within the central breast cancer susceptibility gene 1 product COOH-terminal domain (BRCT1, residues 315-403) that has been shown to contain the poly(ADP-ribose) polymerase binding region and to interact with polynucleotide kinase (37). These findings support the possible involvement of the XRCC1 Arg399Gln variant in repairing damage caused by PAH-DNA adducts (15, 24, 25, 48, 49) and suggest possible mechanisms of XRCC1 399Gln allele interaction with PAH-DNA adducts on breast cancer risk. Because the weak additive interaction was observed only in the never-smoking subgroup, we cannot exclude the possibility that this finding was due to chance.

Without high intake of antioxidants, oxidative DNA damage, mainly repaired by the base excision repair pathway, may flourish. In the present study, we found several significant protective joint effects, with a 33% to 42%
decrease in the risk of breast cancer associated with carrying the XRCC1 194Trp allele, but not the 399Gln allele, coupled with a high intake of fruits and vegetables and high dietary plus supplemental intake of antioxidants. For example, a significant moderate interaction effect between the 194Trp allele and high intake of fruits, fruit juices, and vegetables was observed (ICR, 0.49; 95% CI, 0.03 to 0.95), which has an important public health implication. These results were consistent with the findings from the Nurse’s Health Study, which indicated an interaction between the 194Trp allele (but not the 399Gln allele) and plasma α- and β-carotene levels on breast cancer risk (19). Fruits and vegetables may reduce the risk of breast cancer (3), and this may be in part because they contain antioxidants, which decrease the level of oxidation of DNA. However, there are other possible mechanisms for the beneficial effects of fruits and vegetables, such as fibers, minerals, and several other bioactive compounds (50).

The Arg194Trp variant (at nucleotide 26304) occurs in the linker region that separates the N-terminal domain from the central BRCT1 domain and purportedly binds to the amino terminus of apurinic endonuclease (11). Apurinic endonuclease is a multifunctional rate-limiting enzyme that not only is responsible for repair of AP sites but also functions as a redox factor maintaining transcription factors in an active reduced status (51, 52). The various transcription factors that require reduction to bind to DNA contain a cysteine residue within the DNA-binding motif that, if oxidized, prevents DNA binding and, if reduced, augments DNA binding (52). Therefore, the pronounced decreased risk among the 194Trp carriers may be due to increased redox activity and may have nothing to do with DNA repair. This could also explain why the 399 allele shows no evidence of any interaction with intake of fruits and vegetables or antioxidants.

Our results suggest that the codon 194 and 399 XRCC1 polymorphisms have different effects in combination with PAH-DNA adducts, fruits and vegetables, and antioxidants. Haplotype analysis indicated that the two nonsynonymous single nucleotide polymorphisms were not on the same common haplotype (19), and serve as a basis for explaining the potential functional difference of the two XRCC1 polymorphisms. The most striking feature of XRCC1 is its ability to interact with other DNA repair proteins, although lacking any known enzymatic activity itself (53). A currently favored model, based on biochemical studies, is that XRCC1 primarily interacts with its various partners in a sequential manner at the site of damage (53-55). Thus, altered affinity of XRCC1 for its base excision repair partners, attributed to the different polymorphisms status of XRCC1, may lead to different gene-exposure interaction patterns.

We did not find evidence for effect modification of the XRCC1 genotype-breast cancer association by a number of other factors that could potentially induce DNA damage or oxidative stress: lung alcohol intake or obesity. This is in contrast to several previous cancer studies that have indicated potential interactions between XRCCI polymorphisms and environmental exposure. Three studies observed interactions between the homozygous 194Arg/Arg genotype and smoking and alcohol drinking with lung and bladder cancer risk (56-58). In addition, the 399Gln allele showed consistent interactions with smoking, alcohol drinking, and occupational exposure to ionizing radiation in elevating breast cancer risk (20, 35). Shu et al. reported Gln399Gln was associated with a decreased breast cancer risk among subjects with higher blood levels of sex hormones-binding globulin (34). These inconsistent findings across studies might be attributed to different biological pathways across different cancer sites or specific ethnic background. It is also possible that false-positive associations occurred because of low power to detect a subgroup interaction.

Our study has several strengths. The population-based study design ensured that cases and controls arose from the same source population, and the relatively large sample size allowed examination of the gene-environment interactions of interest. It is unlikely that XRCC1 genotype was related to participation in the study. The inclusion of an internal biomarker of PAH exposure, rather than relying on the subjects’ recall of past exposures, should better reflect how the body would have been likely to respond to such exposures in the past (59). There was also very high (>97%) reliability in the measurement of genotype using the fluorescence polarization method. Although the study is large, some subgroup analyses were limited by small numbers of subjects; results must therefore be interpreted with care.

In conclusion, our data show that neither of the two genetic polymorphisms of XRCCI (Arg194Trp and Arg399Gln) directly influence breast cancer risk. However, there was a suggestive weak additive interaction between the XRCCI 399Gln allele and PAH-DNA adducts on breast cancer risk only among never smokers. Furthermore, XRCCI 194Trp carriers might have a decreased breast cancer risk in interactions with high intake of fruit and vegetable. Because no DNA repair capacity phenotype assay was carried out in this study, and many other functional single nucleotide polymorphisms in DNA repair genes might also be involved in genotype-phenotype associations, additional large population-based studies are needed to test the possible synergistic effect of DNA repair gene-gene and gene-environment interactions based on justified functional investigations in cell or animal model systems.

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Polymorphisms in XRCC1 Modify the Association between Polycyclic Aromatic Hydrocarbon-DNA Adducts, Cigarette Smoking, Dietary Antioxidants, and Breast Cancer Risk

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