Hypothesis

Interaction between Genetic Variations in DNA Repair Genes and Plasma Folate on Breast Cancer Risk

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Abstract

Folate status has been inversely associated with breast cancer risk. Because folate deficiency can cause DNA damage, such as uracil misincorporation, single strand breaks, and double strand breaks, genetic polymorphisms in base excision repair and double strand break repair genes may lead to variation in DNA repair proficiency and modify the effect of folate on breast cancer risk. We prospectively investigated the a priori hypothesized interaction between plasma folate levels and five nonsynonymous polymorphisms in the XRCC1, XRCC2, and XRCC3 genes on breast cancer risk in a nested case-control study within the Nurses’ Health Study (712 case-control pairs). Suggestive evidence of interaction was seen for two of these polymorphisms. Compared with the reference group of non-carriers in the lowest quartile of plasma folate, the reduction in risk (66%) was statistically significant among XRCC1 194Trp carriers in the highest quartile (multivariate odds ratio, 0.34; 95% confidence interval, 0.16–0.72). The inverse association between XRCC1 194Trp and breast cancer risk was attenuated by lower plasma folate status. The inverse association between plasma folate level and breast cancer risk was stronger among 194Trp carriers (P, trend = 0.01) than non-carriers (P, trend = 0.09). We also observed that the positive association between the XRCC2 188His allele and breast cancer risk was only significant in women in the lowest plasma folate quartile (carriers versus non-carriers; multivariate odds ratio, 2.04; 95% confidence interval, 1.05–3.97), and this excess risk was abolished among those with higher plasma folate levels. Moreover, the inverse association between plasma folate level and breast cancer risk was stronger among XRCC2 188His carriers (P, trend = 0.004) than non-carriers (P, trend = 0.09). Although none of the statistical tests for interaction was significant, these data give some support for the hypothesis that genetic variations in DNA repair genes may modify the relation between plasma folate level and breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2004;13(4):520–524)

Introduction

Epidemiological evidence has suggested the role of diminished folate status in the development of breast cancer (1). An inverse association of dietary folate with the risk of breast cancer was observed in three large prospective epidemiological studies, the Nurses’ Health Study, the Iowa Women’s Health Study, and the Canadian National Breast Screening Study (2–4). The disruption of DNA integrity is one potential mechanism by which folate deficiency is involved in carcinogenesis. Folate deficiency reduces the methylation of dUMP to dTMP and thus induces dNTP pool imbalances (5), resulting in excessive uracil misincorporation into human DNA (6) during DNA replication and repair processes. Uracil in DNA is repaired by the base excision repair (BER) pathway, which creates transient single strand breaks (SSB) following the excision of uracil by uracil DNA glycosylase (7). With folate deficiency, uracil misincorporation and excision repair recur due to the limited thymidine pool (8). Simultaneous repair of adjacent uracils on opposite strands can cause double strand breaks (DSB) (9). Elevated levels of chromosome breaks were observed in folate-deficient individuals and were reversed by folate administration (6). Furthermore, the hypomethylation due to diminished folate status increases the sensitivity of mammalian DNA to methyl-sensitive nucleases, thereby leading to an accumulation of DNA strand breaks (5, 10–12). In addition to causing DNA damage, folate deficiency-induced imbalanced nucleotide pool was shown to impair DNA repair capacity in rat colonocytes and Chinese hamster ovary cells (13, 14).

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Because folate deficiency may confer individual susceptibility to breast cancer by causing DNA damage, such as uracil misincorporation, SSB and DSB, and impairing DNA repair capacity, genetic polymorphisms in BER and DSB repair genes may lead to variation in DNA repair proficiency and in turn modify the effect of folate on breast cancer risk. XRCCI is involved in the BER pathway. XRCCI has no known enzymatic activity, but it interacts with DNA polymerase β (15, 16), PARP, and DNA ligase III (17–19). This suggests that XRCCI may act as a nucleating factor in BER by bringing different components together at the site of action to promote the efficiency of the repair machinery. Homologous recombination repair is an important mechanism in the repair of DSB in mammalian cells. XRCC2 and XRCC3, two RAD51 paralogs, are required for the RAD51 focus formation in homologous recombination repair (20, 21).

In a prospective nested case-control study within the Nurses’ Health Study, plasma folate was inversely associated with breast cancer risk (22) (the multivariate odds ratio (OR), 0.73; 95% confidence interval (CI), 0.50–1.07; for highest versus lowest quintile; \( P_{\text{trend}} = 0.06 \)). We prospectively investigated the a priori hypothesis that the beneficial effect of plasma folate on breast cancer risk may vary according to the genetic variation in DNA repair genes. Polymorphisms in XRCCI, XRCC2, and XRCC3 genes in relation to breast cancer risk have been evaluated in this study population (23, 24). In this present analysis, we included all five common nonsynonymous polymorphisms in the coding regions of these three genes (XRCCI Arg194Trp, XRCCI Arg280His, XRCCI Arg399Gln, XRCC2 Arg188His, and XRCC3 Thr241Met) because of their potential functional relevance.

Materials and Methods

Study Population. The Nurses’ Health Study was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55 completed a self-given questionnaire on their medical histories and baseline health-related exposures. Updated information has been obtained by questionnaires every 2 years. Incident breast cancers were identified by self-report and confirmed by medical record review. Between 1989 and 1990, blood samples were collected from 32,826 of the cohort members. As of May 31, 1996, the follow-up rate in this subcohort was 99%.

Eligible cases in this study consisted of women with pathologically confirmed incident breast cancer from the subcohort who gave a blood specimen. Cases with a diagnosis anytime after blood collection up to May 31, 1996 with no previously diagnosed cancer except for non-melanoma skin cancer were included. One or two controls were randomly selected among women who gave a blood sample and were free of diagnosed cancer (excluding non-melanoma skin cancer) up to and including the interval in which the case was diagnosed. Controls were matched to cases on year of birth, menopausal status, postmenopausal hormone use, month and time of day of blood collection, and fasting status at blood draw; menopause was defined as previously described (25). The nested case-control study consists of 727 incident breast cancer cases and 969 matched controls. The study protocol was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women’s Hospital, Boston, MA.

Exposure Data. Information regarding breast cancer risk factors was obtained from the 1976 baseline questionnaire, subsequent biennial questionnaires, and a questionnaire completed at the time of blood sampling. Menopausal status and use of postmenopausal hormones were assessed at blood draw and updated until date of diagnosis for cases and the equivalent date for matched controls. First-degree family history of breast cancer was asked in 1982 and updated in subsequent questionnaires. Information regarding cigarette smoking was asked on the baseline questionnaire and updated biennially.

Laboratory Assays. Plasma folate levels for 712 case control pairs were determined by radioassay kit (Bio-Rad, Richmond, CA) (22). All case control pairs were assayed together; the samples were ordered randomly and labeled within each pair. Plasma quality control samples were interspersed to assess laboratory precision. The mean coefficient of variation for 75 pairs of replicate plasma samples was 6.5%. Laboratory personnel were blinded to case-control status and identity of replicate samples.

Genotyping was performed by the 5’ nuclease assay (TaqMan), using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. TaqMan primers and probes were designed using the Primer Express Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case-control status and blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. The details of genotyping assay for each polymorphism are available from the authors. In the XRCCI gene, the Arg280His polymorphism is in 100% genotype concordance with the C26602T polymorphism among the 90 individuals in the NIH DNA Polymorphism Discovery Resource, according to the XRCCI resequencing data of NIEHS Environmental Genome Project at the University of Washington (http://egp.gs.washington.edu). Due to the difficulty in genotyping the Arg280His polymorphism, C26602T was genotyped as a surrogate marker for the Arg280His.

Statistical Analysis. Plasma levels of folate were categorized into quartiles, with cut points based on the batch-specific distribution of control subjects. Weighted median values for each quartile were based on weighted batch-specific medians of controls by the proportion of subjects in each batch. In multivariate analysis, in addition to the matching variables, we adjusted for standard breast cancer risk factors: body mass index (BMI) [kg/m²] at age 18, weight gain since age 18, age at menarche, age at menopause, parity/age at first birth, first degree family history of breast cancer, personal history of benign breast disease, and duration of postmenopausal hormone use. Alcohol consumption was based on the 1990 dietary questionnaire; the 1986 questionnaire was used for individuals who did not provide this information on the 1990 questionnaire.
Because conditional and unconditional logistic regression analyses yielded similar results, to increase statistical power, we used unconditional logistic regression to calculate OR and 95% CI and to assess whether the relation of plasma folate with the risk of breast cancer was modified by genotype. Tests for trend were conducted by assigning the weighted median values for quartiles of plasma folate level among controls to both cases and controls as continuous variables.

Because of the relatively low allele frequency of XRCC1 Arg194Trp (6.6%), XRCC1 Arg280His (5.2%), and XRCC2 Arg188His (7.2%), we modeled genotype as a dichotomous variable (carriers versus non-carriers). We evaluated XRCC1 Arg399Gln (36.2%) and XRCC3 Thr241Met (38.0%) in three genotype categories. When we modeled genotype as a dichotomous variable and plasma folate as a continuous variable to assess interaction, the test of a single multiplicative interaction term evaluated whether the trend for plasma folate was statistically significantly different according to the genotype. All \( P \) values were two-sided.

### Results

The characteristics of cases and controls were described previously (22). The main effects of plasma folate levels and these five polymorphisms on breast cancer risk were separately evaluated in our study population (22–24). In this present study, we assessed whether the association between plasma folate levels and breast cancer risk differed according to genotype. ORs for breast cancer risk by XRCC1 Arg194Trp and plasma folate level are listed in Table 1. As compared with the reference group of non-carriers in the lowest quartile of plasma folate, the reduction in risk (66%) was statistically significant among 194Trp carriers in the highest quartile (multivariate OR, 0.34; 95% CI, 0.16–0.72). The inverse association between the carriage of 194Trp allele and breast cancer risk was apparent in the high plasma folate categories and was attenuated among women with lower plasma folate levels. The inverse association between plasma folate level and breast cancer risk appeared stronger among 194Trp carriers (\( P, \) trend = 0.01) than non-carriers (\( P, \) trend = 0.09), although the interaction was not statistically significant (\( P, \) interaction = 0.12). Additionally, in the analysis of interactions between XRCC1 Arg194Trp and plasma folate level, the multivariate ORs and tests of interaction did not change materially after controlling for plasma vitamin B6, vitamin B12, homocysteine, \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, \( \alpha \)-tocopherol, \( \gamma \)-tocopherol, and lutein/zeaxanthin, one at a time or all simultaneously.

The inverse association between plasma folate level and breast cancer risk was stronger among XRCC2 188His carriers (\( P, \) trend = 0.004) than non-carriers (\( P, \) trend = 0.09) (Table 2). A significantly positive association of the polymorphism XRCC2 188His with breast cancer risk was limited to women in the lowest quartile of plasma folate levels (carriers versus non-carriers, multivariate OR, 2.04; 95% CI, 1.05–3.97), and this excess risk was abolished among those with higher plasma folate levels. The interaction between plasma folate and XRCC2 Arg188His genotype on breast cancer risk did not approach statistical significance (\( P, \) interaction = 0.31). The multivariate OR remained significant for XRCC2 188His carriers in the lowest quartile after additionally controlling for plasma vitamin B6, vitamin B12, homocysteine, \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, \( \alpha \)-tocopherol, \( \gamma \)-tocopherol, and lutein/zeaxanthin.

No suggestive evidence of effect modification by any of other three polymorphisms (XRCC1 Arg280His, XRCC1 Arg399Gln, or XRCC3 Thr241Met) was observed on the association of plasma folate levels with breast cancer risk. Alcohol is a known folate antagonist (26). We did not observe significant interactions between alcohol intake and the five polymorphisms. In addition, no significant interactions were observed between dietary folate and methionine intakes and the five polymorphisms.

### Discussion

DNA damage induced by folate deficiency evokes two distinct DNA repair systems. The excessive uracil misincorporation induced by folate deficiency may evoke

### Table 1. Breast cancer risk by plasma folate levels and XRCC1 Arg194Trp

<table>
<thead>
<tr>
<th></th>
<th>1 (Lowest)</th>
<th>2</th>
<th>3</th>
<th>4 (Highest)</th>
<th>( P ) for trend</th>
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</thead>
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<tr>
<td>194Trp non-carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/Controls(^a)</td>
<td>182/156</td>
<td>164/150</td>
<td>155/148</td>
<td>136/144</td>
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<tr>
<td>Multivariate OR(^b)</td>
<td>1.00</td>
<td>0.92 (0.67–1.26)</td>
<td>0.89 (0.65–1.21)</td>
<td>0.80 (0.58–1.10)</td>
<td>0.18</td>
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<tr>
<td>Multivariate OR(^c)</td>
<td>1.00</td>
<td>0.98 (0.71–1.35)</td>
<td>0.85 (0.61–1.17)</td>
<td>0.77 (0.55–1.07)</td>
<td>0.09</td>
</tr>
<tr>
<td>194Trp carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/Controls(^a)</td>
<td>25/23</td>
<td>22/22</td>
<td>15/23</td>
<td>12/28</td>
<td></td>
</tr>
<tr>
<td>Multivariate OR(^b)</td>
<td>0.94 (0.51–1.73)</td>
<td>0.86 (0.46–1.62)</td>
<td>0.55 (0.28–1.09)</td>
<td>0.37 (0.18–0.75)</td>
<td>0.02</td>
</tr>
<tr>
<td>Multivariate OR(^c)</td>
<td>0.87 (0.46–1.64)</td>
<td>0.88 (0.46–1.70)</td>
<td>0.50 (0.25–1.02)</td>
<td>0.34 (0.16–0.72)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\)The number of participants does not sum to total women because of missing data on genotype.

\(^b\)Unconditional logistic regression adjusted for the matching variables: age, menopausal status, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.

\(^c\)Unconditional logistic regression adjusted for the matching variables, BMI at age 18 (continuous), weight gain since age 18 (<5 kg, 5–19.9, ≥20 kg), age at menarche (<12 years, 12, 13, >13), age at menopause (<45, ≥45 to <50, ≥50 to <55 or ≥55 years), parity/age at first birth (nulliparous, 1–2 children/age at first birth ≤24 years, 1–2 children/age at first birth >24, 3+ children/age at first birth ≤24, 3+ children/age at first birth >24), first degree family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol intake (0, >0 to <5, ≥5 to <15, ≥15 to <30, ≥30 g/day), and postmenopausal hormone use (never use, past use, current use <5 years, current use ≥5 years).
over-initiated BER, accumulating intermediate AP sites and SSB, and hence imposing greater dependence on the repair capacity of the downstream BER pathway. DSB caused by hypomethylation or adjacent SSB are repaired by the DSB repair pathway. We prospectively investigated the a priori gene-environment interaction hypothesis that the beneficial effect of plasma folate level on breast cancer risk may vary according to genetic variations in the coding regions of DNA repair genes (XRCC1, XRCC2, and XRCC3) in a nested case-control study within the Nurses’ Health Study.

In this prospective nested case-control study, we previously observed that XRCC1 Arg194Trp carriers were at a decreased risk of breast cancer (carriers versus non-carriers, multivariate OR, 0.79; 95% CI, 0.60–1.04) (23), which is consistent with most of the published studies reporting inverse associations between the XRCC1 Arg194Trp allele and cancer risk at breast or other sites (27,28). This suggests a protective role for Arg194Trp in the development of breast cancer potentially by increasing BER capacity. No association has yet been reported between Arg194Trp and altered biomarkers of DNA damage (29,30). Here, we observed a suggestion that the XRCC1 Arg194Trp genotype modified the association between plasma folate levels and breast cancer risk.

Table 2. Breast cancer risk by plasma folate levels and XRCC2 Arg188His

<table>
<thead>
<tr>
<th></th>
<th>1 (Lowest)</th>
<th>2</th>
<th>3</th>
<th>4 (Highest)</th>
<th>P for trend</th>
</tr>
</thead>
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<td>188His carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cases/Controls</td>
<td>170/155</td>
<td>149/147</td>
<td>136/145</td>
<td>126/146</td>
<td>0.18</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>1.00</td>
<td>0.91 (0.66–1.25)</td>
<td>0.84 (0.61–1.16)</td>
<td>0.78 (0.56–1.08)</td>
<td>0.09</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>1.00</td>
<td>0.98 (0.70–1.36)</td>
<td>0.80 (0.57–1.12)</td>
<td>0.74 (0.52–1.05)</td>
<td>0.09</td>
</tr>
<tr>
<td>188His carriers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/Controls</td>
<td>35/15</td>
<td>28/25</td>
<td>24/24</td>
<td>20/23</td>
<td>0.01</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>2.15 (1.13–4.10)</td>
<td>1.00 (0.55–1.79)</td>
<td>0.89 (0.49–1.64)</td>
<td>0.77 (0.41–1.47)</td>
<td>0.004</td>
</tr>
<tr>
<td>Multivariate OR</td>
<td>2.04 (1.05–3.97)</td>
<td>1.00 (0.54–1.84)</td>
<td>0.84 (0.45–1.59)</td>
<td>0.77 (0.39–1.50)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

P, interaction = 0.31 in the multivariate model. C.

The number of participants does not sum to total women because of missing data on genotype.

Unconditional logistic regression adjusted for the matching variables: age, menopausal status, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.

Unconditional logistic regression adjusted for the matching variables, BMI at age 18 (continuous), weight gain since age 18 (<5 kg, 5–19.9, ≥20), age at menarche (<12 years, 12, 13, >13), age at menopause (<45, ≥45 to <50, ≥50 to <55 or ≥55 years), parity/age at first birth (nulliparous, 1–2 children/age at first birth ≤24 years, 1–2 children/age at first birth >24, 3+ children/age at first birth ≤24, 3+ children/age at first birth >24), first degree family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol intake (0, >0 to <5, ≥5 to <15, ≥15 to <30, ≥30 g/day), and postmenopausal hormone use (never use, past use, current use <5 years, current use ≥5 years).

XRCC2-deficient cells, whereas naturally occurring 188H allele displayed a much smaller difference in survival from the wild type, suggesting that the 188H allele had a subtle effect on damage sensitivity (31). Our data showed that the positive association of this variant with breast cancer risk was only apparent among women in the lowest plasma folate quartile, and no apparent effect of this variant was observed among those with higher plasma folate levels. This suggests that adequate folate status may attenuate the elevated breast cancer risk associated with this genetic variation. The data also imply that, among the carriers of this variant, the reduced DNA repair capacity is adequate to maintain the DNA integrity in the presence of normal amount of DNA damage; but the increased DNA DSB due to folate deficiency may overwhelm the partially impaired DNA repair system and in turn increase cancer risk.

Although the interactions were not statistically significant, the present study provided preliminary data to support the novel hypothesis that genetic variations in BER and DSB repair pathway modify the relation between plasma folate level and breast cancer risk. Our data may suggest one potential biological mechanism underlying the beneficial effect of folate in the etiology of breast cancer; that is, the adverse effect of folate deficiency on breast cancer risk may be at least partially due to increased DNA damage. The multivariate ORs did not change materially after controlling for plasma vitamin B6, vitamin B12, homocysteine, and antioxidants, one at a time or all simultaneously, suggesting that the observed interaction was not confounded by these factors.

We did not observe any suggestion of effect modification of any of the other three polymorphisms (XRCC1 Arg280His, XRCC1 Arg399Gln, and XRCC3 Thr241Met) on the relation of plasma folate level with breast cancer risk. None of these three polymorphisms was associated with altered breast cancer risk in this nested case-control study (23,24). Functional effects of the two nonsynonymous variants, XRCC1 Arg399Gln and XRCC3 Thr241Met, have been studied in vitro transfection experiments (33,34). Compared to the corresponding wild-type alleles, the variant alleles had no impact on complementation of the repair defect and correction of...
The findings from our study, if confirmed, suggest that follow-up rates strengthen the validity of this study.

Potential interaction between folate and polymorphisms not associated with radiation-induced G2 phase delay biomarkers, such as DNA adducts, sister 399Gln allele and higher levels of DNA damage, suggest that high plasma folate level would be particularly important for women with certain genotypes of DNA repair genes.

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