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Breast Cancer Res Treat. Author manuscript; available in PMC 2010 February 1.

Published in final edited form as:
Breast Cancer Res Treat. 2009 February ; 113(3): 585–594. doi:10.1007/s10549-008-9962-z.

A prospective study of genetic polymorphism in MPO, antioxidant status, and breast cancer risk

Chunyan He,
Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Building II, Room 200, Boston, MA 02115, USA, Phone: (617)-432-7092; Fax: (617)-432-1722, e-mail: che@hsph.harvard.edu

Rulla M. Tamimi,
Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Building II, Room 200, Boston, MA 02115, USA

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA

Susan E. Hankinson,
Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Building II, Room 200, Boston, MA 02115, USA

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA

David J. Hunter, and
Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Building II, Room 200, Boston, MA 02115, USA

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA

Jiali Han
Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA

Abstract

Oxidative stress may be involved in breast carcinogenesis. Myeloperoxidase (MPO) is an endogenous oxidant enzyme that generates reactive oxygen species (ROS). A single nucleotide polymorphism (SNP) G-463A in the promoter region has been associated with a decrease in risk of breast cancer. We assessed the association between this polymorphism and breast cancer risk in a nested case-control study within the Nurses' Health Study (1269 incident breast cancer cases and 1761 matched controls). We further investigated potential gene-gene and gene-environment interactions. There were no significant associations between MPO or COMT genotypes and risk of breast cancer. However, the combination of *a priori* hypothesized low-risk genotypes in MPO and COMT genes was associated with a marginally significant decrease in breast cancer risk (OR, 0.28; 95% CI, 0.08–1.00). Dietary intake and plasma antioxidant levels may modify the association between the MPO polymorphism and breast cancer risk. Although the test for departure from multiplicative interaction was not significant, inverse associations with MPO genotype were more pronounced among women who consumed higher amounts of total fruits and vegetables (OR, 0.58; 95% CI, 0.30–1.12); this association was not found among the low-consumption group (OR, 1.11; 95% CI, 0.63–1.96). The

Correspondence to: Chunyan He.

relative risk associated with the MPO homozygous variant genotype was 0.44 (95% CI, 0.18–1.09) for women who had the highest level of plasma carotenoids. Results from this study suggest that exogenous and endogenous modulators of oxidative stress may modify the association between the MPO polymorphism and breast cancer risk. Further research is needed to confirm these possible associations.

Keywords

MPO (myeloperoxidase); COMT (catechol-O-methyltransferase); Oxidative stress; Antioxidant; Breast cancer

Introduction

Increasing evidence suggests that oxidative stress is involved in the pathogenesis of breast cancer [1]. Exposure to endogenous and exogenous oxidant sources generates reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals *in vivo*. ROS cause oxidative damage to biomolecules such as DNA, proteins, and lipids, and can cause cellular alteration that may lead to tumorigenesis [2,3]. Meanwhile, multiple antioxidant defenses exist that can neutralize ROS. Oxidative stress occurs when excessive production of ROS overwhelms the antioxidant defense system or when there is a decrease or lack of antioxidant defenses. Fruits and vegetables are rich sources of a number of antioxidants, such as carotenoids, tocopherols, and ascorbic acid. These compounds can decrease oxidative load [4]. ROS are also endogenously generated by enzymes including myeloperoxidase (MPO) and endothelial nitric oxide (NO) synthase (eNOS) [5,6], or neutralized by enzymes including manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase (GPX) [7]. Variability in these enzymes and environmental exposures may determine the level of oxidative stress in the organism and play a role in breast cancer risk.

MPO is a lysosomal enzyme that is present in neutrophils and monocytes to fight against microbial infection [8]. It generates a strong oxidant hypochlorous acid and also activates procarcinogens in tobacco smoke [8,9]. A single nucleotide polymorphism (SNP) G-463A (rs2333227) has been identified in the promoter region of the MPO gene, with the A variant allele associated with reduced mRNA expression and transcriptional activity due to disruption of the SP1 binding site [10]. This polymorphism has been reported to be associated with cancer risk at several sites [11–16]. For breast cancer, two studies have reported a nonsignificant decrease in breast cancer risk associated with the AA genotype [17,18]. Another study found no significant association between the MPO G-463A polymorphism and postmenopausal breast cancer risk [19].

MnSOD, catalase, and GPX are major enzymes that neutralize ROS and form the first line of defense against superoxide and hydrogen peroxide [7]. Non-synonymous polymorphisms in the MnSOD (Val16Ala, rs4880) and GPX-1 (Pro198Leu, rs1050450) genes have been shown to reduce their capacity to quench ROS [20–22]. Ultimate levels of cytotoxic ROS may depend upon the balance among activities of MnSOD, GPX, and MPO. Oxidative stress *in vivo* can also be modulated by enzymes such as catechol-O-methyltransferase (COMT) [23], which catalyzes the addition of a methyl group to reactive catechol estrogens to convert them into stable methyloxyestrogen conjugates [24]. COMT may protect DNA from oxidative damage by preventing quinone formation and redox cycling [25]. A non-synonymous polymorphism in COMT (Val158 Met, rs4680) leads to reduced enzyme activity [26], which is hypothesized to increase breast cancer risk through reduction in protecting DNA damage from ROS and accumulation of estradiol catechol metabolites. Lin et al. reported an interaction between the MPO G-463A and COMT Val158Met polymorphisms on breast cancer risk, with an elevated

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risk associated with the increasing numbers of high-risk genotypes in both genes among women with longer duration between menarche and first full-term pregnancy [18].

We investigated the association between a polymorphism in the MPO gene and breast cancer risk in the Nurses' Heath Study (NHS). We further evaluated potential gene-gene and gene-environment interactions between the MPO polymorphism and factors hypothesized to modulate oxidative stress, including genetic factors such as polymorphisms in the MnSOD, GPX and COMT genes and environmental factors such as dietary antioxidant intake and plasma antioxidant levels.

Materials and Methods

Study Population

We conducted a nested case-control study within the Nurses' Health Study cohort. The Nurses' Health Study was established in 1976, when 121,700 US registered nurses between the ages of 30 and 55 years returned an initial questionnaire on their medical histories and baseline health-related exposures. Updated information has been obtained by questionnaire every 2 years. Incident breast cancer cases were identified through self-report and were confirmed by medical record review. Histopathologic characteristics of breast tumors were obtained from medical records when available. In 1989 and 1990, blood samples were collected from 32,826 (27%) women. Blood samples were returned within 26 hours of being drawn, immediately centrifuged, aliquoted into plasma, red blood cells, and buffy coat components, and stored in liquid nitrogen freezers. Subsequent follow-up has been >98% for this subcohort.

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 after blood collection up to June 1, 2000 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, recent post-menopausal hormone (PMH) use, month of blood return, time of day of blood collection, and fasting status at blood draw. The nested case-control study consists of 1269 incident breast cancer cases and 1761 matched controls.

Exposure Assessment

Information regarding breast cancer risk factors was obtained from the 1976 baseline questionnaire, subsequent biennial follow-up 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 ascertained in 1982 and updated in subsequent questionnaires. Information regarding current and past history of cigarette smoking was ascertained in the 1976 questionnaire and updated in subsequent questionnaires [27].

Dietary Data

In 1980 a dietary component was added to the prospective follow-up questionnaire. The validity and reliability of the food-frequency questionnaire (FFQ) in the NHS have been described elsewhere [28]. Since 1980, the FFQ has been expanded to include approximately 130 individual food items plus vitamin and mineral supplement use, accounting for over 90% of intake of most major nutrients. This expanded questionnaire was administered to the cohort in 1984, 1986, 1990, 1994, 1998, and 2002. Nutrient intakes were computed by multiplying the frequency of response by the nutrient content of the specific portion sizes. We also asked

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questions on the use of specific vitamins and brand and type of multivitamins as well as dose and duration of use; vitamin supplement use was updated biennially. Total daily nutrient intakes were calculated by adding the amounts from multivitamins and specific supplements to the intakes from food. Values for nutrients in foods were derived from USDA sources and supplemented with information from manufacturers. Total energy-adjusted dietary nutrient intakes were used in analyses. Food intakes were adjusted for total energy consumption [29]. To reduce within-person variation and represent long-term dietary intake of participants, we modeled breast cancer risk in relation to the cumulative average of dietary intake up to the questionnaire before the diagnosis [30]. Several validation studies in the NHS cohort indicate that nutrient intakes from the FFQ are valid for ranking subjects in diet analyses. In a comparison of the 1986 FFQ with two 1-week diet records, correlations were 0.79 for vitamin A from foods and 0.76 for vitamin C from foods [31]. In other studies, vitamin E and β-carotene intakes from the FFQ predicted plasma levels of α-tocopherol (r = 0.55) [32] and β-carotene (r = 0.31) [33], respectively. 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.

Genotype and Laboratory Assays

DNA was extracted from buffy coats using Qiagen QIAamp Blood kit (Qiagen, Chatsworth, CA). Genotyping was performed by the 5′ endonuclease assay (TaqMan), using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. The MPO -764T>C (rs2243828) SNP was genotyped in place of the -463G>A SNP (rs2333227) because of technical difficulty in genotyping the -463G>A SNP. The genotype concordance between these two SNPs is 100% in Caucasians and other ethnic groups (African American, Hispanic and Pacific Rim) (http://snp500cancer.nci.nih.gov). TaqMan primers and probes were designed using the Primer Express Oligo Design software v2.0 (ABI PRISM). Cases and their matched controls were genotyped at the same time. A random 10% of the samples were inserted to validate genotyping procedures. Laboratory personnel were blinded to case–control status and quality control samples. The concordance for the blinded quality control samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request.

Plasma antioxidant levels (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, retinol, α-tocopherol, γ-tocopherol) were assessed by reversed-phase high-performance liquid chromatography (HPLC) methods described by El-Sohemy et al. [34]. All case-control pairs were assayed together, with samples ordered randomly within each pair. Plasma quality control samples were interspersed to assess laboratory precision. Laboratory personnel were blinded to case-control status and the identity of replicate samples. Coefficients of variation for the blinded quality control samples in this data set had a median of 7.4% (range 7.1% to 11.0%) [35]. Lutein and zeaxanthin were analyzed together as lutein/zeaxanthin since they were isomers and are not separated by the method used. Total carotenoids score in this analysis were the sum of scores of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin. The score for each antioxidant ranged from 1 to 5, representing quintiles of original concentration. Total carotenoids score could range from 5 to 25. Plasma levels of antioxidants were categorized into quartiles based on the batch-specific cut points of control subjects [35].

Statistical Analysis

We used a χ^2 test to assess whether the genotypes were in Hardy-Weinberg equilibrium among controls. We performed unconditional and conditional logistic regression analyses and observed consistent results. In order to increase the statistical power in stratified analyses where the matching was broken, we employed unconditional logistic regression to calculate odds

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ratios (ORs) and 95% confidence intervals (CIs). In addition to the matching factors (age, menopause status, recent PMH use, fasting status, time and date at blood draw), we controlled in multivariate analyses for the following potential confounders and breast cancer risk factors: body mass index (BMI) at age 18 (kg/m^2, continuous), weight gain since age 18 (<5, ≥5 to <20, ≥ 20 kg), age at menarche (<12, 12, 13, >13 years), parity/age at first birth (nulliparous, one to two children/age at first birth ≤ 24 years, one to two children/age at first birth >24 years, more than two children/age at first birth ≤ 24 years, more than two children age at first birth >24 years), family history of a first-degree relative (yes/no), and personal history of benign breast disease (yes/no). Analyses including postmenopausal women were also adjusted for age at menopause (<45, ≥45 to <50, ≥50 to <55, ≥55 years) and duration of postmenopausal hormone use (never, past use <5 years duration, past use ≥ 5 years duration, current use <5 years duration, current use ≥ 5 years duration). In analyses involving plasma antioxidant levels, we also controlled for smoking status (never, <20, ≥20 to <35, ≥35 years duration) and alcohol intake (0, <5, ≥ 5 to <15, ≥ 15 to <30, ≥ 30 g/day).

To test statistical significance of gene-gene or gene-environment interactions, we used a likelihood ratio test (LRT) to compare nested models that included a single multiplicative interaction term to the models with the main effect only. The genotypes in MPO, MnSOD, GPX, and COMT genes and plasma levels of antioxidants were treated as ordinal variables. The cumulative average intakes of fruits and vegetables and specific sources of dietary antioxidants were dichotomized as high versus low based on the medians in controls. All reported p-values are two-sided. We performed all statistical analyses using SAS version 9.1 (SAS Institute, Cary, NC).

Results

The characteristics of the study population have been published previously [36]. Cases and controls had similar mean BMI at blood draw and weight gain since age 18 years. Cases were more likely to have a personal history of benign breast disease and a family history of breast cancer than controls. Approximately 63%, 33%, and 4% of controls had TT, TC, and CC genotypes, respectively, in the MPO gene. The genotype distribution in controls was under Hardy-Weinberg equilibrium (p= 0.99).

The associations between MPO T-764C genotypes and breast cancer risk are shown in Table 1. The CC genotype was associated with a non-significant 17% reduction in breast cancer risk (OR, 0.83; 95% CI, 0.55–1.26), after adjusting for matching factors and other covariates. The OR was 0.66 (95% CI, 0.23–1.94) among premenopausal women, and 0.86 (95% CI, 0.54–1.39) among postmenopausal women (Table 1).

Because enzymes such as COMT, MnSOD and GPX modulate oxidative stress, we investigated the gene-gene interactions between polymorphisms of these genes and MPO genotype on breast cancer risk. There was no association between the COMT Val158Met genotype and breast cancer risk (Table 2), and stratification by menopause status did not change the results (data not shown). Based on *a priori* hypothesis, we defined the MPO TT genotype and COMT Met/Met genotype as high-risk genotypes. Compared to women with high-risk genotype combination (MPO TT genotype and COMT Met/Met genotype), women with a low-risk genotype combination (MPO CC genotype and COMT Val/Val genotype) had a marginally significant 72% reduction in risk (OR, 0.28; 95% CI, 0.08–1.00), suggesting a combined effect (Table 2). No significant pairwise interactions or significant stratum-specific joint effects were found between the MPO T-764C polymorphism and the MnSOD Val16Ala, GPX-1 Pro198Leu polymorphisms (Supplementary Table 1).

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We found no significant gene-diet interaction between the MPO polymorphism and dietary intake on breast cancer risk. However, there was some suggestion that the association between MPO polymorphism and breast cancer was modified by the consumption of fruits and vegetables (Table 3). The CC genotype was associated with non-significant 42% reduction in risk among women who consumed higher amount of fruits and vegetables (OR, 0.58, 95%CI, 0.30–1.12). We did not observe an association between the CC genotype and breast cancer risk among women who consumed lower amount of fruits and vegetables (OR, 1.11; 95% CI, 0.63–1.96). Similar results were observed for fruits and vegetables separately and for sources of specific antioxidants such as carotenoids and Vitamin C (Table 3).

Plasma antioxidant levels may serve as a better marker of bioavailable antioxidants than dietary intake levels. Therefore, we also evaluated the association between the MPO genotype and breast cancer risk according to plasma antioxidant levels. We observed a non-significant 56% reduction in risk (OR, 0.44; 95% CI, 0.18–1.09) for women with the CC genotype in the highest quartile of plasma levels of carotenoids compared with women with the TT genotype in the lowest quartile (Table 4). The association between the MPO genotype and breast cancer risk was not modified by plasma levels of α-tocopherol or γ-tocopherol. No statistically significant interactions were observed between the MPO genotypes and other individual plasma antioxidant levels, including α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin (data not shown). Since lifestyle factors such as alcohol consumption and smoking contribute to exogenous oxidative stress [37,38], we also investigated the interaction between these two exposures and the MPO polymorphism. No significant interactions were observed between MPO genotypes and smoking or alcohol consumption on breast cancer risk (Supplementary Table 2).

Discussion

In this nested case-control study, we observed no significant association between a functional polymorphism in the MPO gene and risk of breast cancer. We observed a tendency towards risk reduction with the MPO variant among women who had high dietary antioxidant intake or high plasma antioxidant levels. We also observed a marginally significant decrease in risk associated with the combination of *a priori* hypothesized low-risk genotypes of the MPO and COMT genes. No significant interactions between MPO and MnSOD, GPX, smoking, and alcohol consumption were observed in this study.

MPO is a major enzyme involved in generating ROS and plays a role in oxidative stress and DNA damage [8]. A G→A polymorphism at −463 was identified at the SP1 binding site. Piedrafita et al. reported that the A variant allele was associated with decreased mRNA expression and transcription activity due to disruption of the SP1 binding site [39]. The G allele has been associated with increased MPO mRNA and protein levels in myeloid leukemia cells [40]. MPO GA and AA genotypes were associated with reduced MPO activity, and reduced smoking-related DNA adduct levels in bronchoalveolar lavage cells [41]. It is hypothesized that carrying A alleles is associated with decreased risk of breast cancer through reduced MPO activity and ROS production. We found a non-significant inverse association between MPO homozygous variant genotype and breast cancer risk, providing weak support for this biologically plausible hypothesis. This result was consistent with two previous studies, which both reported a decreased breast cancer risk associated with MPO AA genotype [17,18]. In the Long Island Breast Cancer Project, Ahn et al. observed a non-significant 17% reduction in risk associated with being homozygous for the A allele [17]. Lin et al. found that women with the MPO AA genotype had an OR of 0.64 (95% CI, 0.11–3.76) in a Chinese population [18].

We observed a tendency that the inverse association between MPO genotype and breast cancer risk was more pronounced among premenopausal women, although the power to detect the

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heterogeneity was limited. Similar results were reported in two previous studies by Ahn et al. and Yang et al. [17,19]. Several lines of evidence suggested an association between MPO activity and estrogen levels. Intracellular MPO activity in neutrophils was higher in premenopausal women than in postmenopausal women [42], and circulating variation in MPO is dependent on estradiol levels during the menstrual cycle [43]. Reynolds et al. proposed that estrogen might regulate MPO gene expression in a genotype-dependent fashion [44]. The −463 polymorphism site may be a part of estrogen response element [45], and the estrogen receptor may preferentially bind to the G allele more than to the A allele [46]. Our finding supported the potential role of estrogen in the regulation of MPO activity.

COMT plays a critical role in estrogen metabolism in breast tissue [47]. It catalyzes the methylation of reactive catechol estrogens to convert them into stable methyloxyestrogen conjugates [24]. A SNP in codon 158 results in a valine to methionine amino acid substitution, known to alter enzyme thermolability [26] and lower methylation activity [48,49]. The decrease in COMT activity may increase the accumulation of carcinogenic catechol estrogen [50]. Catechol estrogen can then undergo oxidation to catechol quinones catalyzed by peroxidase and cytochrome p450. A redox cycling between quinones and catechols generates ROS that can cause oxidative damage to DNA and lipids. Such quinones can also react with DNA to form a stable adduct that may lead to breast carcinogenesis [51]. It is conceivable that the genetic polymorphism resulting in a decrease in COMT activity may increase the breast cancer risk. However, epidemiological studies have found inconsistent associations between the COMT Val158 Met polymorphism and breast cancer risk. Two Asian studies have reported a significantly increased risk of breast cancer among women carrying at least one low-activity COMT-L allele (Met allele) compared to non-carriers [52,53]. On the other hand, several studies failed to observe an association between the COMT-L allele and breast cancer risk [54–58]. A few reports suggest a significant increased risk in premenopausal women [59,60]. A recent meta-analysis of 8286 cases and 7322 controls reported no evidence of an association between the COMT Val158 Met polymorphism and breast cancer risk [55]. However, multiple variants in related biological pathways may interact to alter risk. Lin et al. reported an elevated breast cancer risk associated with the increasing numbers of high-risk genotypes of MPO and COMT genes in women with a longer duration between menarche and first full-term pregnancy [18]. In our study, we did not observe an association between the COMT Val158Met polymorphism and breast cancer risk in all women or subgroups stratified by menopausal status. We found that the combination of the *a priori* hypothesized low-risk genotypes in MPO and COMT genes was associated with a marginally significant decrease in breast cancer risk, although the number of cases and controls in the stratum was small. Studies with sufficiently large sample sizes are warranted to confirm this interaction and further elucidate the exact genetic interaction model.

The consumption of fruits and vegetables, specific dietary antioxidants, and plasma antioxidant levels may modify the relationship between MPO genotype and breast cancer risk. The reduced risk associated with MPO genotype was more apparent among women with higher dietary intakes or higher levels of plasma antioxidants, although none of the interactions were statistically significant. This finding was consistent with the study of Ahn et al., which reported an inverse association between the GA or AA genotypes and breast cancer risk among women who consumed higher amount of fruits and vegetables (OR, 0.75; 95% CI, 0.58–0.97) [17]. Fruits and vegetables are rich in antioxidants such as carotenoids, vitamin C, and vitamin E, acting as exogenous modulators of oxidative stress. In addition, carotenoids can prevent DNA damage [61–64]. Our results are consistent with *a priori* hypothesis that a decrease in ROS level, resulting from neutralization by exogenous antioxidants rich in dietary intakes and reduced ROS production associated with MPO variant genotype, would lead to a decreased risk of breast cancer. Our plasma antioxidant data, which reflect both genetic and dietary variation, are consistent with the results from the dietary data.

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The strengths of this study include its nested case-control design, relatively large size, high follow-up rate, prospectively collected dietary data with repeated measurements, and prediagnostic plasma levels of antioxidants. In summary, we observed no significant association between the MPO polymorphism and breast cancer risk in this study. An inverse association was more pronounced among premenopausal women, suggesting the potential role of estrogen in the regulation of MPO activity. Marginal significant decrease in breast cancer risk associated with the combination of the *a priori* hypothesized low-risk genotypes in MPO and COMT genes, together with the tendency towards risk reduction with the MPO variant among women who had high dietary antioxidant intakes or high plasma antioxidant levels, suggest that exogenous and endogenous modulators of oxidative stress may interact with each other to affect breast cancer risk. Further studies examining more genes (such as catalase, eNOS) in the oxidative pathway, as well as exogenous environmental factors that influence oxidative stress, may be helpful in elucidating the role of oxidative stress in the development of breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Dr. Hardeep Ranu, Craig Labadie, and Pati Soule for their genotyping assistance, Carolyn Guo and Karen Corsano for their programming support. We also thank the participants in the Nurses' Health Study for their outstanding dedication and commitment. This work was supported by National Institutes of Health grants CA098233, CA118447, CA065725, USA.

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Table 1

Risk of breast cancer associated with the MPO T-764C polymorphism[§]

	Cases (%)	Controls (%)	OR (95%CI)[a]	OR (95%CI)[b]
All women*	1209 (100)	1678 (100)		
TT	762 (63.0)	1062 (63.3)	1.00 (Ref)	1.00 (Ref)
TC	405 (33.5)	546 (32.5)	1.05 (0.89, 1.23)	1.06 (0.90, 1.26)
CC	42 (3.5)	70 (4.2)	0.84 (0.56, 1.25)	0.83 (0.55, 1.26)
TT	762 (63.0)	1062 (63.3)	1.00 (Ref)	1.00 (Ref)
TC+CC	447 (37.0)	616 (36.7)	1.02 (0.88, 1.20)	1.04 (0.88, 1.22)
Premenopausal women*	241 (100)	299 (100)		
TT	154 (63.9)	196 (65.6)	1.00 (Ref)	1.00 (Ref)
TC	81 (33.6)	90 (30.1)	1.14 (0.79, 1.65)	1.17 (0.78, 1.75)
CC	6 (2.5)	13 (4.3)	0.59 (0.22, 1.60)	0.66 (0.23, 1.94)
TT	154 (63.9)	196 (65.6)	1.00 (Ref)	1.00 (Ref)
TC+CC	87 (36.1)	103 (34.4)	1.07 (0.75, 1.53)	1.11 (0.75, 1.64)
Postmenopausal women*	852 (100)[†]	1239 (100)		
TT	527 (61.9)	773 (62.4)	1.00 (Ref)	1.00 (Ref)
TC	292 (34.3)	414 (33.4)	1.05 (0.86, 1.26)	1.05 (0.86, 1.28)
CC	33 (3.9)	52 (4.2)	0.92 (0.59, 1.46)	0.86 (0.54, 1.39)
TT	527 (61.9)	773 (62.4)	1.00 (Ref)	1.00 (Ref)
TC+CC	325 (38.2)	466 (37.6)	1.03 (0.86, 1.24)	1.03 (0.85, 1.24)

[§]MPO polymorphisms T-764C and G-463A are 100% concordant in Caucasian and other ethnic groups.

[a]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 at blood draw.

[b]Unconditional logistic regression adjusted for the matching factors and BMI at age 18, weight gain since age 18, age at menarche, personal history of benign breast disease, first-degree family history of breast cancer, age at first birth/parity, age at menopause, and duration of postmenopausal hormone use.

*Numbers do not add to the total number of women due to missing values in covariates.

[†]The sum of percentage does not add up to 100 due to rounding.

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Table 2

The main effect of COMT Val158Met polymorphism and its interaction with MPO T-764C polymorphism on breast cancer risk.

	Cases (%)	Controls (%)	OR (95%CI)[a]
COMT Genotype[*]	1212(100)[†]	1683(100)	
Val/Val	271(22.4)	400(23.8)	1.00(Ref)
Val/Met	607(50.1)	837(49.7)	1.02(0.83, 1.24)
Met/Met	334(27.6)	446(26.5)	1.02(0.82, 1.28)
Val/Val	271(22.4)	400(23.8)	1.00(Ref)
Val/Met + Met/Met	941(77.6)	1283(76.2)	1.02(0.84, 1.23)
COMT | MPO Genotype[*¶]	1175(100)[†]	1631(100)[†]	
Met158 Met | MPO T-764T	204 (17.4)	280 (17.2)	1.00 (Ref)
Met158 Met | MPO T-764C	105 (8.9)	141 (8.7)	0.98 (0.71, 1.37)
Met158 Met | MPO C-764C	11 (0.9)	13 (0.8)	1.21 (0.51, 2.86)
Met158 Val | MPO T-764T	369 (31.4)	500 (30.7)	1.03 (0.81, 1.31)
Met158 Val | MPO T-764C	202 (17.2)	266 (16.3)	1.11 (0.84, 1.46)
Met158 Val | MPO C-764C	25 (2.1)	39 (2.4)	0.88 (0.51, 1.53)
Val158 Val | MPO T-764T	169 (14.4)	252 (15.5)	0.99 (0.74, 1.31)
Val158 Val | MPO T-764C	86 (7.3)	123 (7.5)	1.08 (0.76, 1.54)
Val158 Val | MPO C-764C	4 (0.3)	17 (1.0)	0.28 (0.08, 1.00)

[a]Unconditional logistic regression adjusted for the matching factors and BMI at age 18, weight gain since age 18, age at menarche, personal history of benign breast disease, first-degree family history of breast cancer, age at first birth/parity, age at menopause, and duration of postmenopausal hormone use.

[*]Numbers do not add to the total number of women due to missing values in genotyping or covariates.

[†]The sum of percentage does not add up to 100 due to rounding.

[¶]P-interaction = 0.57

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Table 3

Breast cancer risk associated with MPO polymorphism and dietary intakes

	Low consumption[§]		High consumption[§]		
	Cases/ Controls	OR (95% CI)[a]	Cases/ Controls	OR (95% CI)[a]	P for interaction[¶]
Fruit and vegetable[*]					
TT	360/490	1.00 (Ref)	330/492	0.89 (0.71, 1.11)	0.26
TC	188/242	1.11 (0.87, 1.41)	180/258	0.88 (0.68, 1.14)	
CC	25/32	1.11 (0.63, 1.96)	15/31	0.58 (0.30, 1.12)	
TT	360/490	1.00 (Ref)	330/492	0.89 (0.71, 1.11)	0.37
TC+CC	213/274	1.11 (0.87, 1.40)	195/289	0.84 (0.65, 1.08)	
Fruit[*]					
TT	349/490	1.00 (Ref)	341/492	0.98 (0.79, 1.22)	0.86
TC	170/233	1.04 (0.81, 1.34)	198/267	1.03 (0.80, 1.33)	
CC	23/33	0.97 (0.54, 1.73)	17/30	0.77 (0.41, 1.46)	
TT	349/490	1.00 (Ref)	341/492	0.98 (0.79, 1.22)	0.98
TC+CC	193/266	1.03 (0.81, 1.31)	215/297	1.01 (0.79, 1.29)	
Vegetable[*]					
TT	372/499	1.00 (Ref)	318/483	0.86 (0.69, 1.07)	0.23
TC	206/236	1.18 (0.93, 1.51)	162/264	0.78 (0.60, 1.01)	
CC	24/35	0.92 (0.52, 1.61)	16/28	0.70 (0.36, 1.35)	
TT	372/499	1.00 (Ref)	318/483	0.86 (0.69, 1.07)	0.16
TC+CC	230/271	1.15 (0.91, 1.45)	178/292	0.77 (0.60, 1.00)	
Carotenoids[*]					
TT	386/534	1.00 (Ref)	367/525	0.95 (0.78, 1.16)	0.46
TC	211/260	1.12 (0.88, 1.42)	192/282	0.96 (0.75, 1.22)	
CC	24/37	0.94 (0.54, 1.62)	18/33	0.68 (0.36, 1.27)	
TT	386/534	1.00 (Ref)	367/525	0.95 (0.77, 1.16)	0.50
TC+CC	235/297	1.10 (0.87, 1.38)	210/315	0.93 (0.73, 1.17)	
Vitamin C[*]					
TT	374/523	1.00 (Ref)	379/536	0.91 (0.74, 1.11)	0.36
TC	182/270	0.92 (0.72, 1.17)	221/272	1.11 (0.87, 1.40)	
CC	22/33	0.93 (0.52, 1.67)	20/37	0.67 (0.37, 1.22)	

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	Low consumption[§]		High consumption[§]		
	Cases/ Controls	OR (95% CI)[a]	Cases/ Controls	OR (95% CI)[a]	P for interaction[¶]
TT	374/523	1.00 (Ref)	379/536	0.91 (0.74, 1.11)	0.17
TC+CC	204/303	0.92 (0.73, 1.16)	241/309	1.05 (0.84, 1.32)	
Vitamin E[*]					
TT	381/529	1.00 (Ref)	372/530	0.82 (0.67, 1.01)	0.10
TC	203/281	0.94 (0.74, 1.20)	200/261	0.99 (0.78, 1.26)	
CC	19/38	0.67 (0.38, 1.21)	23/32	0.85 (0.47, 1.53)	
TT	381/529	1.00 (Ref)	372/530	0.82 (0.67, 1.01)	0.11
TC+CC	222/319	0.91 (0.72, 1.15)	223/293	0.97 (0.77, 1.23)	

[§] Low and high consumption are based on median values of control group: fruit and vegetable, 5.43 servings/day; fruit, 2.18 servings/day; vegetable, 3.14 servings/day; carotenoids, 8962 IU/day; vitamin C, 227 mg/day; vitamin E, 15 mg/day.

[a] ORs and 95% CIs are calculated by unconditional logistic regression adjusted for the matching factors and BMI at age 18, weight gain since age 18, age at menarche, personal history of benign breast disease, first-degree family history of breast cancer, age at first birth/parity, age at menopause, and duration of postmenopausal hormone use.

[¶] LRT comparing the nested models with interaction terms to the main effect models.

[*] Numbers in the cells are smaller than the total sample size due to missing values in covariates.

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Table 4

Breast cancer risk associated with MPO polymorphism and plasma antioxidants[§]

	Q1 (low)		Q2		Q3		Q4 (high)		
	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[a]	P for interaction[¶]
Carotenoids[*]									
TT	153/133	1.00(Ref)	133/127	0.96(0.67, 1.38)	136/145	0.83(0.58, 1.19)	149/168	0.78(0.54, 1.11)	0.91
TC	76/65	1.05(0.68, 1.62)	67/68	0.81(0.53,1.25)	91/76	1.06(0.70, 1.60)	76/90	0.74(0.49, 1.12)	
CC	7/9	0.80(0.28, 2.31)	5/7	0.79(0.23, 2.72)	16/10	1.39(0.59, 3.31)	9/17	0.44(0.18, 1.09)	
TT	153/133	1.00(Ref)	133/127	0.96(0.67, 1.38)	136/145	0.84(0.58, 1.20)	149/168	0.78(0.54, 1.11)	0.99
TC+CC	83/74	1.02(0.67, 1.55)	72/75	0.81(0.53, 1.23)	107/86	1.10(0.74, 1.64)	85/107	0.69(0.46, 1.04)	
α-tocopherol[*]									
TT	166/146	1.00(Ref)	147/138	0.94(0.66, 1.33)	129/142	0.82(0.58, 1.17)	129/147	0.73(0.51, 1.05)	0.44
TC	83/75	0.97(0.65, 1.47)	63/76	0.70(0.46, 1.08)	81/71	0.95(0.63, 1.44)	83/77	0.93(0.62, 1.40)	
CC	8/10	0.92(0.34, 2.48)	11/10	1.00(0.39, 2.61)	9/11	0.71(0.27, 1.86)	9/12	0.60(0.23, 1.56)	
TT	166/146	1.00(Ref)	147/138	0.94(0.66, 1.33)	129/142	0.83(0.58, 1.17)	129/147	0.73(0.51, 1.05)	0.27
TC+CC	91/85	0.96(0.65, 1.43)	74/86	0.74(0.49, 1.11)	90/82	0.92(0.62, 1.37)	92/89	0.88(0.59, 1.32)	
γ-tocopherol[*]									
TT	154/145	1.00(Ref)	130/142	0.93(0.66, 1.32)	130/147	0.87(0.61, 1.24)	157/139	1.12(0.79, 1.60)	0.73
TC	87/76	1.11(0.74, 1.67)	71/72	0.95(0.62, 1.45)	71/71	0.92(0.60, 1.41)	81/80	1.02(0.67, 1.54)	
CC	10/10	1.07(0.41, 2.83)	6/12	0.51(0.18, 1.45)	9/9	0.98(0.36, 2.66)	12/12	1.08(0.44, 2.63)	
TT	154/145	1.00(Ref)	130/142	0.93(0.66, 1.32)	130/147	0.87(0.61, 1.24)	157/139	1.12(0.78, 1.60)	0.61
TC+CC	97/86	1.10(0.75, 1.63)	77/84	0.89(0.59, 1.33)	80/80	0.92(0.61, 1.40)	93/92	1.02(0.69, 1.53)	

[§]Plasma levels of antioxidants were categorized into quartiles based on the batch-specific cut points of control subjects.

[a]ORs and 95% CIs are calculated by unconditional logistic regression adjusted for the matching factors and smoking status, alcohol intake, BMI at age 18, weight gain since age 18, age at menarche, personal history of benign breast disease, first-degree family history of breast cancer, age at first birth/parity, age at menopause, and duration of postmenopausal hormone use.

[*]Numbers in the cells are smaller than the total sample size due to missing values in covariates.

[¶]LRT comparing the nested models with interaction terms to the main effect models.