Sulfotransferase 1A1 Polymorphism, Endogenous Estrogen Exposure, Well-done Meat Intake, and Breast Cancer Risk

Wei Zheng, Dawen Xie, James R. Cerhan, Thomas A. Sellers, Wanqing Wen, and Aaron R. Folsom

Vanderbilt-Ingram Cancer Center and Vanderbilt Center for Health Services Research, Vanderbilt University, Nashville, Tennessee 37232-8300 [W. Z., W. W.]; School of Public Health, University of South Carolina, Columbia, South Carolina [D. X.]; Mayo Clinic Cancer Center, Rochester, Minnesota 55905 [J. R. C., T. A. S.]; and School of Public Health, University of Minnesota, Minneapolis, Minnesota 55454 [A. R. F.]

Abstract

Sulfotransferase (SULT) 1A1 is involved in the inactivation of estrogens and bioactivation of heterocyclic amines and polycyclic aromatic hydrocarbons. A G→A transition at codon 213 (CGC/Arg to CAC/His) of the SULT1A1 gene was reported recently, and individuals homozygous for the His allele have a substantially lower activity of this enzyme than those with other genotypes. We hypothesized that the His allele may be a risk factor for breast cancer, particularly among women who had risk factors related to higher endogenous estrogen exposure. This hypothesis was investigated in a case-control study conducted in a cohort of postmenopausal Iowa women who completed a mailed questionnaire in 1986 on lifestyle factors including information on major breast cancer risk factors. DNA samples and information related to well-done meat intake were obtained from breast cancer cases diagnosed between 1992 and 1994 and a random sample of cancer-free cohort members. Multivariate analysis was performed on data from 156 cases and 332 controls who donated a blood sample. The frequency of the His allele was 41.6% in cases and 34.1% in controls (P = 0.03), and the risk of breast cancer was increased with the number of His alleles (P for trend = 0.02). Compared with women with the Arg/Arg genotype, an 80% elevated risk was observed among women homozygous for the His allele (95% confidence interval, 1.0–3.2; P = 0.04). This positive association was more pronounced among women who drank alcohol and had a high body mass index, early age at menarche, and late age at menopause, factors related to high endogenous estrogen exposure, than among those who did not have these risk factors. The risk of breast cancer was elevated with increasing doneness level of red meat intake among women with the Arg/Arg genotype (P for trend, 0.01) or the Arg/His genotype (P for trend, 0.10), whereas this association was not evident for women with the His/His genotype. The results from this study suggest that homozygosity for the SULT1A1 His213 allele may be a risk factor for breast cancer, and its effect may be modified by the exposure level of endogenous estrogens and heterocyclic amines.

Introduction

Sulfonate conjugation is an important pathway in the metabolism of a variety of endogenous and exogenous compounds, including estrogens and other mammary carcinogens (1–3). This reaction is catalyzed by SULTs, a superfamily of multifunctional enzymes including six cytosolic SULTs that have been identified in human tissues (1–3). Some of these enzymes share substantial amino acid sequence identity and overlap considerably in substrate specificity and tissue distribution (1–3). SULT1A1 is one of the most important members in this enzyme family due to its extensive tissue distribution and abundance (1–3). This enzyme has a substantially higher activity than other SULTs in catalyzing the sulfonation of 4-nitrophenol, a commonly used assay in biochemical pharmacogenetic studies for testing the activity of thermostable phenol SULTs (1–3). SULT1A1 catalyzes the sulfonation of estrogens to form water-soluble and biologically inactive estrogen sulfates, reducing the level of estrogen exposure in their target tissues (1, 2). Although sulfonation is, in general, considered a detoxification reaction, several SULTs, particularly SULT1A1, are involved in the bioactivation of certain procarcinogens, including heterocyclic amines and PAHs (3–6), well-documented mammary carcinogens (7, 8). The sulfate esters of these compounds are highly reactive and can bind to DNA to form adducts (3, 6). Therefore, it is possible that SULTs may be related to the risk of breast cancer through both their role in the inactivation of estrogens and bioactivation of environmental mammary carcinogens.

The polymorphic nature of SULTs has long been recognized (1–3, 9). Because of a strong correlation of enzyme activity and thermal stability between SULTs from platelets and those from other organs such as liver, brain, and intestine, platelets were used, for convenience, in many previous biochemical pharmacogenetic studies of human SULTs (1, 2, 9). These studies have demonstrated a large individual variation (about 50-fold in some studies) in the activity of platelet SULTs in humans (1, 2, 9, 10). It was reported recently that a large portion of this variability could be explained by a newly identified common polymorphism (a G→A transition) in the coding...
region (nucleotide 638) of the SULT1A1 gene (10). This base change in the gene sequence results in an arginine to histidine substitution at codon 213, and individuals homozygous for the His allele had only about 15% of the SULT activity in platelets of those with other genotypes. The His allele is common in the Caucasian population, and the frequency of this allele was reported to be about 30–35% (11, 12). Given the role of SULT1A1 in the metabolism of estrogens and environmental carcinogens, we hypothesized that the SULT1A1 polymorphism and its interaction with relevant lifestyle factors may be associated with the risk of breast cancer. We have designed a PCR-based RFLP assay to investigate the association of the SULT1A1 polymorphism with breast cancer risk in a case-control study conducted in the Iowa Women’s Health Study.

Materials and Methods

The Iowa Women’s Health Study is a prospective cohort study of 41,836 women, ages 55–69 years, who completed a self-administered questionnaire in January 1986. This cohort of women has been followed for mortality and cancer incidence through computer linkage of study participants with Iowa death certificate files, the National Death Index, and cancer diagnosis data collected by the Iowa State Health Registry, part of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute (13–16). The baseline questionnaire focused on anthropometric, dietary, and other major risk factors for cancer, including family history of cancer, prior medical conditions, cigarette smoking, reproductive factors, and hormone use. Circumferences of the waist and hip were measured according to a validated protocol. These were used to construct a WHR. Details on the methodology of the cohort study have been published elsewhere (13–16).

From 1995 to 1996, a case-control study was conducted in the Iowa Women’s Health Study to collect DNA samples and additional information on meat consumption habits (17–21). Eligible cases for this case-control study were cohort members who were diagnosed with breast cancer between January 1, 1992 and December 31, 1994 (n = 456). Controls were randomly selected from women who were cancer free as of January 1, 1992 (n = 900). Of the 900 controls, 24 were excluded from the control group because they were later found either to have a breast cancer diagnosis (n = 3) or to have been selected to participate in other Iowa Women’s Health Study ancillary projects (n = 21). All eligible women were asked to complete a self-administered questionnaire on meat consumption habits during the reference year (1991, 1992, or 1993). The reference year for cases was the year immediately before breast cancer diagnosis. Controls were divided randomly into three approximately equal groups for each reference year and answered the same questionnaire. In addition, a series of color photographs used to represent the various doneness levels of hamburger, beefsteak, and bacon was included in the questionnaire to facilitate the assessment of usual doneness levels of meat consumed by the study participants (17). Of all women selected for the study, 273 cases and 657 controls responded, representing approximately 60% and 75% response rates, respectively. The major reasons for nonparticipation were refusal (29.1% of cases and 18.7% of controls), inability to locate subject (4.9% of cases and 3.8% of controls), and subject death before contact (5.7% of cases and 2.5% of controls).

Exfoliated buccal cell samples were collected through the mail as a source of genomic DNA for genotyping assays for this study. About 96.6% of women (267 cases and 631 controls) who completed the supplemental questionnaire provided a sample of exfoliated buccal cells using the cytobrush method (22). Genomic DNA from buccal cells were extracted using the NaOH method described by Richards et al. (22). Blood samples were also collected through the mail from 488 women (156 cases and 332 controls), reflecting an overall response rate of 53% (57% for cases and 50% for controls; Refs. 18–20). Specifically, a blood collection kit including vacutainer tubes, biological specimen packaging containers and envelopes, and instructions was mailed to all women who agreed to donate a blood sample. Study participants were instructed to contact their physicians to have their blood drawn and to return samples via overnight express mail using preaddressed, prepaid envelopes provided by the study. Because of an unsatisfactory quality and quantity of DNA extracted from buccal cells, this study was restricted to those whose blood samples were collected.

Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol and stored at a low temperature for subsequent assays (18–20). Most assays for the SULT1A1 genotypes were completed in the summer of 1998 using a PCR RFLP-based assay. According to the published sequence of the human SULT1A1 gene (10, 23), we designed two primers (forward primer, 5′-GGGTCTCTAGAGAGGAGTCGGC; reverse primer, 5′-GCTTGTTGTTCACTGAACCTCC) to amplify a 270-bp fragment of exon 7 that included the polymorphic site (codon 213, His/CAC to Arg/GCG) of the gene. The PCR reactions were performed on a Perkin-Elmer GeneAmp System 9700 according to the manufacturer’s protocol. Specifically, these reactions were carried out in 50 μl of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM deoxyribonucleotide triphosphates, and 1 unit of Taq polymerase. The reactions were heated to 94°C for 1 min followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s. At the end, the reactions were extended 7 min at 72°C. The PCR products (270 bp) were digested with HhaI and analyzed by gel electrophoresis [3% (2:1) NuSieve/SeaKem agarose]. Digestion of each PCR product with HhaI gives rise to 155- and 115-bp fragments for the Arg/GCG allele and a single 270-bp fragment for the His/CAC allele. The SULT1A1 genotypes were successfully identified for all but one case and four controls, resulting in 155 cases and 328 controls for data analysis.

ORs were calculated to measure the strength of the association of breast cancer risk with SULT1A1 genotypes. Unconditional logistic regression was used to control for potential confounding variables assessed in the baseline survey in 1986 and to derive adjusted ORs and 95% CIs. In addition to the study variables, covariates included in multiple logistic models were age (continuously), WHR (<0.85, ≥0.85), and live births (<4, ≥4), variables that were significantly associated with the risk of breast cancer in this study population. Trend tests for dose-response relationships across levels of the exposure variables were performed by treating ordinal score variables (with values of 1, 2, 3, . . . ) as continuous variables in logistic regression models. Tests for interaction used the likelihood ratio test by adding the interaction term(s) (genotype by exposure) to the model that already included the main effect variables (genotype, exposure) and confounding variables. Reported P values are based on two-tailed probability tests.

BMI, age at menarche, age at menopause, and alcohol consumption were evaluated as potential modifiers of SULT1A1 genotypes because they are related to endogenous estrogen exposure. Although WHR, age at first live birth, and parity are also hormonally related, their association with breast cancer risk is thought to be mainly through mechanisms other than cumulative endogenous estrogen exposure (24, 25). These
variables were also considered in stratified analyses. Exposure to well-done meat was measured by assessing usual doneness levels of hamburgers, beefsteaks, and bacon. Doneness levels of rare or medium, well-done, and very well-done meat were given scores of 1, 2, or 3, respectively, for each food to describe the eating habits of participants on the basis of their responses to the color photographs in the questionnaire. A doneness score, defined as the sum of the doneness levels of hamburgers, beefsteaks, and bacon.

### Results

Case-control differences in the distribution of selected demographic and major risk factors are shown in Table 1. Information related to well-done meat intake, a relevant environmental exposure for the current analyses, is also presented in Table 1. The risk of breast cancer was found to be positively associated with age, education, family history of breast cancer, WHR, BMI, and reproductive factors, although with this sample size, only the ORs for WHR and number of live births were statistically significant. A clear dose-response relationship between meat doneness level and breast cancer risk was observed. The results were similar to those observed from all study participants, including those who did not provide a blood sample.

Table 2 presents the frequencies of SULT1A1 alleles and genotypes with selected lifestyle factors, stratified analyses were performed (Table 3). With a few exceptions, the risk of breast cancer was elevated for those who carried the His allele compared to women with the Arg/Arg genotype in virtually all strata defined by the lifestyle factors. The positive association of breast cancer with the His/His genotype was stronger among women who had factors reflecting higher endogenous estrogen exposure, such as high BMI, later age at menopause, earlier age at menarche, and regular alcohol consumption, than those who did not have these factors. The trend tests for the gene dose effect were statistically significant only among those who had these risk factors. Although age at first live birth, parity, and WHR are also established risk factors for breast cancer, their association is believed to be mainly through mechanisms (e.g., promoting mammary cell differentiation or insulin resistance) other than cumulative estrogen exposure. The positive
association with the His/His genotype was stronger in women who had an early age at first live birth, lower parity, or low WHR than it was in those who did not have these factors. Although these data suggested an interactive effect, tests for multiplicative interaction were not statistically significant, perhaps due to the small sample size.

To evaluate potential modifying effects of SULT1A1 genotype on the association between well-done meat intake and breast cancer risk, cases and controls were tabulated according to the joint distribution of these two factors (Table 4). In the stratified analysis by meat doneness level, a substantially elevated risk of breast cancer was found to be associated with the His/His genotype (low enzyme activity) only among women who consumed meat rare or medium. The ORs for the association with the His/His genotype were 5.1 (95% CI, 1.7–15.7) among women who consumed meats rare/medium and only 1.6 (95% CI, 0.5–4.6) among those who consumed meats consistently well or very well done. The effect of meat doneness level was evaluated in the stratified analysis by SULT1A1 genotypes. The risk of breast cancer was elevated only among women who carried the Arg/Arg genotype (high enzyme activity). These data again suggested an effect modification, although the interaction test based on the multiplicative model was not statistically significant.
Discussion
To our knowledge, no study has been published thus far on the association between the SULT1A1 polymorphism and breast cancer risk. The data from this study suggest that the R213H polymorphism of the SULT1A1 gene may influence the risk of breast cancer. Homozygosity for the His allele was associated with an 80% elevated risk of breast cancer, and the risk was further elevated among women who had some risk factors related to higher endogenous estrogen exposure. We also found that the SULT1A1 polymorphism may modify the association between well-done meat intake and breast cancer risk; the positive association with usual doneness level of meat intake was more evident among women who carried the Arg allele than those who carried the His allele. Our findings are new and consistent with the role of SULT1A1 in the metabolism of estrogens and environmental carcinogens, such as heterocyclic amines and PAH (1–6), well-documented mammary carcinogens in animal models (4, 7, 8).

Our finding of a positive association between the His allele (encoding a low activity allozyme) and breast cancer risk is consistent with the role of SULT1A1 in the inactivation of estrogens, the hormones believed to play a central role in the etiology of breast cancer (24, 25). This finding was further supported by the observation that the positive association with the His allele was more evident among women who had risk factors related to higher endogenous estrogen exposure. It has been reported that the level of endogenous estrogen is positively related to age at menopause and inversely related to age at menarche (24, 25). Alcohol drinking also increases blood estrogen levels (24–26). After menopause, adipose tissue is the major source of estrogen production from androgens (24, 25, 27), and high body weight, often measured using BMI, has been demonstrated to be a risk factor for postmenopausal breast cancer (19, 23). It was intriguing to find that the association of breast cancer with the His/His genotype was stronger among women who had a high BMI, earlier age at menarche, later age at menopause, and a regular alcohol consumption history than it was among those who did not have these factors.

The strong association of breast cancer risk with the His allele observed in this study among women with a low WHR is puzzling because a high WHR is a risk factor for breast cancer. In a cross-sectional study of 88 participants of the Iowa Women’s Health Study, we found that WHR was positively correlated with blood level of insulin and inversely related to the blood level of sex hormone-binding globulin (28). However, WHR was not correlated with blood estrogens ($r = -0.16$ for estrone; $r = 0.13$ for estradiol), even free estradiol ($r = 0.14$). Recently, Tchernof et al. (29) reviewed published data from previous epidemiological studies and concluded that there is an increase in central body fatness during the transition period of menopause, and this increase may be due to estrogen deficiency as a result of a progressive loss of ovarian function in estrogen production. Intervention studies have shown that estrogen replacement therapy reduces central fat distribution in postmenopausal women (29). The association of breast cancer with the His/His genotype also appears to be stronger among women who had an earlier age at first live birth and lower parity than it is among those who did not have these factors. It appears that this observation cannot simply be explained by the cumulative estrogen exposure hypothesis. During a pregnancy, particularly during the first pregnancy, breast cells proliferate rapidly and differentiate into mature cells prepared for lactation (24, 25). Mature breast cells have a longer cell cycle and spend more time in $G_1$, the phase that allows for DNA repair, and are thus less susceptible to carcinogens (24). Early age at first live birth is believed to reduce the susceptibility of mammary tissue at an early stage of a woman’s life, and additional live births may confer further benefits by increasing mature cells (and thus decreasing susceptible cells) of the breast.

Burned meat contains a wide variety of potentially carcinogenic compounds (4, 7, 8, 30), including heterocyclic amines and PAH procarcinogens that can be activated by SULT1A1 and other SULT variants (2–4). Despite some inconsistent findings (31), high intakes of fried foods and a preference for well-done meats have been shown to be associated with an increased risk of breast cancer in several epidemiological studies (17, 21, 32–37), including a prospective study reported recently (37). We recently reported a dose-response relationship between doneness levels of meat consumption and the risk of breast cancer, with a nearly 5-fold elevated risk observed among women who consistently consumed very well-done meat as compared with controls, recall intake of well-done meat as compared with controls, particularly because consumption of well-done hamburger is recommended in the news media to reduce the risk of Escherichia coli infection.

In summary, this case-control study found that postmenopausal women who were homozygous for the His allele at codon 213 of the SULT1A1 gene may be at an increased risk of breast cancer, particularly if they have risk factors related to higher endogenous estrogen exposure. Our data also suggest a potentially modifying effect of the SULT1A1 polymorphism on the association of well-done meat intake and breast cancer. The dual effect of SULT1A1 in the inactivation of estrogens and activation of environmental carcinogens complicates the association between SULT1A1 genotype and breast cancer risk. Because endogenous estrogen exposure is believed to play a
more important role in the etiology of breast cancer than heterocyclic amine and PAH exposure, it is conceivable that low activity of SULT1A1 (the His allele) may be a risk factor for breast cancer for most women. The high frequency of this risk allele and the involvement of this enzyme in estrogen, heterocyclic amine, and PAH metabolism suggest that SULT1A1 polymorphism may be important in the etiology of breast cancer. The sample size of this study is small, and additional studies will be needed to replicate the findings.

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