Short Communication

Association of CYP1B1 Polymorphisms and Breast Cancer Risk

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Abstract

Cytochrome P450 1B1 catalyzes the conversion of 17β-estradiol (E2) to the catechol estrogen metabolites 2-OH-E2 and 4-OH-E2 that have been postulated to be involved in mammary carcinogenesis. We sought to determine whether two common functional polymorphisms in Cytochrome P450 1B1, V432M (m1) and A453S (m2) are related to breast cancer risk. Using a nested case control design within the Nurses’ Health Study cohort, we genotyped 453 cases and 456 controls and found no significant association between m1[val/leu and leu/leu versus val/val, OR = 1 (CI, 0.72–1.45)] or m2 [asn/ser versus ser/ser, OR = 0.8 (CI, 0.62–1.15)] and breast cancer risk. However, we did observe women with the Val/Val (m1) genotype to have a higher percentage of estrogen receptor-positive tumors (P = 0.03). We did not observe any correlation with the m2 genotypes and estrogen receptor status. The association of the m1 and m2 genotypes on plasma hormone levels in postmenopausal control women not using hormone replacement therapy was also evaluated. Carriers of the m1 leu and m2 ser alleles had modestly higher estradiol levels but similar estrone and estrone sulfate levels. The results presented do not support a strong association between m1 and m2 and the risk of breast cancer.

Introduction

E2 is thought to have a role in mammary carcinogenesis. E2 is metabolized by either formation of the catechol estrogen derivatives 2-OH-E2 and 4-OH-E2 or by C-16α hydroxylation. The CYP1B1 enzyme predominately catalyzes the formation of 4-OH-E2 (1–3), the most carcinogenic estrogen in animal models (4). Unlike the 2-OH-E2 derivative, 4-OH-E2 induces uterine adenocarcinoma (5) and can induce DNA single-strand breaks (6). In one study, human breast cancer tissue had a significantly higher ratio of 4-OH-E2/2-OH-E2 compared with adjacent normal tissue (7). In human breast cancer cell lines, the formation of 4-OH-E2 is inducible by dioxin, a common environmental contaminant (8). Although CYP1B1 is expressed in a wide variety of tissues, expression is particularly high in the breast, prostate, and uterus (9, 10), supporting a role for CYP1B1 in hormone-mediated cancer. These findings underscore the importance of the CYP1B1 with regard to metabolism of environmental carcinogens and estrogens and its potential role in the initiation of tumors in estrogen-responsive organs, like the breast.

Two polymorphisms have been examined in relation to breast cancer risk, the m1 allele (Val to Leu at codon 432) and the m2 allele (Asn to Ser at codon 453). Recently, biochemical studies determined that the Val allele and the Asn allele had higher catalytic efficiency for the 4-hydroxylation of estradiol compared with their wild-type counterparts (11, 12). Changes in 4-hydroxylation of 17β-estradiol are of particular interest because of the potential carcinogenicity and estrogenic activity of the 4-OH-E2.

In a case control study, Bailey et al. (13), found no association with the m1 and m2 alleles and breast cancer risk. They did, however, find an association between the m1 Val/Val genotype and Caucasian breast cancer patients who had ER-positive breast cancer (P = 0.02); no correlation with the m2 allele was noted (13). In a second case control study of 186 Asian breast cancer cases and 200 Asian controls, the authors found that women with the m1 Leu/Leu genotype had a 2-fold elevated risk of breast cancer compared with women with the Val/Val genotype (14). This lack of consistency may be attributable to ethnic differences among studies. These authors were unable to evaluate receptor status.

In this study, we evaluated, among primarily Caucasian women, the relationship between the CYP1B1 alleles and breast cancer risk in a nested case control study within the NHS cohort. Given the role of CYP1B1 in estradiol metabolism, we also evaluated the relationship between the m1 and m2 alleles and circulating estrogen levels.

Materials and Methods

Study Population. The NHS was initiated in 1976, when 121,700 United States registered nurses between the ages of 30 and 55 returned an initial questionnaire reporting medical histories and baseline health-related exposures. Between 1989 and 1990, blood samples were collected from 32,826 women. Incident breast cancers are identified by self-report and confirmed by medical record review. Eligible cases in this study consisted of women diagnosed with pathologically confirmed incident breast cancer after giving a blood specimen up to June 1, 1994. Controls were matched to cases on year of birth, menopausal status, and postmenopausal hormone use, as well as age at menarche, age at first birth, age at menopause, and body mass index.

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3 The abbreviations used are: E2, 17β-estradiol; ER, estrogen receptor; CYP1B1, cytochrome P450 1B1; NHS, Nurses’ Health Study; OR, odds ratio; CI, confidence interval; BMI, body mass index.
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(13, 17). Genotype was also evaluated using dichotomous variables, Val/Leu + Leu/Leu combined and Asn/Ser + Ser/Ser combined, as a gene dosage effect on breast cancer risk was not apparent. Unconditional multivariate models controlling for the matching factors enabled all controls to be included in analyses, limiting the cases to specified histopathological characteristics. Interactions between genotypes and breast cancer risk factors were evaluated by including interaction terms in multivariate logistic regression models. The likelihood ratio test was used to assess the statistical significance of these interactions.

Mixed regression models were used to evaluate the association between genotype and circulating hormone levels among postmenopausal controls not currently using postmenopausal hormones, controlling for BMI at blood draw and the matching variables. Hormone fractions were measured in three different batches; laboratory batch was treated as a random effect in all hormone analyses. We calculated least square mean hormone levels to evaluate differences in hormone levels between the genotypes. The natural logarithm of the plasma hormone values was used in the analyses to reduce the skewness of the regression residuals. We used the SAS statistical package for all analyses (Ref. 18; SAS Institute, Inc.).

Results

Our study included 453 incident breast cancer cases and 456 cancer controls. A total of 348 cases and 345 controls were postmenopausal, and 64 cases and 69 controls were premenopausal, whereas menopausal status was uncertain for 41 cases and 42 controls. Self-reported major ethnicity/ancestry was similar between cases and controls (cases 16% NHS cases and 18% NHS women with the Val/Val genotype, women with the Asn/Asn genotype, women with the Asn/Asn and Ser/Ser genotype did not have elevated levels of estrone-sulfate or estrone; however, there was an increased level of estradiol (8.37%, P = 0.05). Similarly, compared with women with the Asn/Asn genotype, women with the Asn/Asn and Ser/Ser genotype did not have elevated levels of estrone-sulfate or estrone; however, there was an increased level of estradiol (16.5%, P = 0.03). We further evaluated the combination of m1 and m2 on breast cancer risk and observed no association.

Discussion

CYP1B1 has an important role in estrogen metabolism. It catalyzes the formation of 4-hydroxyestradiol, a carcinogenic metabolite that retains significant estrogenic activity (2). Several polymorphisms have been identified in CYP1B1, two of which have been studied in relation to breast cancer. The Val343Leu (m1) and the Asn453Ser (m2) polymorphisms both result in an amino acid change in exon 3, which encodes the heme-binding domain, the region critical to the catalytic function of CYP1B1 (13). Recent functional studies show differences between wild-type CYP1B1 and variants in estrogen hydroxylation; specifically, the ratio of 4-OH-E2:2-OH-E2 was increased, compared with treatment with 2-OH-E2 (19, 20). In our study, the high activity alleles are not associated with increased breast cancer risk, which is consistent...
with one other case control study that included 164 Caucasian cases and controls and 59 African-American cases and controls (13). Additionally consistent with the previous study, and with the recent biochemical data suggesting an increase in the enzymatic activity of the Val allele, is the association between the m1 Val polymorphism and ER-positive status. We found a marginally significant decrease in estradiol levels in women with the Val allele. Assays under development for estrogen metabolites, produced by CYP1B1, could be more informative, as these markers may serve as a more direct measure of CYP1B1 enzymatic activity.

Our results suggest that despite a potential association with estradiol levels, neither the V432L nor the A453S polymorphisms in the CYP1B1 gene alone or in combination are sufficient to substantially influence breast cancer risk in Caucasian women.

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References

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