Null Results in Brief

Association of *CYP1B1* Haplotypes and Breast Cancer Risk in Caucasian Women

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Abstract

CYP1B1 is a key enzyme involved in estrogen metabolism and may play an important role in the development and progression of breast cancer. In a population-based case-control study, we examined eight *CYP1B1* haplotype-tagging single nucleotide polymorphisms in relation to invasive breast cancer risk. Analyses were based on 1,655 cases and 1,470 controls; all women were Caucasian. Among the individual single nucleotide polymorphisms, one (rs9341266) was associated with increased risk of breast cancer (P_{trend} = 0.021), although the association was no longer significant after adjusting for multiple tests. A marginally significant haplotype effect was identified (P_{global} = 0.015), with significant associations identified for 2 uncommon haplotypes comprising 4% of the controls. Results suggest that genetic variation in *CYP1B1* has at most a minor influence on breast cancer susceptibility among Caucasian women. (Cancer Epidemiol Biomarkers Prev 2009;18(4):1321–3)

Introduction

Cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) is a key member of the cytochrome P450 (CYP) superfamily of enzymes (1). The encoded estrogen-metabolizing protein catalyzes the hydroxylation of estradiol (2), an estrogenic hormone that plays an important role in the development and progression of hormone-related cancers such as breast cancer. Overexpression of CYP1B1 may cause accumulation of estrogen metabolites that directly or indirectly damage DNA (3). A recent meta-analysis found no association between the *CYP1B1* Leu432Val polymorphism (rs1056836) and risk of breast cancer among Caucasian women (4). Associations with breast cancer have been examined for other putative functional polymorphisms in *CYP1B1* (5, 6). One previous analysis in Polish women showed no significant haplotype effects among eight *CYP1B1* tagging single nucleotide polymorphism SNPs (7). We undertook a comprehensive evaluation of common genetic variations in *CYP1B1* with breast cancer risk in a large U.S. population–based case-control study.

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Subjects and Methods

Study Population. The study population has been previously described (8). Briefly, the subjects enrolled in this population-based study were all English-speaking females, residing in Massachusetts, New Hampshire, and Wisconsin. Case women had a recent diagnosis of invasive breast cancer, were ages 20 to 69 y, and were identified through state-wide cancer registries. Controls were randomly selected from lists of licensed drivers (if <65 y) and from a roster of Medicare beneficiaries (if 65-69 y) in each state. DNA samples were collected through the mail from 70% of interviewed cases and 61% of controls using an oral rinse protocol (9).

SNP Genotyping. We genotyped the same haplotype-tagging SNPs in *CYP1B1* that were examined in the study by Gaudet et al. (7) of Polish women. The SNP identification process, based on strategies used in the Breast and Prostate Cohort Consortium (10), was described in the earlier report (7). In brief, SNPs were identified by a search of public databases and by resequencing exonic regions of the gene in a multiethnic panel of 190 patients with advanced breast or prostate cancer enrolled in the Multiethnic Cohort. This sample size was chosen to provide >85% power to discover variants with a minor allele frequency of >5% present in a single population group. Using the TagSNP program (11), a minimum set of 8 SNPs was selected to capture common haplotype diversity in *CYP1B1* among Causcasian women specifying a pairwise r^2 of >0.8. We

Table 1. Polymorphisms in *CYP1B1* and associations with invasive breast cancer

	No. invasive cases (%) $n = 1655$ [‡]	No. controls (%) $n = 1470$ [‡]	OR* (95% CI)*	P_{trend} [†]
rs163077				
CC	862 (52.4)	778 (53.4)	1.00 (Reference)	
CT	595 (36.2)	527 (36.2)	1.00 (0.86-1.16)	
TT	121 (7.4)	98 (6.7)	1.08 (0.81-1.44)	0.73
rs163086				
CC	1007 (61.2)	883 (60.6)	1.00 (Reference)	
CT	528 (32.1)	470 (32.3)	0.99 (0.85-1.15)	
TT	63 (3.8)	75 (5.2)	0.74 (0.52-1.04)	0.24
rs9341266				
CC	1487 (90.2)	1333 (91.1)	1.00 (Reference)	
CT	128 (7.9)	92 (6.3)	1.27 (0.96-1.68)	
TT	8 (0.5)	2 (0.1)	4.36 (0.92-20.72)	0.021
rs162562				
AA	969 (58.9)	871 (59.8)	1.00 (Reference)	
AC	556 (33.8)	476 (32.7)	1.03 (0.89-1.21)	
CC	81 (4.9)	84 (5.8)	0.86 (0.62-1.18)	0.75
rs1800440				
AA	1070 (64.9)	934 (63.8)	1.00 (Reference)	
AG	483 (29.3)	430 (29.4)	1.00 (0.86-1.17)	
GG	65 (3.9)	53 (3.6)	1.08 (0.74-1.57)	0.81
rs162557				
CC	901 (54.8)	837 (57.5)	1.00 (Reference)	
CT	609 (37.0)	489 (33.6)	1.14 (0.98-1.33)	
TT	88 (5.4)	98 (6.7)	0.82 (0.61-1.11)	0.80
rs162556				
TT	421 (25.6)	392 (26.9)	1.00 (Reference)	
CT	829 (50.4)	727 (49.9)	1.05 (0.89-1.25)	
CC	354 (21.5)	311 (21.4)	1.05 (0.86-1.29)	0.61
rs10175368				
GG	836 (50.7)	765 (52.3)	1.00 (Reference)	
AG	660 (40.1)	552 (37.7)	1.09 (0.94-1.26)	
AA	122 (7.4)	114 (7.8)	0.98 (0.75-1.29)	0.58

* ORs adjusted for reference age (categorical) and state of residence.
† Tests for trend in breast cancer risk for each SNP were conducted by inclusion of an indicator term representing the number of minor alleles (0, 1, 2).
‡ Differences between the total numbers of cases and controls and frequencies shown in the table are due to missing genotype data.

genotyped all the eight haplotype-tagging SNPs, including one SNP (rs1800440), which causes an amino acid change (Asn453Ser).

Isolation and preparation of the genomic DNA samples have been previously described (9). Genotypes were evaluated using validated Taqman or MGM Eclipse assays as described in detail at the snp500cancer Website[9] (12). Genotyping concordance among 192 duplicate pairs for the 8 SNPs ranged from 93% to 100%, with a median of 99% (concordance rates were <97% for rs9341266, rs1800440, and rs10175368).

We restricted the analysis to Caucasian women who comprised 98% of the study population (1,655 invasive cases and 1,470 controls). Genotypes for 7 of the 8 SNPs were consistent with Hardy-Weinberg equilibrium ($P \geq 0.05$) based on an exact test (13); 1 SNP (rs162557) had a borderline departure from Hardy-Weinberg equilibrium ($P = 0.03$).

Statistical Analysis. For each individual SNP, multivariable unconditional logistic regression was used to obtain age- and state-adjusted odds ratio (OR) estimates and 95% confidence intervals (CIs). Tests for linear trend in breast cancer risk for each individual SNP were conducted by including an ordinal coding of the genotypes of the SNP in the logistic regression models. Haplotype frequencies and effects were examined using the statistical program Haplo.stats (14). The function

Table 2. Haplotypes of *CYP1B1* polymorphisms and risk of invasive breast cancer

CYP1B1 haplotype*	Invasive breast cancer		
	Frequency (%)		OR (95% CI) [†]
	Cases ($n = 1655$)	Controls ($n = 1470$)	
CCCAACTA	23.4	23.4	1.00 (Reference)
CTCAACTG	17.7	19.7	0.91 (0.78-1.07)
CCCAGCCG	18.3	18.1	1.03 (0.88-1.21)
TCCCATCG	17.5	17.2	1.01 (0.86-1.19)
TCCAACCG	8.2	7.8	1.08 (0.88-1.33)
CCCCATTG	3.8	3.9	1.02 (0.77-1.35)
CCTAACTA	4.2	3.1	1.40 (1.05-1.87)
CTCAATTG	1.8	0.9	2.00 (1.23-3.27)
Rare haplotypes [‡]	5.1	5.9	0.86 (0.66-1.13)
			$P_{global} = 0.015$

*Polymorphic bases include rs163077, rs163086, rs9341266, rs162562, rs1800440, rs162557, rs162556, and rs10175368.
† ORs based on an additive effect model with adjustment for reference age (categorical) and state of residence.
‡ Haplotypes with <1% frequency.

[9] http://www.snp500cancer.nci.nih.gov/home_1.cfm

Haplo.score was used to estimate haplotypes and assess differences in haplotype frequencies between cases and controls. A global score test was used to evaluate the overall significance, with adjustment for age group and residence. Effects of individual haplotypes were also examined by comparing the breast cancer risk associated with each inferred haplotype to the risk associated with the highest estimated frequency haplotype. The estimated ORs and 95% CIs were obtained using the function Haplo.glm. Rare haplotypes with frequencies of <1% were pooled into a single category to reduce the burden of sparse table cells.

Results

The results of the association analysis for individual *CYP1B1* SNPs are summarized in Table 1. Results for one uncommon SNP (rs9341266) had a significant test for trend with increasing number of minor alleles (P_{trend} = 0.021). Results were essentially unchanged after adjustment for established breast cancer risk factors.

One LD block was identified in our study population (data not shown). Eight *CYP1B1* haplotypes were identified with a frequency >1% (Table 2). One haplotype comprising 3.1% of controls (CCTAACTA) was associated with a modestly elevated relative risk (OR, 1.40; 95% CI, 1.05-1.87). One other haplotype found in 1% of controls (CTCAATTG) was associated with an approximate doubling in the relative risk (OR, 2.00; 95% CI, 1.23-3.27) when compared with the most common reference haplotype. A marginally significant haplotype effect was found when comparing breast cancer cases with the controls (P_{global} = 0.015).

We considered results for individual SNPs according to menopausal status, body mass index, and use of hormone replacement therapy, and no differences were noted (data not shown).

Discussion

To our knowledge, this is one of the first studies that used haplotype-tagging SNPs to examine the influence of *CYP1B1* haplotypes on breast cancer risk. Using this approach, we found no evidence that Caucasian women with any common haplotype in *CYP1B1* have a substantially altered risk of developing invasive breast cancer. Despite a relatively large sample size, the study had limited power to examine uncommon haplotypes and interactions with potential effect modifiers, and we lacked information on steroid receptor status. Results are in line with one previous study that included an identical panel of haplotype-tagging SNPs (7), which found no significant associations with breast cancer among inferred *CYP1B1* haplotypes. A genome-wide association study based on the Cancer Genetic Markers of Susceptibility Project also did not identify breast cancer associations with markers at chromosome 2p22-p21 where *CYP1B1* resides (15). The study identified associations of individual *CYP1B1* variants with breast cancer risk and does not rule out a minor haplotype effect in Caucasian women.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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