

Alcohol Dehydrogenase Genetic Polymorphisms, Low-to-Moderate Alcohol Consumption, and Risk of Breast Cancer

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Background: In vitro, human isoenzymes encoded by genes homozygous for the *ADH1C**1 or *ADH1B**2 alleles metabolize ethanol to acetaldehyde at a faster rate than those homozygous for the *ADH1C**2 or *ADH1B**1 allele. Because alcohol is a known risk factor for breast cancer, we evaluated the joint association of genetic variants in *ADH* and alcohol consumption in relation to breast cancer.

Methods: A nested case-control study of 321 cases and matched controls was conducted. Five single nucleotide polymorphisms (SNPs) in the *ADH1C* and *ADH1B* genes were genotyped. Logistic regression was used to assess odds ratios (ORs) and 95% confidence limits (CIs) for each SNP. Haplotype analysis of all 5 SNPs was also undertaken.

Results: Among drinkers, the median intake of total alcohol was 13 g/wk (10th–90th percentiles; 4.5–135.9) in cases and 18 g/wk (10th–90th percentiles; 4.5–104.1) in controls. Women who drank alcohol tended to be at an increased risk of developing breast cancer compared with those who did not drink (OR = 1.40%, 95% CI 0.97–2.03), particularly those who were premenopausal at the time of breast cancer diagnosis (OR = 2.69%, 95% CI: 1.00–7.26). Of the known functional alleles, breast cancer risk was not significantly increased among carriers of at least 1 *ADH1C**1 or *ADH1B**2 allele, when compared with those homozygous for the genotype at each locus. However, breast cancer risk tended to be lower among women who inherited the *G* allele at *ADH1B* IVS1+896A > G (OR = 0.62, 95% CI 0.37–1.04). Overall haplotype frequencies were not significantly different between cases and controls.

Conclusions: In this study low levels of alcohol are associated with a modest increase in breast cancer risk that is not altered by known functional allelic variants of the *ADH1B* and *1C* gene. The protective association conferred by the *G* allele at *ADH1B* IVS1+896A > G needs further evaluation.

Key Words: Alcohol Dehydrogenase, Genotypes, Breast Cancer.

A NUMBER OF epidemiological studies have demonstrated that regular alcohol intake may be associated with an increase in the incidence of breast cancer in women (Hamajima et al., 2002; Smith-Warner

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et al., 1998; Tjønneland et al., 2003; Willett et al., 1987) with some evidence that there is a dose-response relationship independent of beverage type (Singletary and Gapstur, 2001). A pooled analysis of 6 prospective studies in Europe, Canada, and the United States, reported a 9% increase in the risk of breast cancer for every 10 g (0.75–1 drink) increase in alcohol intake per day (Smith-Warner et al., 1998). However, a consistent increase in the risk of breast cancer has not been reported at lower consumption levels (Kropp et al., 2001; Petri et al., 2004; Smith-Warner et al., 1998). In animal models ethanol intake has been demonstrated to cause mammary tumors (Singletary et al., 1991).

Despite evidence of a positive association between alcohol intake and breast cancer among humans and animals, the underlying biological mechanisms have yet to be clearly defined. In humans, 80% of ethanol is primarily oxidized to acetaldehyde by alcohol dehydrogenase (ADH) in the liver (Bosron and Li, 1986). Acetaldehyde can damage DNA by: (1) forming DNA adducts; (2) causing single and double strand breaks; (3)

forming cross-links and protein cross-links; and (4) inhibiting DNA repair (Grafstrom et al., 1994; Kuykendall and Bogdanffy, 1994; Ristow et al., 1995; Vaca et al., 1995). Chronic alcohol consumption can induce the cytochrome P450 enzymes, in particular CYP2E1, which assists in the conversion of alcohol to acetaldehyde (Gonzalez, 2005). CYP2E1 is also involved in the metabolism of various procarcinogens to carcinogens (Gonzalez, 2005). Further, both CYP2E1 and xanthine oxidoreductase (XOR), an enzyme involved in the metabolism of acetaldehyde to acetate, generate reactive oxygen species that have been implicated in carcinogenesis (McManaman et al., 1999; Wright et al., 1999). In MCF-7 breast cancer cell lines, alcohol at moderate doses down-regulates mRNA expression and protein levels of BRCA1, a known breast cancer tumor suppressor gene, while stimulating estrogen receptor expression (Fan et al., 2000). Moderate alcohol intake has also been associated with increased levels of circulating estrogens and DHEAS, and decreased levels of sex hormone binding globulin (SHBG) in premenopausal and postmenopausal women (Dorgan et al., 1994; Garcia-Closas et al., 2002; Hankinson et al., 1995; Hines et al., 2000; Reichman et al., 1993; Sierksma et al., 2004).

There are at least 5 different classes of human ADH isoenzymes based on differences at the molecular level (Bosron and Li, 1986; Smith et al., 1973). Class I ADH polypeptide subunits are encoded by 3 specific gene loci, *ADH1A* (α), *ADH1B* (β), and *ADH1C* (γ), previously known as *ADH1*, *ADH2*, and *ADH3*, respectively. These loci are in close proximity to one another. Single nucleotide polymorphisms (SNPs) in the *ADH1C* and *ADH1B* gene have identified that result in functional differences in the kinetic properties of the γ and β subunits, respectively (Hoog et al., 1986). The substitution of Isoleucine to Valine at position 349 and Arginine to Glutamine at position 271 correspond to the *ADH1C**2 allele versus the *ADH1C**1 allele (Xu et al., 1988). Similarly the substitution of Arginine for Histidine at position 47 corresponds to the *ADH1B**2 allele versus the *ADH1B**1 allele. In vitro, the γ -1 polypeptide subunits encoded by the *ADH1C**1 allele metabolize alcohol to acetaldehyde 2.5 times faster than the *ADH1C**2 allele and the β -1 polypeptide subunit, a product of the *ADH1B**2 allelic variant, oxidizes ethanol 100 times faster than products of the *ADH1B**1 variant (Bosron and Li, 1986; Hurley et al., 1990; Matsuo et al., 1989).

One prospective study and 3 retrospective case-control studies have examined the association between the *ADH1C**1 allele, alcohol intake, and the risk of breast cancer with conflicting results (Coutelle et al., 2004; Freudenheim et al., 1999; Hines et al., 2000; Terry et al., 2006). Two published studies, a case series, and a case-control study, have reported a protective association between alcohol drinkers who were also carriers of the *ADH1B**2 variant and the development of breast cancer (Lilla et al., 2005; Sturmer et al., 2002). Using prospectively gathered data,

our aims were to extend these recent analyses by conducting a prospective nested case-control study to examine the association of alcohol consumption, 5 SNPs of the *ADH1C* and *ADH1B* genes and the risk for breast cancer. These polymorphisms include 3 known functional variants of the *ADH1C* and *ADH1B* genes and 2 that are common in Caucasians but not associated with an amino acid change.

MATERIALS AND METHODS

In 1989, as part of the Campaign Against Cancer and Heart Disease (CLUE II) in Washington County, 32,898 individuals donated a blood sample and completed a brief questionnaire after signing an informed consent. This study is nested within the cohort comprised of the 14,625 women who were residents of Washington County and took part in the CLUE II Campaign. The brief questionnaire administered at the time of blood donation, before the diagnosis of cancer, included information on age, race, sex, height, weight, education, marital status, and smoking. Participants were asked to complete and return an extensively validated 60-item block Food Frequency Questionnaire (FFQ; Block G., 1987) along with a toe-nail clipping. A total of 11,112 women returned the FFQ. Cases were women who donated blood in 1989, and who were diagnosed with breast cancer as their first cancer up through 2002. Incident breast cancer cases were identified by linkage to the Washington County Cancer Registry and, since 1992, also the Maryland State Registry. Each case was matched to one control by race, freeze/thaw status, age (within 1 year), availability of FFQ, and menopausal status at baseline. If premenopausal, that is that they had menses in the prior 12 months, they were also matched by day of phase of menstrual cycle (0–11, 12–16, 17–31 days). Information on cancer stage and grade were based on the AJCC TNM staging guidelines [American Joint Committee on Cancer (AJCC), 2002]. Estrogen and progesterone receptor status were available from pathology records and the cancer registry. Controls were not known to be deceased at the time of diagnosis of the cases or to have been diagnosed with cancer other than cervical cancer in situ, or basal or squamous cell skin cancer.

Information on self-reported alcohol intake in the prior year was available at baseline for 82% of the cases and 82% of the controls. Study participants selected from 1 of 9 questionnaire categories (never or less than once a month up to 5 or more per day) regarding how many drinks were consumed for beer, wine, and liquor intake. Information on BMI, smoking, education, marital status, hormone therapy use, and oral contraceptive use at the time of blood donation were also available. Information on known breast cancer risk factors such as menopausal status, age of menarche, age of first birth, years of lactation, and family history (female first-degree relative or grandmother who had breast cancer) were obtained from subsequent follow-up questionnaires. Genotyping was attempted on 321 cases and 313 controls for *ADH1C*, *ADH1B* genotypes. In 8 controls, DNA was found to be of insufficient quality to perform the Taqman assay. The study was approved by the Committee on Human Research at The Johns Hopkins Bloomberg School of Public Health.

Laboratory Assays and Genotyping

Plasma, buffy coat, and red blood cells were separated and stored at -70°C within 24 hours of collection. The alkaline lysis method was used to extract DNA from peripheral buffy coat (Klitsch and Neuhuber, 2000). DNA concentration was set at 100 $\mu\text{g}/\text{mL}$. The *ADH1C* Ex8-56A>G—rs 698, *ADH1C* Ex6-14G>A—rs1693482, *ADH1C* IVS6 +10G>A—rs 1789912, and the *ADH1B* Ex3 +23 A>G—rs 1229984, *ADH1B* IVS1 +896A>G—rs 1353621 were assessed using the Taqman[®] or 5' nuclease assay (Applied Biosystems

Division, Perkin-Elmer, Foster City, CA). Previously 217 samples, 106 controls, and 111 cases were genotyped for the *ADH1C* Ex8-56A>G polymorphism using a modified version of the PCR/RFLP method of Groppi et al. (1990). There was 98% concordance between the 53 samples that had been analyzed by PCR and Taqman. A prior PCR/RFLP result was used for those 93 cases and 70 controls that were unable to be genotyped by Taqman for the *ADH1C* Ex8-56A>G polymorphism.

Statistical Analysis

Differences in the distribution of demographic, lifestyle, and breast cancer risk factors were compared between cases and controls using chi-square tests for categorical variables and *t*-tests for continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between these factors and breast cancer were also calculated using conditional logistic regression. Wine, beer, and liquor intake were converted to g/wk based on information from the USDA national nutrient database (a 12-ounce can of beer is equal to 13 g of ethanol, 1 medium glass of table wine has 9.6 g of ethanol, and 1 shot of liquor has 14 g of ethanol; USDA National Nutrient Database, 2005). Total alcohol intake was calculated based on the sum in g/wk of wine, beer, and liquor intake for each individual. The median, 10th and 90th percentiles for total alcohol intake were calculated for cases and controls. Given the high proportion of nondrinkers and the narrow distribution of alcohol consumption among those who drank alcohol information on wine, beer, and liquor was condensed to 2 categories: nondrinkers and drinkers. Different cutoff points were also explored among drinkers such as 25%, and 10% the results were not significantly different and therefore not reported here.

To minimize loss of cases logistic regression adjusting for age and menopausal status (matching factors) was used to assess the associations of alcohol and *ADH1C*, *ADH1B* genotypes with the risk of breast cancer. Because the results were similar to those obtained from conditional logistic regressions, we report only findings from the unmatched analyses. Characteristics assessed as potential confounders include years of education, smoking history, family history of breast cancer in mother, sister, grandmother or children, age at menarche, age at first birth, duration of lactation, oral contraceptive pill use, hormone replacement use and body mass index (BMI). BMI was calculated using information on weight and height obtained in 1989. Likelihood ratio tests were used to assess the effect of adding each variable to the model on the parameter estimates of the main association being tested. None of these variables altered the parameter estimates by $\geq 10\%$ and therefore were not included in the model. The cut-off was chosen a priori.

The association between alcohol intake and menopausal status at diagnosis, and stage and hormone receptor status of the tumor were also examined, given the potential biological differences between these groups. In women who were premenopausal at baseline, menopausal status at diagnosis was determined based on their age at the diagnosis of their breast cancer. Two cutoff points were examined (age ≤ 51 years and age ≤ 55 years) as a surrogate for menopausal status at diagnosis based on the average age of menopause in the United States. Women with hormone receptor positive tumors (estrogen and/or progesterone receptor positive) were analyzed separately to hormone receptor negative (estrogen and/or progesterone receptor negative) tumors. The controls of the matched case were included in the analysis. To assess for dose response, when more than 2 categories were involved, a trend test was performed across all levels of exposure by treating categorical variables as continuous ordinal variables in a logistic regression model. The median value among controls for that category was used.

Consistency with Hardy-Weinberg proportions was assessed for each polymorphism among controls using a chi-square test ($p > 0.05$). Measures of linkage disequilibrium (D' and r^2) were

calculated between each pair of SNPs. Known functional genotypes *ADH1C**2 and *ADH1B**1 were designated as slow alleles and *ADH1C**1 and *ADH1B**2 as fast alleles based on in vitro data (Bosron and Li, 1986; Hurley et al., 1990). In assessing the association between genotypes and breast cancer, the reference group was defined as women with no fast alleles. Women homozygous and heterozygous for the fast alleles were assessed separately and then also combined into 1 category. For the other 2 SNPs, *ADH1C*+10G>A and *ADH1B*+896A>G, the most prevalent homozygous genotype was used as the reference group. To assess the combined effect of *ADH* genotype and alcohol on breast cancer risk new variables were created with the reference group being nondrinkers homozygous for the slow allele. Effect modification by genotype, menopausal status, and BMI of the estimated ORs was assessed by the statistical significance of the product term in the logistic regression model.

Haplotype analyses were conducted for all 5 SNPs genotyped. Haplotypes were estimated using an estimation-maximization algorithm (Excoffier and Slatkin, 1995) and overall differences in haplotype frequencies between cases and controls were assessed using the global score test implemented in HaploStats (R Version 1.2.2), adjusting for age and menopausal status (Lake et al., 2003; Schaid et al., 2002). A logistic regression model was used to estimate the effect of individual haplotypes, assuming an additive model by using posterior probabilities of the haplotypes as weights to update the regression coefficients in an iterative manner.

As our data had missing observations in some covariates, including alcohol and genotypes, we used multiple imputations to generate 10 replicates of complete data sets. Decision trees were used to model the distributions of the missing data given the observed data, including the response (Dai et al., 2006). Models were fit on all 10 replicate data sets, and the results for the parameter estimates and standard errors were obtained (Little and Rubin, 1987; Schafer, 1997). The imputed results were then compared with the results without imputed data. Using chi-square and *t*-tests, we also assessed whether there were differences in other characteristics between those missing alcohol and genotype data and those who were not. Analyses were conducted using both STATA Software version 8.0 (Stata Corporation, College Station, TX, 2004) and R version 2.01 (The R Project for Statistical Computing, <http://www.r-project.org/>).

RESULTS

Characteristics of the study sample are shown in Table 1. The mean age was 56.8 and 56.6 years among cases and controls, respectively. The majority of the participants were Caucasian which was reflective of the residential area from where the population was sampled. A maternal family history of breast cancer which included first and second degree relatives was associated with an increased risk of breast cancer (OR = 2.32, 95% CI 1.35–3.97). A statistically significant dose response was observed between increasing BMI and breast cancer risk (p trend = 0.02). Women with a BMI ≥ 30 had 1.6 times the risk of developing breast cancer relative to women with a BMI < 25 (OR = 1.60, 95% CI 1.04–2.45). When stratified by menopausal status at baseline, breast cancer risk associated with BMI was only significantly increased among postmenopausal women (OR = 2.01, 95% CI 1.18–3.43). Further, the interaction between BMI (< 25 vs ≥ 25) and menopausal status was statistically significant ($p = 0.05$).

Table 1. Percentage Distribution of Descriptive Characteristics of Breast Cancer Cases and Controls at Study Baseline in 1989, Washington County, MD

	Cases (%), N = 321	Controls (%), N = 321	OR (95% CI)
<i>Age (y) at baseline</i>			
Mean (SD)	56.8 (12.4)	56.6 (12.3)	1.16 (0.98,1.37)
<i>Menopausal status at baseline</i>			
Premenopausal	26	29	1.0
Postmenopausal	71	70	2.00 (0.37,10.9)
Missing	3	1	
<i>Race</i>			
White	99	99	N/A
Black	1	1	
Other	<1	0	
<i>BMI at baseline (kg/m²)</i>			
<25	42	50	1.0
25–29.9	34	31	1.39 (0.96,1.99)
≥30	24	19	1.60 (1.04,2.45)
<i>Smoking</i>			<i>p</i> trend = 0.02
Never	62	65	1.0
Former	25	20	1.27 (0.87,1.85)
Current	13	15	0.84 (0.52,1.36)
<i>Education (grade)</i>			<i>p</i> trend = 0.91
<12	24	29	1.0
=12	42	41	1.23 (0.82,1.84)
>12	34	30	1.39 (0.91,2.11)
<i>Marital status</i>			<i>p</i> trend = 0.13
Never married	5	5	1.0
Married now	73	68	1.09 (0.53,2.27)
Other	22	27	0.84 (0.39,1.79)
Missing	0	<1	<i>p</i> trend = 0.27
<i>Ever pregnant</i>			
No	12	8	1.0
Yes	68	68	0.61 (0.33,1.11)
Missing	20	24	
<i>Age at first birth (y)</i>			
Nulliparous	12	8	1.0
<20	18	17	0.61 (0.30,1.24)
20–24	32	31	0.62 (0.32,1.20)
25–29	15	14	0.71 (0.34,1.49)
≥30	3	5	0.41 (0.13,1.29)
Missing	20	25	<i>p</i> trend = 0.26
<i>Months breast feeding</i>			
None	43	40	1.0
1–6	9	10	0.87 (0.42,1.77)
>6	15	18	0.79 (0.45,1.41)
Missing	33	32	<i>p</i> trend = 0.43
<i>Age at menarche (y)</i>			
<12	13	15	1.0
12–13	48	39	1.80 (1.02,3.16)
>13	19	22	1.17 (0.63,2.16)
Missing	20	24	<i>p</i> trend = 1.00
<i>Oral contraceptive use</i>			
Never	74	75	1.0
Former	25	21	1.26 (0.80,2.00)
Current	1	3	0.57 (0.17,1.95)
Missing		1	<i>p</i> trend = 0.30
<i>Other hormone use</i>			
Never	79	78	1.0
Former	9	12	0.79 (0.46,1.35)
Current estrogen and/or progesterone use	11	8	1.30 (0.71,2.37)
Missing	1	2	<i>p</i> trend = 0.4
<i>Maternal family history (first and second degree relatives)</i>			
No	63	71	1.0
Yes	20	9	2.32 (1.35,3.97)
Missing	17	20	

OR, odds ratios that were calculated using conditional logistic regression; CI, confidence interval; SD, standard deviation; BMI, body mass index (kg/m²) calculated from self-reported height and weight measurements.

Fifty-two percent of cases and 58% of controls did not drink. Among those women who drank alcohol the median consumption was 13.0 g/wk for cases (10th–90th percentiles; 4.5–135.9) and 18 g/wk (10th–90th percentiles; 4.5–104.1) for controls. Among controls alcohol intake varied by education, but not by age at baseline or BMI. Women with a 12th grade education or better were more likely to drink alcohol than those with less (32 vs 18%).

Women who drank alcohol were at an increased risk of breast cancer compared with those who did not (OR = 1.40, 95% CI 0.97–2.03; Table 2). The association did not significantly change when alternate cutoff points were used. When stratified by menopausal stage at breast cancer diagnosis, the odds of developing breast cancer was 2.69 (95% CI 1.00–7.26) in women ≤ 51 years of age who drank alcohol, relative to nondrinkers, and 1.25 (95% CI 0.84–1.87) among older female drinkers > 51 years compared with nondrinkers (Table 2). The interaction was not statistically significant ($p = 0.16$). Similar results were obtained when the cut point of ≤ 55 years was used (data not shown). No significant associations were observed between alcohol intake and estrogen or progesterone hormone receptor status (Table 2) or grade of tumor (data not shown). When stratified by education, a significant association between total alcohol intake and breast cancer risk was only observed among women drinkers with ≥ 12 th grade education compared with nondrinkers (OR = 1.49, 95% CI 0.99–2.24).

The association between different types of alcohol and breast cancer risk was also examined. Women who drank wine were 1.6 times more likely to develop breast cancer than nonwine drinkers (OR = 1.60, 95% CI 1.01–2.54).

However, no association was observed for women who drank beer (OR = 0.95, 95% CI 0.56–1.63) or liquor (OR = 1.10, 95% CI 0.65–1.86).

The genotype distribution among the control subjects reflect frequencies previously reported for the SNPs among Caucasians (<http://snp500cancer.nci.nih.gov>) and all were consistent with Hardy–Weinberg proportions ($p > 0.05$). Fifteen percent of cases and 11% of controls were missing information on all 5 genotypes. The *ADH1C* Ex8-56A $> G$ and *ADH1C* Ex16-14G $> A$ polymorphisms were in strong linkage disequilibrium ($D' = 1$) and almost completely correlated ($R^2 = 0.99$). Carriers of at least 1 *ADH1C**1 (Ex8-56A $> G$) allele were not at significantly higher risk of developing breast cancer than women homozygous for the *ADH1C**2 (Ex8-56A $> G$) allele in a multivariate analysis adjusted for matching factors (OR = 1.16, 95% CI 0.77–1.76). Results of a similar magnitude were observed for carriers of at least 1 *ADH1C**1 (Ex16-14G $> A$) allele (OR = 1.23, 95% CI 0.73–2.07) or at least 1 *ADH1B**2 (Ex3+23 A $> G$) allele (OR = 1.55, 95% CI 0.68, 3.56; Table 3). However, in women homozygous for the *G* allele at *ADH1B* IVS1+896A $> G$ there was a borderline trend toward a reduced risk of breast cancer when compared with women homozygous for the *A* allele at the locus (Table 3). The presence of an *A* allele at *ADH1C* IVS6+10G $> A$ did not confer any additional breast cancer risk (Table 3).

Power was limited to assess gene–gene interactions. Exploratory analyses revealed no significant associations. Five haplotypes were identified among the 5 SNPs (Figure 1). Overall, the difference between cases and controls was not statistically significant for either the global

Table 2. Association Between Alcohol Intake and Breast Cancer Risk in Washington County, MD, 1989–2002

Alcohol Intake	Cases, N = 321	Median (grams/week) (10th, 90th, pctile)	Controls, N = 321	Median (grams/week) (10th, 90th, pctile)	Adjusted OR, 95% CI
<i>Total</i>					
Nondrinkers	167	0	187	0	1.00 (ref.)
Drinkers	95	13.0 (4.5, 135.9)	76	18.0 (4.5, 104.1)	1.40 (0.97, 2.03)
Missing	59		58		
<i>Premenopausal at BC diagnosis</i>					
Nondrinkers	26	0	35	0	1.00 (ref.)
Drinkers	15	6.5 (4.5, 106.8)	9	17.5 (4.5, 52.8)	2.69 (1.00, 7.26)
Missing	9		8		
<i>Postmenopausal at BC diagnosis</i>					
Nondrinkers	141	0	152	0	1.00 (ref.)
Drinkers	80	13.0 (4.5, 139.1)	67	18.5 (4.5, 166.3)	1.25 (0.84, 1.87)
Missing	50		50		
<i>Estrogen receptor negative</i>					p interaction = 0.16
Nondrinkers	25	0	30	0	1.00 (ref.)
Drinkers	19	11.0 (4.5, 105.8)	14	12.8 (4.5, 102.5)	1.84 (0.75, 4.51)
<i>Estrogen receptor positive</i>					
Nondrinkers	112	0	129	0	1.00 (ref.)
Drinkers	64	14.8 (4.5, 142.3)	48	23.5 (4.5, 174.1)	1.47 (0.93, 2.31)
					p interaction = 0.75

Pctile, percentile; OR, odds ratios, adjusted for matching factors (baseline menopausal status and age); CI, confidence interval; BC, breast cancer; women ≤ 51 were categorized as premenopausal and women > 51 as postmenopausal in women in whom menopausal status at diagnosis was unknown; 51 cases and controls missing information on estrogen receptor status and therefore the cases and controls do not add up to 321.

Table 3. Associations Between *ADH* Genotypes and Breast Cancer Risk in Washington County, MD, 1989–2002

Genotypes	Controls N = 321	Controls N = 321	Adjusted OR (95% CI)
<i>Functional</i>			
ADH1C (Ex8-56A > G)			
A,A (2,2)	50	60	1.00 (ref.)
A,G (1,2)	133	137	1.14 (0.73,1.78)
G,G (1,1)	120	115	1.18 (0.75,1.86)
A,G/G,G (1,2, 1,1)	253	252	1.16 (0.77,1.76)
Missing	18	9	
			<i>p</i> trend = 0.50
ADH1C (Ex6-14G > A)			
G,G (2,2)	29	39	1.00 (ref.)
A,G (1,2)	100	105	1.24 (0.71,2.17)
A,A (1,1)	98	99	1.21 (0.69,2.13)
A,G/G,G (1,1/2,2)	198	204	1.23 (0.73,2.07)
Missing	94	78	
			<i>p</i> trend = 0.62
ADH1B (Ex3+23 A > G)			
A,A (1,1)	246	270	1.00 (ref.)
A,G (1,2)	14	10	1.43 (0.62,3.34)
G,G (2,2)	1	0	N/A
A,G/G,G (1,2/2,2)	15	10	1.55 (0.68,3.56)
Missing	60	41	
			<i>p</i> trend = 0.24
<i>Other</i>			
ADH1C (IVS6 + 10G > A)			
G,G	101	101	1.00 (ref.)
A,G	103	100	1.03 (0.69,1.53)
A,A	48	35	0.79 (0.47,1.34)
A,G/A,A	151	135	0.96 (0.66,1.38)
Missing	69	85	
			<i>p</i> trend = 0.49
ADH1B (IVS1 + 896A > G)			
A,A	96	106	1.00 (ref.)
A,G	117	108	0.87 (0.59,1.28)
G,G	52	33	0.62 (0.37,1.04)
A,G/G,G	169	141	0.79 (0.55,1.14)
Missing	56	74	
			<i>p</i> trend = 0.08

The rs numbers used by the NCI SNP 500 database (<http://snp500cancer.nci.nih.gov/home>) are as follows; *ADH1C* Ex8-56A > G—rs 698, *ADH1C* Ex6-14G > A—rs 1693482, *ADH1B* Ex3-+23 A > G—rs 1229984, *ADH1C* IVS6+10G > A—rs 1789912, *ADH1B* IVS1 + 896A > G—rs 1353621. OR, odds ratio, adjusted for matching factors (baseline menopausal status and age); CI, confidence interval.

test or individual haplotypes. There was a borderline association with 1 haplotype containing the *G* allele at *ADH1B* IVS1+896A > G.

Table 4 reports on the association between *ADH* genotype status, alcohol intake, and the risk of breast cancer. A nonstatistically significant increase in breast cancer risk was consistently observed among carriers of at least 1 *ADH1C**1 or *ADH1B**2 allele who drank alcohol when compared with women who did not drink and were homozygous for the *ADH1C**1 and *ADH1B**2 alleles, respectively (Table 4). The interaction between genotype and alcohol intake with respect to breast cancer risk for each association was not statistically significant (Table 4). The protective effect of the *G* allele at *ADH1B* IVS1+896A > G on breast cancer risk was not observed among drinkers when compared with nondrinkers homozygous for the *A* allele at the same locus. The association between the *ADH1C* IVS6+10 G > A polymorphism and breast cancer risk did not vary by alcohol intake (Table 4).

All the analyses reported here were reanalyzed with imputed results and then compared with the data generated without imputation. No meaningful differences were observed between the 2 sets of data. Further, based on available data with regard to lifestyle, demographic, and known breast cancer risk factors, there was no statistically significant difference in these factors between individuals with and without missing alcohol or genotype data.

DISCUSSION

In this prospective study, we observed a small but statistically significant increase in the risk of breast cancer only among premenopausal women who would be considered light to moderate drinkers. The presence of functional variants of the *ADH1C* or *ADH1B* gene, known to increase ADH activity in vitro, did not modify this association. However, in this study breast cancer risk tended to be lower in carriers of at least 1 *G* allele at *ADH1B*

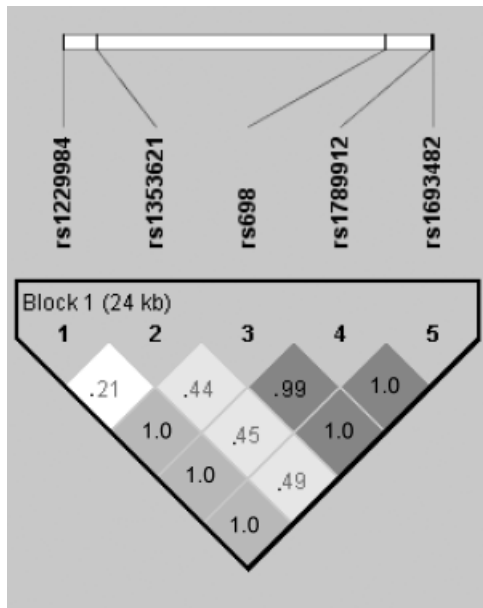


Fig. 1. Linkage disequilibrium between selected single nucleotide polymorphisms (SNPs) in *ADH1B* and *ADH1C* (Haploview 3.2, Barrett et al., 2005).

IVS1+896A>G when compared with women homozygous for the A allele at the same locus.

Three other prospective studies have reported increased breast cancer risk of a similar magnitude among light to moderate drinkers, 2 of which included premenopausal women (Friedenreich et al., 1993; Holmberg et al., 1994; van den Brandt et al., 1995). In one study, a dose response was observed only among premenopausal women

(*p* trend = 0.07). Similar results were also reported in a large case-control study where information of lifetime alcohol intake was collected (Freudenheim et al., 1999). The biological mechanism behind a possible difference in breast cancer risk from alcohol consumption based on menopausal status is unclear. Age-related differences in ADH and CYP2E1 enzyme activity in breast tissue is a possible explanation as such changes in enzyme activity have been observed in gastric tissue and blood (Bebia et al., 2004; Moreno et al., 1994; Pozzato et al., 1995).

The evaluation of the functional variants in the *ADH1C* gene, alcohol intake, and breast cancer risk was based on sound biological rationale from in vitro studies that reported differences in ADH enzyme activity arising from modifications in the γ polypeptide subunits. Further these enzymes have been detected in breast epithelial cells where 85% of breast cancers originate (Jelski et al., 2006; Triano et al., 2003). In 3 case-control studies, 2 that measured lifetime intake (Freudenheim et al., 1999; Terry et al., 2006), the *ADH1C*1* allele has been shown to significantly modify the association of alcohol and breast cancer particularly in premenopausal women (Coutelle et al., 2004; Freudenheim et al., 1999; Terry et al., 2006). In all 3 studies, the risk of breast cancer was at least 2-fold greater among women homozygous for the *ADH1C*1* allele who drank alcohol, compared with nondrinkers. These results were not reproduced in a prospective study of 465 incident breast cancer cases and 621 controls (Hines et al., 2000). Using nondrinkers as the reference group, they observed a small increase in breast cancer risk among women who drank alcohol greater than or equal to 10 g/d (OR = 1.1,

Table 4. Association Between *ADH* Genotype Status, Alcohol Intake, and the Risk of Breast Cancer, Washington County, MD, 1989–2002

Genotype	Nondrinker			Drinker		
	Cases/controls	OR	95% CI	Cases/controls	OR	95% CI
<i>Functional</i>						
<i>ADH1C</i> (Ex8-56A>G)						
2,2	25/30	1.00(ref.)		14/19	0.86	(0.36, 2.05)
1,2/1,1	132/154	0.96	(0.53,1.72)	75/52	1.63	(0.86, 3.11)
			<i>p</i> interaction = 0.16			
<i>ADH1C</i> (Ex6-14G>A)						
2,2	12/16	1.00(ref.)		9/15	0.75	(0.24,2.32)
1,2/1,1	101/123	0.98	(0.44,2.21)	56/42	1.58	(0.67,3.74)
			<i>p</i> interaction = 0.23			
<i>ADH1B</i> (Ex3+23A>G)						
1, 1	120/152	1.00(ref.)		77/67	1.44	(0.95,2.17)
1,2/2,2	5/6	0.90	(0.25,3.26)	5/1	6.00	(0.69,52.3)
			<i>p</i> interaction = 0.23			
<i>Other</i>						
<i>ADH1C</i> (IVS6 +10G>A)						
G,G	58/55	1.00(ref.)		24/21	1.09	(0.53,2.21)
A,G/A,A	57/88	0.67	(0.40,1.11)	47/40	1.17	(0.66,2.06)
			<i>p</i> interaction = 0.30			
<i>ADH1B</i> (IVS1 +896A>G)						
A,A	55/49	1.00(ref.)		31/26	1.04	(0.54,2.02)
A,G/G,G	64/104	0.57	(0.34,0.94)	45/35	1.08	(0.65,2.14)
			<i>p</i> interaction = 0.12			

OR, odds ratio, adjusted for matching factors (baseline menopausal status and age); CI, confidence interval; rs, identifier used by the NCI SNP 500 data base (<http://snp500cancer.nci.nih.gov/home>).

95% CI 0.7–1.6) that was unchanged by *ADH1C* genotype (Hines et al., 2000). The lack of association seen in our study and that by Hines et al. may reflect the relatively low level of alcohol intake reported by women in these studies. Coutelle et al. reported a significant increase in breast cancer risk among homozygous carriers of the *ADH1C* allele with a daily intake of >20 g of ethanol when compared with <20 g of ethanol (Coutelle et al., 2004). Epidemiological studies of other cancers such as head and neck also suggest that functional variants of *ADH* may only modify cancer risk among heavy drinkers and not among light drinkers (Harty et al., 1997; Olshan et al., 2001; Schwartz et al., 2001). An alternate explanation for the modest breast cancer risk observed at low levels of alcohol intake may be due to reported elevations in circulating endogenous hormones such as estradiol and DHEAS (Dorgan et al., 1994; Hines et al., 2000). The strong correlation observed between the 2 functional polymorphisms in the *ADH1C* gene is consistent with recent resequencing (<http://egp.gs.washington.edu/data/adh1c/>) that supports the likelihood that these 2 genotypes are in linkage disequilibrium (Edman and Maret, 1992).

Few studies have examined the association between the functional *ADH1B*2* variant and cancer in Caucasians because of its low prevalence. In a case-control study of German women, a reduction in breast cancer risk was reported in carriers of the *ADH1B*2* variant who on average consumed 12 or more grams of alcohol per day (Lilla et al., 2005). A protective association was also reported in a case-only study of 274 women with invasive breast cancer (Sturmer et al., 2002). These results were not replicated in our study but we did confirm the low prevalence of the *ADH1B*2* variant among Caucasians women (Brennan et al., 2004; Lilla et al., 2005). In Asians, the presence of the *ADH1B*2* variant indirectly limits their alcohol consumption due to toxic side effects such as flushing produced by high levels of acetaldehyde (Borras et al., 2000; Seitz et al., 2001).

There are a number of possible explanations for the observed protective association between carriers of the *G* allele at *ADH1B* IVS1B+896, and breast cancer risk. The polymorphism may be in linkage disequilibrium with another known or yet to be identified functional variant in the *ADH1B* gene or other genes in close proximity. Another possibility, although less likely, given its location, is that the polymorphism has a protective function of its own. Future studies may benefit from genotyping multiple polymorphisms within the *ADH1B* region and conducting haplotype analyses.

Strengths of our study include the prospective collection of information on alcohol intake before the diagnosis of breast cancer (minimizing bias due to differential reporting by cases and controls), long-term follow-up (up to 13 years) and the population-based study sample. In addition, the associations between other potential risk factors and breast cancer were comparable to published studies,

suggesting good internal validity. Further, the similar results obtained from our imputed data sets suggest that significant bias was not introduced by the missing data. Limitations of our study include the large number of nondrinkers, limited sample size to analyze gene-gene interactions and alcohol intake data from a single time point.

In conclusion, the results of this study support prior studies that suggest that even low levels of alcohol may modestly influence breast cancer risk. Further, the *ADH* genotypes that have been observed to increase ethanol oxidation and elimination in vitro appear to be at best only weak modifiers of breast cancer risk in Caucasian women. Our results also support the further evaluation of the *ADH1B* IVS1+896A>G polymorphism in women. Given the modest association between low levels of alcohol consumption and breast cancer risk, the identification of highly susceptible groups within the general population will enable us to better target preventive strategies.

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