

***ADH3* genotype, alcohol intake and breast cancer risk**

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Moderate alcohol consumption of ~1–2 drinks per day has been associated with a 30–50% increase in breast cancer risk. Individuals differ in their ability to metabolize alcohol through genetic differences in alcohol dehydrogenase (*ADH*), the enzyme that catalyzes the oxidation of ~80% of ethanol to acetaldehyde, a known carcinogen. Individuals differ in their *ADH* genotype, and one locus in particular (*ADH3*) is polymorphic in Caucasian populations. Using data from the Long Island Breast Cancer Study Project, we examined whether fast metabolizers of alcohol, as measured by the *ADH3*¹⁻¹ genotype, have a higher risk of breast cancer from alcohol intake compared with those individuals who are slow metabolizers, but consume similar amounts of alcohol. We combined genotyping information with questionnaire data on 1047 breast cancer cases and 1101 controls and used unconditional logistic regression methods to estimate multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) between alcohol intake and breast cancer risk. Among individuals homozygous for the fast metabolizing allele (*ADH3*¹⁻¹), a lifetime alcohol consumption of 15–30 g/day (~1–2 drinks per day) increased breast cancer risk by 2-fold (OR = 2.0, 95% CI = 1.1–3.5). In contrast, the increase in risk from a lifetime alcohol consumption of 15–30 g/day was less pronounced in the intermediate and slow metabolizing groups, respectively: *ADH3*¹⁻² (OR = 1.5, 95% CI 0.9–2.4) and *ADH3*²⁻² (OR = 1.3, 95% CI 0.5–3.5). Fast metabolizers who drank 15–30 g/day of alcohol had 2.3 times (95% CI 1.3–4.0) greater risk of breast cancer than non-drinkers who were intermediate or slow metabolizers. This association for fast metabolizers who drank 15–30 g/day was particularly pronounced among premenopausal women (premenopausal women OR = 2.9,

95% CI = 1.2–7.1; postmenopausal women OR = 1.8, 95% CI = 0.9–3.8). These population-based data support the hypothesis that fast metabolizers of alcohol have a higher risk of breast cancer risk, from alcohol intake than slow metabolizers.

Introduction

Despite the increasing epidemiologic evidence that alcohol intake may be associated with an elevated risk of breast cancer (1), the overall magnitude of the association has been small with a range of 1.3–1.5 in relation to 1–2 drinks per day (2–11). It is more difficult to rule out confounding and bias as alternative explanations for the findings with low magnitude associations even if the findings have been consistently replicated (12). Added support for the role of alcohol in breast cancer may come from large studies that consistently support underlying biological mechanisms for the association.

One plausible biological mechanism for alcohol to influence breast cancer risk is through acetaldehyde, a carcinogen, and a metabolite of ethanol. Ethanol is the main type of alcohol and ~80% of it is metabolized by the enzyme alcohol dehydrogenase (*ADH*) (1). Acetaldehyde induces sister chromatid exchange, mutations and chromosomal aberrations in cell cultures and in human lymphocytes (13,14) and is carcinogenic in animal models. Approximately 96–98% of the activity of *ADH* in the body is in the liver but it is expressed and regulated in a number of tissues including breast tissue (15,16). The *ADH* gene has several polymorphisms: *ADH2* polymorphisms have been primarily found among Asians whereas *ADH3* polymorphisms are commonly seen among whites, Asians and Africans (17,18). Presence of the high risk *ADH3*¹ allele increases metabolism of alcohol. Individuals with *ADH3*¹⁻¹, *ADH3*¹⁻² and *ADH3*²⁻² are classified as fast, intermediate and slow metabolizers, respectively. The nomenclature for *ADH3* has been changed recently to *ADH1C* but for purposes of comparison with the previously published literature we will use the older nomenclature (*ADH3*) (19).

We undertook a study of alcohol intake, alcohol metabolism (as measured by *ADH3* genotype) and breast cancer risk using data from the Long Island Breast Cancer Study Project (LIBCSP). We hypothesized the association between alcohol intake and breast cancer risk would be most pronounced among the fast metabolizers of alcohol.

Materials and methods

Study population

We conducted a population-based case-control study of breast cancer, the LIBCSP in Nassau and Suffolk counties, New York. Details of the overall study design are provided in prior publications and summarized briefly here (20). Cases were women with *in situ* or invasive cancer newly diagnosed between August 1, 1996 and July 31, 1997, who were English-speaking. There were no age or race restrictions. Controls were randomly selected

Abbreviations: *ADH*, alcohol dehydrogenase; BMI, body mass index; CI, confidence interval; LIBCSP, Long Island Breast Cancer Study Project; OR, odds ratio.

through random digit dialing methods (for subjects under 65 years) and Health Care Finance Administration lists (for subjects 65 years of age and older), and frequency-matched to the expected distribution among cases in 5 year age-groups. In-person interviews were completed for 1508 cases (82.1% of eligible cases) and 1556 controls (62.8% of eligible controls). Of those who completed an interview, 73.1% of cases and 73.3% of controls donated a blood sample. Of those who donated a blood sample, we were unable to genotype 5% of cases and 3.5% of controls, mainly due to lack of sufficient quality DNA to complete the assay. Thus, our final sample size for this molecular epidemiology project was 1047 cases and 1101 controls. The Institutional Review Boards of all the participating institutions approved the study protocol and the individual subjects all signed informed consent forms.

As previously published (20), an increase in breast cancer among women on Long Island was found to be associated with lower parity, late age at first birth, little or no breastfeeding, a family history of breast cancer, and increasing income and education. Results were similar when the analyses were restricted to respondents who donated blood, or for those with DNA available for these analyses (data not shown). Factors that were found to be associated with a decreased likelihood that a respondent, regardless of case-control status, would donate blood (20) include increasing age (1% decrease for each yearly increase in age) and past active smoking (25% decrease); factors associated with an increased probability include white or other race (65 and 74% increase, respectively, versus black race), ever consuming alcohol (28% increase), ever breastfeeding (47% increase), ever using hormone replacement therapy (63% increase), ever using oral contraceptives (21% increase) and ever having had a mammogram (51% increase). Case-control status was not a predictor of blood donation.

Exposure assessment

Subjects were asked to report on their alcohol intake by type, quantity and frequency for specific age periods. Unlike many other studies, the LIBCSP questionnaire requested information not just on current intake of beer, wine and liquor, but also at different periods of life. Women who answered 'no' to ever consuming alcoholic beverages such as beer, wine or liquor at least once a month for 6 months or more were classified as non-drinkers. Women who answered 'yes' to this question were asked to report their consumption separately for beer, wine and liquor for the following time periods: under 20 years old, 20–29 years, 30–39 years, 40–49 years, 50–59 years, and 60 years and older. Women were asked to report the frequency of consumption for any of the following intervals (day, week, month, year or <1 year) for each alcohol type. They were also asked how many drinks they consumed each time they drank in units appropriate for each alcoholic beverage type (12 oz bottle of beer, 4 oz glass of wine and 1.5 oz shot of liquor). We used information on type, frequency and quantity to calculate total grams of alcohol consumed per day for each time period. We used standard conversions applied by others (2) of 13.2, 11.6 and 14.1 g of ethanol for one 12 ounce bottle of beer, one 4 ounce glass of wine and one 1.5 ounces of hard liquor, respectively. For example, if a woman reported that during her 20s she usually consumed beer three times per week, wine four times a month and hard liquor six times a year, and usually drank two bottles of beer, one glass of wine and three shots of liquor each time, her estimated intake of alcohol would be 13.2 g/day for her 20s. To construct the lifetime measure of alcohol intake, we applied weights to each age period where the weights were equivalent to the number of years spent in the age interval.

Other data collection

We also used other detailed data obtained during the interviewer administered 2 h main questionnaire including reproductive history, exogenous hormone use, menopausal status, body mass index (BMI), cigarette smoking, family history of breast cancer and demographic information (<http://epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html>). Information on estrogen receptor and progesterone receptor status and stage of disease (*in situ* versus invasive) was obtained from the pathology reports in the medical records of the breast cancer cases (20).

Genotyping

DNA isolated from blood cells was genotyped using template-directed primer extension with detection of incorporated nucleotides by fluorescence polarization in a 96-microwell-based format essentially as described previously (21). All analyses were performed blinded to case-control status. Master DNA 96-well plates containing 10 ng/ μ l were used to make replica plates containing 25 ng DNA/well. For PCR amplification, the primers (forward 5'-CCC AAA CTT GTG GCT GAC TT-3', reverse 5'-TCA CAC TTA CTT ATA TGA CAG GCA G-3') gave a 493 bp product. Conditions for amplification were 0.2 μ l (8 pmol/ μ l) forward and reverse primers, 0.4 μ l 25 mM MgCl₂, 1 μ l 10 $\times$ PCR buffer, 0.1 μ l (5 U/ml) *Taq* polymerase (Roche Molecular Biochemicals, Indianapolis, IN), 0.25 μ l (10 mM) dNTPs (Roche) and 5.35 μ l water. Denaturation at 94°C for 5 min 30 s was followed by 34 cycles of 94°C

for 30 s, 60°C for 45 s and 72°C for 1 min, followed by 4 min at 72°C. Primers and dNTPs were digested with 1 U of shrimp alkaline phosphatase (1 U/ μ l, Roche) after addition of 1 μ l of 10 $\times$ buffer and 1 U *Escherichia coli* exonuclease I (10 U/ μ l, United States Biochemical, Cleveland, OH) and 7.9 μ l of water for 45 min at 37°C followed by heating at 95°C for 15 min. The reverse extension primer was 5'-TTC ACT GGA TGC ATT ATT AAC AAA T-3'. Acycloprime FP SNP Detection kit G/A contained the ddNTPs labeled either with R110 or TAMRA (Perkin Elmer Life Sciences, Boston, MA). To 7 μ l of reaction mixture was added 0.05 μ l Acycloprimer enzyme, 1 μ l G/A Terminator mix, 2 μ l 10 $\times$ reaction buffer, 0.5 μ l extension primer (10 pmol/ μ l) and 9.45 μ l water. Extension was carried out by heating at 95°C for 2 min followed by 30 cycles of 95°C for 15 s and 55°C for 30 s. Plates were read on a Perkin Elmer Victor instrument. In addition to assay specific quality control samples, 10% of samples were reassayed after relabeling to keep laboratory personnel blinded to identity. Of the 112 duplicate samples, 98.2% were concordant for genotype result (two pairs were discordant).

Statistical methods

We first compared differences between genotypes and breast cancer risk factors using the χ^2 -test for categorical variables, and the analysis of variance test for continuous variables (22). Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the main effects of genotype on breast cancer risk (23). Based on detailed analyses of alcohol and breast cancer risk in the same study population (24), we selected two measures of alcohol intake—average lifetime intake and current intake—to assess the interaction with genotype. Lifetime intake was categorized into the following categories: none, <15 g/day, 15–30 g/day and $\geq$ 30 g/day. Current alcohol intake was categorized into the following categories: none, <5 g/day, 5–15 g/day and $\geq$ 15 g/day. Cutpoints were selected after testing for linearity with breast cancer risk using finer classification of alcohol doses (24).

All models included the frequency-matching factor of age. We also examined confounding by the following factors, which were selected *a priori*: years of education, income, race, active smoking status, total caloric intake and BMI. In addition, we examined confounding by other known risk factors for breast cancer including history of benign breast disease, parity, age at first birth, age at menarche, menopausal status and lactation status. We compared the change in estimate for the exposure coefficient between statistical models with and without the potential confounder. Variables were kept in the final model if they altered the parameter estimates on the exposure by at least 10% (25). In addition, the final column of each table shows results from a saturated model to illustrate the absence of further confounding.

Effect modification by genotype was first examined through use of stratified analysis, running separate models for each genotype subgroup, and second by comparing the log-likelihood statistic for models that included a multiplicative interaction term in the logistic regression model to those without (23). We also further evaluated additive interaction by using indicator terms for those with the genotype only, exposure only and those with both the genotype and exposure of interest (25). Analyses were also stratified by menopausal status. Because we previously found statistically significant differences between lifetime alcohol intake and breast cancer risk by BMI (<25 and $\geq$ 25) (24), we further examined whether the interaction between alcohol intake and genotype was modified by BMI.

In addition to the main analyses, we performed analyses to examine the extent selection bias may have contributed to our findings as those with available data for the genotyping analyses were more likely to have consumed alcohol than those subjects who did not have available genotype data (20). To do so, we used an econometric method developed to adjust for sample selection bias known as a Heckit (26). Essentially this is a two-stage technique that models predictors of blood donation in the first stage and uses this information in the second stage model of the primary outcome (in this case a logistic model for breast cancer risk). In the second stage, we use an estimate of the fitted coefficients to form an 'inverse mills ratio'. This ratio is a function of the probability density function divided by the cumulative density function based on the first model and is entered into the second model as an independent variable providing an adjustment for sample selection.

Results

Allele and genotype frequencies for *ADH*₃ are reported in Table I. There were no statistically significant differences between breast cancer cases and controls by allele frequency (*ADH*₃*1: 66.3% for cases and 65.2% for controls; *ADH*₃*2: 33.7% for cases and 34.8% for controls, *P* = 0.9). The genotypes among controls are in Hardy-Weinberg equilibrium (χ^2 -value = 0.16 with 1 df). The ORs for breast cancer

Table I. Allele and genotype frequency for *ADH3*, LIBCSP

Characteristics	Cases (N = 1047)	Controls (N = 1101)	
Allele frequency (%)			
<i>ADH</i> ₃ ^{3*1}	66.3%	65.2%	
<i>ADH</i> ₃ ^{3*2}	33.7%	34.8%	χ^2 (P-value) = 0.87
Genotype N (%)			OR ^a (95% CI)
<i>ADH</i> ₃ ²⁻²	103 (9.8%)	136 (12.4%)	1.00
<i>ADH</i> ₃ ¹⁻²	500 (47.8%)	494 (44.9%)	1.29 (0.97–1.71)
<i>ADH</i> ₃ ¹⁻¹	444 (42.4%)	471 (42.8%)	1.21 (0.91–1.62)
Premenopausal women			
<i>ADH</i> ₃ ²⁻²	38 (11.4%)	50 (13.4%)	1.00
<i>ADH</i> ₃ ¹⁻²	158 (47.3%)	163 (43.6%)	1.24 (0.77–2.00)
<i>ADH</i> ₃ ¹⁻¹	138 (41.3%)	161 (43.1%)	1.10 (0.68–1.78)
Postmenopausal women			
<i>ADH</i> ₃ ²⁻²	64 (9.3%)	79 (11.6%)	1.00
<i>ADH</i> ₃ ¹⁻²	330 (47.9%)	314 (46.1%)	1.25 (0.86–1.80)
<i>ADH</i> ₃ ¹⁻¹	295 (42.8%)	288 (42.3%)	1.23 (0.85–1.78)

^aAdjusted for reference age.

were modestly elevated among women categorized as intermediate (*ADH*₃¹⁻² = 1.29, 95% CI = 0.97–1.71) and fast metabolizers (*ADH*₃¹⁻¹ = 1.21, 95% CI = 0.91–1.62) relative to slow (*ADH*₃²⁻²) metabolizers of alcohol, but the associations were not statistically significant.

Table II summarizes associations between genotype and various breast cancer risk factors including age, menopausal status, first-degree family history of breast cancer, race, education, BMI, age at menarche, age at first birth, cigarette smoking, hormone replacement use and alcohol intake. Among controls, there was a higher prevalence of slow metabolizers (*ADH*₃²⁻²) among women who were younger and had a lower BMI and a higher prevalence of fast metabolizers (*ADH*₃¹⁻¹) among the non-white subjects. Slow metabolizers were also more likely to consume more alcohol, both for current and lifetime average consumption. Other risk factors did not differ by genotype status.

Multivariate-adjusted estimates stratified by genotype category are presented in Table III. Lifetime alcohol intake of 15–30 g/day was associated with a 2-fold increase in breast cancer risk among fast metabolizers (OR = 1.97, 95% CI = 1.10–3.34). In contrast, slow metabolizers had a more modest increase in breast cancer risk, which was not statistically significant (OR = 1.34, 95% CI = 0.51–3.54), from consumption of 15–30 g/day. The increase in risk for the intermediate metabolizers (*ADH*₃¹⁻²) was between these two estimates (OR = 1.49, 95% CI = 0.91–2.41). However, the test for multiplicative interaction was not significant (*P* = 0.2). Neither lighter nor heavier consumption of alcohol were associated with breast cancer risk in any genotype category. Table III also reports the results for current alcohol consumption. The magnitude of the association between current consumption of ≥ 15 g was also higher among fast metabolizers (OR = 1.51, 95% CI = 0.85–2.66) than among the intermediate (OR = 1.12, 95% CI = 0.72–1.75) or slow metabolizers (OR = 1.34, 95% CI = 0.55–3.29), though none of the effect estimates were statistically significant.

Table IV presents the results compared with a common referent group of subjects who were never drinkers and either slow or intermediate metabolizers. The OR for the joint effect of both a fast metabolizing genotype and consumption of

15–30 g/day was 2.30 (95% CI = 1.31–4.04) versus those non-drinkers who were intermediate or slow metabolizers. The separate effects of the fast metabolizing genotype and consumption of 15–30 g/day were less pronounced (OR = 1.16, 95% CI = 0.86–1.57 and OR = 1.46, 95% CI = 0.96–2.24, respectively).

This association with breast cancer for fast metabolizers who drank 15–30 g/day was particularly pronounced among premenopausal women (OR = 2.92, 95% CI = 1.20–7.13). The separate effects of the fast metabolizing genotype and consumption of 15–30 g/day were less pronounced (OR = 1.09, 95% CI = 0.62–1.94 and OR = 1.21, 95% CI = 0.59–2.48, respectively). In contrast, among postmenopausal women the association with breast cancer for fast metabolizers who drank 15–30 g/day was 1.82 (95% CI = 0.86–3.84) which was similar to those intermediate and slow metabolizers who drank 15–30 g/day (OR = 1.58, 95% CI = 0.92–2.72). Results for current consumption were similar to the patterns reported in Table IV for lifetime consumption (data not shown) but were no longer elevated after adjusting for lifetime alcohol consumption.

We further examined these differences by menopausal status by BMI. Both fast metabolizing genotype and alcohol consumption of ≥ 15 g per day were associated with increased risk of premenopausal breast cancer (OR = 1.54, 95% CI = 0.58–4.10 for BMI < 25 and OR = 3.29, 95% CI = 1.06–10.23 for BMI ≥ 25), although the effect was only significant in heavier women. However, among postmenopausal women, only women with BMI < 25 had increased risk from fast metabolizing genotype and alcohol consumption of ≥ 15 g per day (OR = 2.67, 95% CI = 1.09–6.56 for BMI < 25 and OR = 0.72, 95% CI = 0.31–1.64 for BMI ≥ 25). However, postmenopausal women with BMI < 25 who were intermediate or slow metabolizers also faced an increased risk from alcohol consumption of ≥ 15 g per day (OR = 2.06, 95% CI = 1.04–4.10).

In addition to the primary analyses, we examined whether selection bias contributed to our findings using econometric procedures (26). These analyses modeled differences between those with available genotyping data and those without and used this information in a second stage estimation of gene–environment interaction. These analyses suggest that if we were to quantitatively adjust for selection bias the joint effect of genotype and alcohol consumption presented in Table IV would be of similar magnitude. The OR for the joint effect of both a fast metabolizing genotype and consumption of 15–30 g/day was 1.96 (95% CI = 1.12–3.41) versus those with intermediate or slow metabolizing genotypes and non-drinker. The ORs for the separate effects of the fast metabolizing genotype and consumption of 15–30 g/day were 1.17 (95% CI = 0.87–1.57) and 1.25 (OR = 1.46, 95% CI = 0.82–1.92), respectively. These findings from the sensitivity analyses, where we statistically adjust for the potential selection bias associated with DNA availability, are similar to those from the analyses from which no adjustments were made.

Discussion

Overall, we found non-significant, modest associations between those subjects with at least one high risk *ADH*₃¹ allele and breast cancer risk (OR = 1.21, 95% CI = 0.91–1.62 for *ADH*₃¹⁻¹ and OR = 1.29, 95% CI = 0.97–1.71 for *ADH*₃¹⁻² relative to *ADH*₃²⁻²). The frequency of the high risk *ADH*₃¹ allele

Table II. Selective descriptive characteristics by *ADH3* genotype category among controls in the LIBCSP

Characteristic	<i>ADH</i> ₃ ¹⁻¹ (N = 471)	<i>ADH</i> ₃ ¹⁻² (N = 494)	<i>ADH</i> ₃ ²⁻² (N = 136)	P-value
	Numbers			
Age at diagnosis				
20–29	7 (1.49%)	6 (1.21%)	1 (0.74%)	
30–39	38 (8.07%)	34 (6.88%)	16 (11.76%)	
40–49	115 (24.42%)	114 (23.08%)	38 (27.94%)	
50–59	143 (30.36%)	135 (27.33%)	33 (24.26%)	
≥60	168 (35.67%)	205 (41.50%)	48 (35.29%)	
(μ ± SD)	55.38 ± 12.10	57.19 ± 13.02	54.46 ± 12.16	P = 0.02
Menopausal status				
Premenopausal	161 (35.86%)	163 (34.17%)	50 (38.76%)	
Postmenopausal	288 (64.14%)	314 (65.83%)	79 (61.24%)	P = 0.61
Family history of breast cancer				
None	390 (84.78%)	423 (87.04%)	115 (87.79%)	
First-degree	70 (15.22%)	63 (12.96%)	16 (12.21%)	P = 0.51
Race				
White	409 (86.84%)	480 (97.17%)	130 (95.59%)	
Black	39 (8.28%)	9 (1.82%)	1 (0.74%)	
Asian and other	23 (4.88%)	5 (1.01%)	5 (3.68%)	P < 0.0001
Education				
High school	183 (39.02%)	220 (44.53%)	46 (33.82%)	
College	213 (45.24%)	203 (41.09%)	65 (47.79%)	
Post college	73 (15.57%)	71 (14.37%)	25 (18.38%)	P = 0.17
BMI (kg/m ²)				
>25	230 (49.57%)	242 (49.69%)	77 (57.04%)	
≥25	234 (50.43%)	245 (50.31%)	58 (42.96%)	
(μ ± SD)	26.46 ± 5.97	26.37 ± 5.56	25.78 ± 5.69	P = 0.48
Age at menarche (μ ± SD)	12.40 ± 1.64	12.64 ± 1.68	12.54 ± 1.54	P = 0.07
Age at first birth (parous only) (μ ± SD)	24.92 ± 4.27	25.18 ± 4.74	25.04 ± 4.37	P = 0.69
Active cigarette smoking				
Never	228 (48.51%)	211 (42.80%)	61 (44.85%)	
Current	81 (17.23%)	99 (20.08%)	26 (19.12%)	
Former	161 (34.26%)	183 (37.12%)	49 (36.03%)	P = 0.50
Hormone Replacement use				
Never	350 (74.31%)	356 (72.06%)	93 (68.38%)	
Ever	121 (25.69%)	138 (27.94%)	43 (31.62%)	P = 0.37
Lifetime alcohol intake (g/day)				
Never	190 (40.34%)	181 (36.71%)	33 (24.26%)	
<15	230 (48.83%)	232 (47.06%)	77 (56.62%)	
15–30	26 (5.52%)	42 (8.52%)	15 (11.03%)	
≥30	25 (5.31%)	38 (7.71%)	11 (8.09%)	P = 0.01
Current alcohol intake(g/day)				
Never	256 (54.58%)	239 (48.78%)	56 (41.18%)	
<0.5	64 (13.65%)	63 (12.86%)	15 (11.03%)	
0.5–5	67 (14.29%)	67 (13.67%)	19 (13.97%)	
5–15	56 (11.94%)	71 (14.49%)	29 (21.32%)	
≥15	26 (5.54%)	50 (10.20%)	17 (12.50%)	P = 0.01

was 65.2% among control population is similar to the frequency reported previously in other populations: 58% of European Whites, 91% of Asians and 88% of Africans (27). The frequency of the three genotypes in the Long Island controls was 12.4, 44.9, and 42.8% for slow, intermediate and fast metabolizers, respectively. Similar genotype frequencies were reported in the two other studies: 16.6–18% for slow, 48–49% for intermediate and 34–35% for fast (27,28). Among individuals homozygous for the fast metabolizing allele (*ADH*₃¹⁻¹) a lifetime alcohol consumption of 15–30 g/day (~1–2 drinks per day) increased breast cancer risk 2-fold (OR = 2.0, 95% CI 1.1–3.5). In contrast, the increase in risk from a lifetime alcohol consumption of 15–30 g/day was more modestly elevated in the other two groups: *ADH*₃¹⁻² (OR = 1.5, 95% CI 0.9–2.4) and *ADH*₃²⁻² (OR = 1.3, 95% CI 0.5–3.5). We did

not find any association between alcohol consumption of >30 g/day and breast cancer risk irrespective of genotype as we reported in (24). Pooled analyses (29) as well as studies of alcoholics (30) have suggested a leveling of risk; very heavy drinking has not been associated with increased breast cancer risk relative to moderate drinking. We cannot explain why we have not observed an increase in risk from heavy lifetime consumption of alcohol. However, the confidence intervals were sufficiently wide that increased risk could not be excluded.

Two other studies have investigated alcohol intake, *ADH*₃, and breast cancer risk with conflicting results (27,28). Freudenheim *et al.* (27) reported an increased risk for fast metabolizers with high lifetime alcohol consumption among premenopausal, but not postmenopausal, women

Table III. Multivariate-adjusted ORs and 95% CI for lifetime alcohol intake and breast cancer risk, stratified by *ADH*₃ genotype, LIBCSP

	Among subjects with <i>ADH</i> ₃ ¹⁻¹				Among subjects with <i>ADH</i> ₃ ¹⁻²				Among subjects with <i>ADH</i> ₃ ²⁻²			
	Cases	Controls	OR ^a (95%CI)	OR ^b (95%CI)	Cases	Controls	OR ^a (95%CI)	OR ^b (95%CI)	Cases	Controls	OR ^a (95% CI)	OR ^b (95%CI)
Life time alcohol intake (g/day)												
Never ^c	196	190	1.00	1.00	167	181	1.00	1.00	29	33	1.00	1.00
<15	183	230	0.77 (0.57–1.05)	0.81 (0.59–1.12)	254	232	1.24 (0.92–1.65)	1.25 (0.93–1.69)	52	77	0.85 (0.45–1.62)	0.88 (0.45–1.74)
15–30	46	26	1.99 (1.14–3.47)	1.97 (1.10–3.54)	55	42	1.55 (0.97–2.48)	1.49 (0.91–2.41)	16	15	1.52 (0.60–3.87)	1.34 (0.51–3.54)
≥30	19	25	0.82 (0.43–1.58)	0.84 (0.42–1.68)	24	38	0.70 (0.39–1.25)	0.80 (0.44–1.45)	6	11	0.89 (0.27–2.90)	0.90 (0.24–3.39)
Current alcohol intake (g/day)												
Never ^c	255	256	1.00	1.00	244	239	1.00	1.00	46	56	1.00	1.00
<5	89	131	0.63 (0.45–0.89)	0.65 (0.45–0.93)	121	130	0.92 (0.67–1.27)	0.95 (0.68–1.32)	20	34	0.74 (0.37–1.50)	0.75 (0.36–1.58)
5–15	58	56	1.08 (0.70–1.68)	1.05 (0.67–1.66)	73	71	1.07 (0.73–1.57)	1.12 (0.75–1.66)	21	29	1.12 (0.54–2.32)	1.15 (0.53–2.52)
≥15	40	26	1.70 (0.98–2.95)	1.51 (0.85–2.66)	61	50	1.15 (0.75–1.77)	1.12 (0.72–1.75)	15	17	1.31 (0.56–3.05)	1.34 (0.55–3.29)

^aORs are adjusted for age at diagnosis, education level, race, caloric intake, active smoking status and BMI.

^bORs are additionally adjusted for history of benign breast disease, parity, age at first birth, age at menarche, menopausal status and lactation status.

^cAs reference group.

(OR = 3.6, 95% CI = 1.5–8.8). In contrast, Hines *et al.* (28) found no association with current alcohol among fast metabolizers of alcohol and breast cancer risk. These studies differed in their assessment of alcohol intake (lifetime versus current, respectively) and also were relatively small for gene–environment interaction studies (315 cases and 465 cases, respectively). Our larger study, which includes 1047 cases, supports the overall findings among premenopausal women of Freudenheim *et al.* (27). We also observed the association with genotype to be stronger among premenopausal women. Among postmenopausal women, moderate alcohol drinking of 15–30 g/day was associated with an increase in breast cancer risk irrespective of genotype. We did not see any gene–environmental interaction with current alcohol intake after accounting for lifetime intake. Thus, the differences among these three studies may be because of the measure of alcohol. Other studies examining upper respiratory cancer (31) and oral cavity and pharynx cancer (32) also suggest modification of the alcohol and cancer association by *ADH*₃ genotype.

Possible alternative explanations for our findings should be considered. For recall bias to be a likely explanation for our findings, any bias associated with the reporting of alcohol intake would have to vary by genotype that is unlikely to have occurred. Thus, differences between associations by genotype status cannot be explained by recall bias. We were also able to assess confounding by a number of variables included in the main study questionnaire. For an unmeasured confounder to explain the interaction results we found, it would have to be differentially distributed in the separate genotype exposure strata. It is unlikely that this occurred. There was very high (98%) reliability in the measurement of genotype. Measurement error in exposure classification may have created the appearance of interaction, although our findings with respect to the main effects of alcohol (24) agree with the overall literature suggesting that moderate alcohol of 1–2 drinks per day is associated with breast cancer risk (2–11). We did find, as has been reported previously (18,27),

that fast metabolizers had lower lifetime and current alcohol intake. The lower intake among fast metabolizers will affect the statistical power within strata but should not influence our overall gene–environment findings.

In addition, we considered the possible implications selection bias may have on our findings, given that participants in our study who reported drinking alcohol were also more likely to be willing to donate blood, and thus have DNA available for the analyses reported here (20). It should be noted that unlike many gene–environment interaction studies, the blood collection rate was high (~73%) minimizing the chance of selection bias. For example, the overall percent of eligible subjects with blood samples in another study investigating *ADH*₃, alcohol intake and breast cancer risk was ~30% (27). The results of our analyses adjusting for differences between those who had genotyping results available and those who did not, however, suggested a similar gene–environment interaction. Thus it is unlikely that recall, confounding, measurement issues or selection bias can explain the associations that we have found in this study.

A stronger association between alcohol intake and breast cancer risk among fast metabolizers of alcohol than among intermediate or slow metabolizers helps lend support to a proposed underlying biological mechanism linking alcohol to breast cancer risk. Possible mechanisms of the role of alcohol in carcinogenesis include the influence of alcohol on nutrient metabolism, detoxification of other carcinogens, activation of other enzymes, alteration of hormonal status, immune function, cellular proliferation, DNA repair, lipid peroxidation and promotion of cell invasion and migration (1,13,33–37). In addition to these potential mechanisms, ADH metabolizes ethanol into acetaldehyde, a known carcinogen. Acetaldehyde induces sister chromatid exchange, mutations and chromosomal aberrations in cell cultures and in human lymphocytes (13,14) and is carcinogenic in animal models. Higher levels of acetaldehyde may also result in systematic effects that can affect the development of cancer at many tumor sites that are not necessarily in direct contact with ethanol

Table IV. Multivariate-adjusted ORs and 95% CI of gene–environment interactions for *ADH3* and lifetime alcohol intake, LIBCSP

Lifetime alcohol intake (g/day)	Genotype	Cases	Controls	OR ^a (95% CI)	OR ^b (95% CI)
Among all women					
Never ^c	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	196	214	1.00	1.00
Never	<i>ADH3</i> ¹⁻¹	196	190	1.18 (0.88–1.59)	1.16 (0.86–1.57)
<15	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	306	309	1.16 (0.90–1.51)	1.19 (0.91–1.55)
<15	<i>ADH3</i> ¹⁻¹	183	230	0.93 (0.70–1.24)	0.97 (0.72–1.30)
15–30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	71	57	1.51 (1.00–2.28)	1.46 (0.96–2.24)
15–30	<i>ADH3</i> ¹⁻¹	46	26	2.32 (1.35–4.00)	2.30 (1.31–4.04)
≥30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	30	49	0.73 (0.44–1.22)	0.81 (0.47–1.39)
≥30	<i>ADH3</i> ¹⁻¹	19	25	0.98 (0.51–1.86)	0.95 (0.49–1.86)
Premenopausal women					
Never ^c	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	55	63	1.00	1.00
Never	<i>ADH3</i> ¹⁻¹	48	54	1.11 (0.64–1.95)	1.09 (0.62–1.94)
<15	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	107	108	1.16 (0.72–1.85)	1.16 (0.72–1.87)
<15	<i>ADH3</i> ¹⁻¹	57	89	0.74 (0.44–1.24)	0.71 (0.41–1.20)
15–30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	25	23	1.25 (0.62–2.53)	1.21 (0.59–2.48)
15–30	<i>ADH3</i> ¹⁻¹	23	9	3.13 (1.30–7.55)	2.92 (1.20–7.13)
≥30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	9	19	0.65 (0.26–1.60)	0.54 (0.20–1.42)
≥30	<i>ADH3</i> ¹⁻¹	10	9	1.34 (0.49–3.65)	1.39 (0.50–3.90)
Postmenopausal women					
Never ^c	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	135	145	1.00	1.00
Never	<i>ADH3</i> ¹⁻¹	143	129	1.20 (0.85–1.71)	1.17 (0.82–1.68)
<15	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	193	192	1.17 (0.85–1.61)	1.19 (0.86–1.64)
<15	<i>ADH3</i> ¹⁻¹	122	129	1.10 (0.77–1.57)	1.13 (0.79–1.62)
15–30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	46	31	1.72 (1.02–2.92)	1.58 (0.92–2.72)
15–30	<i>ADH3</i> ¹⁻¹	22	15	1.92 (0.91–4.03)	1.82 (0.86–3.84)
≥30	<i>ADH3</i> ²⁻² + <i>ADH3</i> ¹⁻²	21	24	1.00 (0.52–1.94)	1.03 (0.53–2.00)
≥30	<i>ADH3</i> ¹⁻¹	8	15	0.68 (0.27–1.70)	0.67 (0.26–1.69)

^aORs are adjusted for age, education level, race, active smoking status, caloric intake and BMI.

^bORs are additionally adjusted for history of benign breast disease, parity, age at first birth, age at menarche, menopausal status and lactation status.

^cAs reference group.

(e.g. breast) (35). One possible direct mechanism for alcohol metabolism in carcinogenesis is the role ADH has in producing reactive oxygen species (15). Recent laboratory evidence also suggests that ADH, which is highly expressed in normal mammary tissue, may be functioning as a tumor suppressor (38).

Given both our observed differences by menopausal status and BMI as well as the magnitude of the effect estimates between alcohol metabolism and alcohol intake are much smaller for breast cancer than for oral and pharyngeal cancer, further exploration of the underlying biological mechanism is needed. It may be that with breast cancer alcohol may be working not through aldehyde or other genetic damage but rather through modifying endogenous hormone levels (39). High activity of ADH in the adrenal glands may be related to steroid hormone metabolism (27,40). This may be particularly relevant for breast cancer risk as alcohol intake has been shown to increase estrogen levels, particularly estrone sulfate, and may increase the rate at which androgen is aromatized to estrogen (1,41–43). In a controlled feeding study of premenopausal women consuming 30 g of alcohol daily, there were increases in plasma dehydroepiandrosterone (7%) in the follicular phase, plasma estrone (21%), estradiol (28%), urinary estradiol (32%) in the peri-ovulatory phase, urinary estrone, estradiol and estriol (at least 15% or more) in the luteal phase (44). In addition, while the Hines study did not find an interaction between current intake and *ADH3* status, it did report differences in some plasma hormone levels and *ADH3* genotype status (28). The relationship between *ADH3*, alcohol intake and endogenous hormonal levels needs to be further investigated.

There are a number of strengths to this study including a large sample size. Further, it is unlikely that confounding, recall and selection bias can explain the associations we found in this study. We had detailed alcohol history data with which to construct measures of lifetime alcohol intake so that we could evaluate interactions with lifetime intake rather than just current intake. The smaller group of studies with alcohol data from multiple time periods suggest that it is overall lifetime alcohol consumption rather than a specific period of risk that is associated with breast cancer risk (45–48). Our reliability analyses suggest that the genotyping assay had excellent reliability (98%). Finally, our data agrees with our *a priori* hypothesis that fast metabolizers would face a higher risk from alcohol consumption than slow metabolizers, particularly among premenopausal women.

Overall, this study lends support to a potential underlying biological mechanism to explain the association between alcohol intake and breast cancer risk. As such, it helps support alcohol as an underlying causal factor in breast cancer carcinogenesis. In sum, these data suggest that fast metabolizers of alcohol may face a higher risk of breast cancer risk, particularly premenopausally, from alcohol intake than slow metabolizers and that the overall association seen between alcohol and breast cancer may not be explained by bias.

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