A Prospective Study of the Effect of Alcohol Consumption and *ADH3* Genotype on Plasma Steroid Hormone Levels and Breast Cancer Risk[1]

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Abstract

One suggested mechanism underlying the positive association between alcohol consumption and breast cancer risk is an influence of alcohol on steroid hormone levels. A polymorphism in alcohol dehydrogenase type 3 (*ADH3*) affects the kinetics of alcohol oxidation and thereby could influence the effect of alcohol consumption on hormone levels. We investigated the *ADH3* polymorphism, alcohol intake, and risk of breast cancer in a nested case-control study. Among women in the Nurses' Health Study who gave a blood sample in 1989–1990, 465 incident breast cancer cases were diagnosed before June 1994 and were matched to 621 controls. Using conditional logistic regression, we calculated relative risks and confidence intervals to assess breast cancer risk for *ADH3* genotype. Among postmenopausal controls not using hormones at time of blood collection, partial Pearson correlation coefficients were calculated to assess the association between alcohol intake and plasma hormone levels according to *ADH3* genotype. No association was observed between *ADH3* genotype and overall breast cancer risk (relative risk = 0.9 for slow oxidizers compared with fast; 95% confidence interval = 0.6–1.3). Among postmenopausal women, *ADH3* genotype did not modify the weak association observed between alcohol intake and breast cancer risk (*P* for interaction = 0.45). Statistically significant trends in the relationship between alcohol consumption and hormone level dependent on oxidative capacity (*ADH3* genotype) were observed for dehydroepiandrosterone sulfate and sex hormone-binding globulin ($P < 0.05$). These data suggest that the *ADH3* polymorphism modestly influences the response of some plasma hormones to alcohol consumption but is not independently associated with breast cancer risk and does not modify the association between alcohol and breast cancer risk.

Introduction

Epidemiological studies suggest a modest direct association between alcohol consumption and the risk of breast cancer (1–5). In a recent pooled analysis of six large prospective studies, a statistically significant linear increase in breast cancer incidence with increasing alcohol consumption was observed (5). However, the mechanism by which alcohol increases breast cancer risk is unclear. One plausible mechanism is the exposure to toxic metabolites of alcohol. *In vitro* and *in vivo* studies have demonstrated that acetaldehyde, a metabolite of ethanol (a constituent of alcoholic beverages), is carcinogenic (6, 7). An alternative hypothesis is that alcohol may influence the levels of reproductive steroid hormones that play a critical role in breast carcinogenesis by either decreasing metabolic clearance or increasing the production of estrogens (8, 9). Many epidemiological studies have found that alcohol intake is significantly correlated with individual plasma or urinary endogenous steroid hormone levels (10–13), whereas several have not (13–15).

The oxidation of ethanol is catalyzed predominantly by the ADHs[3], and to a lesser extent by cytochrome P450IIE1 and H_2O_2-dependent catalase (16). ADH is a zinc-containing cytosolic enzyme that oxidizes short-chain alcohols to aldehydes, mainly formaldehyde and acetaldehyde (17). Animal studies suggest that this is the rate-limiting step of ethanol metabolism (18). ADH, a dimeric protein consisting of two 40-kDa enzyme subunits, is found primarily in the liver but also in other tissues, including the kidney, lung, and gastric mucosa (16, 17). There are a total of five enzyme subunits (α, β, γ, π, and χ) encoded at five separate gene loci: *ADH1*, *ADH2*, *ADH3*, *ADH4*, and *ADH5*, respectively. The ADH enzymes are divided into three classes determined by the preferential substrates of the isoenzymes. The class I ADH enzymes, encoded by *ADH1*, *ADH2*, and *ADH3*, are mainly involved in the oxidation of ethanol and other small, aliphatic alcohols. Polymorphic variants exist among the class I *ADH* genes, specifically *ADH2* and *ADH3*, and are known to produce enzymes with distinct kinetic properties. These functional differences produce variability in alcohol metabolic capacity between individuals (16, 17).

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[3] The abbreviations used are: *ADH3*, alcohol dehydrogenase type 3; RR, relative risk; 95% CI, 95% confidence interval; NHS, Nurses' Healthy Study; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.

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At the *ADH3* locus, the allele coding for the γ_1 allele differs from the γ_2 by two amino acids at positions 271 and 349 (19). The substitution of Gln for Arg at position 271 is believed to affect enzyme kinetics by changing the NAD(H) dissociation rate, producing a γ_2 subunit that is less metabolically active (slower oxidizer) than γ_1 (19). Pharmacokinetic studies have shown that there is a 2.5-fold difference in V_{max}, the maximum achievable velocity of the enzyme, for ethanol oxidation between the homodimeric γ_1 isoenzyme compared with the homodimeric γ_2 isoenzyme (16).

Epidemiological studies have investigated the *ADH3* polymorphism as a potential modifier of alcohol-cancer relationships. In a recent case-control study, Harty *et al.* (20) found that fast oxidizers who consumed very high levels of alcohol (>56 drinks per week) have a 5.3-fold increased risk of oral-pharyngeal cancer (95% CI = 1.0–28.8) relative to slow and intermediate oxidizers who consumed similar levels of alcohol. The authors hypothesize that heavy drinkers who are homozygous for the fast oxidizing allele receive more exposure to the carcinogenic effects of acetaldehyde. However, this result was not confirmed in another study (21). Freudenheim *et al.* (22) suggested an increased risk of breast cancer for homozygotes with the fast oxidizing allele compared with the intermediate and slow oxidizers among premenopausal women only (odds ratio = 2.3; 95% CI = 1.2–4.3). We investigated the *ADH3* polymorphism as both a potential risk factor for breast cancer and a potential modifier of the association between alcohol consumption and breast cancer in a case-control study nested within the prospective NHS. To identify a potential mechanism, we assessed the relationships among *ADH3* genotype, alcohol consumption, and plasma levels of steroid hormones in a subset of these women.

Materials and Methods

Study Population. In 1976, 121,700 married, female registered nurses between the ages of 30 and 55, from 11 states in the United States, were enrolled in the NHS by completing a self-administered questionnaire. This cohort has been prospectively followed with biennial questionnaires to update exposure information on a number of breast cancer risk factors, as well as disease status.

In 1989–1990, blood samples were collected from 32,826 of the cohort members as described previously (12). Each woman arranged to have her blood drawn and returned via overnight courier; 97% of the samples were received within 26 h of collection. The stability of estrogens in whole blood within this time period has been documented previously (23). Upon arrival, the samples were centrifuged and aliquoted into plasma, buffy coat, and RBC components and archived in continuously monitored liquid nitrogen freezers. The subcohort for this nested case-control study consisted of women who returned a blood sample and were free of diagnosed cancer (excluding non-melanoma skin cancer) at the time of blood draw. Cases were women who had a confirmed diagnosis of breast cancer anytime after blood collection through May 31, 1994. During this period, 465 eligible cases of incident breast cancer were identified. The estimated follow-up rate for the sub-cohort, as a proportion of potential person-years of observation through 1994, is over 97%. Controls (n = 465) were randomly selected among women who had provided a blood sample and were matched to cases (1:1) on year of birth, menopausal status (postmenopausal *versus* not), postmenopausal hormone use, month of blood return, time of day of blood collection, and fasting status at blood draw. For postmenopausal cases who were not taking postmenopausal hormones at least 3 months prior to blood draw, a second matched control was randomly selected to increase the power for analyses with plasma steroid hormone levels. The total number of controls was 621.

Laboratory Techniques. Blinded to case-control status, we used a slightly modified version of the PCR-restriction fragment length polymorphism method described by Hardy et al. (20) to genotype at the *ADH3* locus; DNA samples were digested with *Nla*III after amplification rather than prior to amplification. In addition, blinded quality controls (~10% of the sample size) were included and were genotyped with 100% concordance.

Hormone Analysis. Plasma hormone levels of estradiol, estrone, estrone sulfate, testosterone, androstenedione, percentage of free estradiol, bioavailable estradiol (*i.e.,* unbound plus albumin-bound), percentage of free bioavailable estradiol, DHEA, and DHEAS were assayed in up to three separate batches (12). Estrone sulfate from batches 1 and 2 and SHBG were assayed in the laboratory of Dr. C. Longcope (University of Massachusetts Medical Center). The Nichols Institute (San Juan Capistrano, CA) performed all other hormone assays. Within-batch laboratory coefficients of variation were ≤13.6%, with the exception of SHBG, which was 21.9%. The total number of postmenopausal controls with measured plasma hormone levels was 307, with the exception of SHBG (n = 298).

Statistical Analysis. We used conditional logistic regression to calculate RRs and 95% CIs to assess the risk of breast cancer for the three different genotypes: $ADH3_{1,1}$ (fast), $ADH3_{1,2}$ (intermediate), and $ADH3_{2,2}$ (slow). The following breast cancer risk factors were adjusted for in the multivariate models to control for any potential confounding: alcohol consumption (nondrinkers, >0 to ≤10, >10 g/day) body mass index (<22, 22–25, >25–29, >29 kg/m^2), age of menarche (<12, 12–14, >14 years), the interaction between parity and age at first birth (0; 1–2 children, ≤24 years; >2 children, ≤24 years; 1–2 children, >24 years; >2 children, >24 years), weight gain since 18 years of age (<10, 10–25, >25 kg), family history of breast cancer (yes/no), and history of benign breast disease (yes/no). Among postmenopausal women, duration of postmenopausal hormone use (none, <5, ≥5 years) and age at menopause (<48, 48 to <52, ≥52 years) were also adjusted for in the multivariate models. Prospective information regarding breast cancer risk factors were obtained from the 1976 baseline questionnaire, subsequent biennial questionnaires, and a questionnaire completed at blood draw. Menopausal status and use of postmenopausal hormones were assessed at blood draw and updated until date of diagnosis for cases and matched controls. The relationship between alcohol consumption and risk of breast cancer within genotype was determined by including interaction terms between each alcohol category (nondrinkers, >0 to ≤10, >10 g/day) and *ADH3* genotype. Current alcohol consumption was based on data collected prior to blood draw (1986 questionnaire); the 1990 questionnaire was used for individuals who did not provide this information on the 1986 questionnaire. Nineteen individuals were missing alcohol data from both questionnaires and were excluded from the alcohol analysis. In addition, we assessed the relationship between *ADH3* genotype, alcohol consumption at ages 18–22 (nondrinkers, <3, ≥3 drinks/week), and breast cancer risk.

Separate analyses were conducted for premenopausal and postmenopausal women. For postmenopausal women, conditional logistic regression was used to compute RRs and 95% CIs. Because of the relatively small number of premenopausal

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women, unconditional logistic regression was used to compute RRs and 95% CIs controlling for the matching factors, body mass index, age of menarche, the interaction between parity and age of first birth, family history of breast cancer, and history of benign breast disease. For subgroup analyses with small sample sizes, conditional logistic regression did not provide stable estimates when adjusting for all breast cancer risk factors, but they did provide similar estimates to unconditional analyses when these covariates were not included in the models.

To test for trends and interactions, current alcohol consumption was modeled as a continuous variable derived by assigning the median alcohol values of the controls for each consumption category to all individuals in that category. The *P* for trend was based on the Wald test. Conditional logistic regression was used for the combined genotypes. Because of small sample sizes, unconditional logistic regression controlling for the matching factors, as well as the previously mentioned risk factors, was used for determining trend within each genotype. For alcohol-*ADH3* interactions, *ADH3* genotype was modeled as a three-category ordinal variable. Statistical significance of *ADH3*-alcohol interactions was determined by a likelihood ratio test comparing the model with the interaction term to the model with the main effects only.

ADH3 genotype was also assessed as a modifier of the relationship between alcohol and plasma hormone levels in the postmenopausal controls who were not using hormones at the time of blood draw. Women with hormone levels below detectable limits (n = 8) were assigned the lowest detectable level. Within each batch, hormone values >3 interquartile ranges were treated as outliers and excluded. Various hormone fractions were not assayed for all women because of an insufficient quantity of plasma. In addition, five women with missing alcohol data were excluded from all hormone analyses.

To adjust for potential confounders, we regressed alcohol consumption as well as the natural log of the hormone value on the following variables: body mass index (kg/m^2), age (years), smoking (never, past, or current), and indicators for two of the three laboratory batches. These variables were selected because they were found to be associated with hormone levels in an overlapping subset of the NHS cohort (12). Pearson correlation coefficients between the two sets of adjusted residuals were used to assess the linear association between alcohol consumption and the natural logarithm of plasma hormone levels for each *ADH3* genotype. To test for trends in the relationship between alcohol consumption and plasma hormone levels across *ADH3* genotypes, we regressed hormone levels on alcohol consumption (g/day), *ADH3* genotype (ordinal), the interaction between alcohol and genotype [*ADH3* ordered times alcohol consumption (g/day)], as well as the potential confounders listed above. The *P* for trend was based on the Wald test of the interaction term.

Results

Seventy-seven percent of the breast cancer cases were postmenopausal at the time of diagnosis. Cases were more likely to have a history of benign breast disease (62% *versus* 42%), a first-degree family history of breast cancer (18% *versus* 13%), and a longer duration of postmenopausal hormone use (34% *versus* 28% have used for 5 or more years). Among parous women, controls were more likely to have three or more full-term pregnancies (63% *versus* 58%). Other case-control comparisons included (cases *versus* controls) mean age at menarche (12.4 *versus* 12.5 years), age at first birth (25.2 *versus* 25.0 years), body mass index (25.5 *versus* 25.5 kg/m^2), and alcohol consumption (22% *versus* 24% consume ≥10 g/day). Eighty-five percent of cases and 85% of controls reported that they were Caucasian. The *ADH3* genotype distribution in this study population was consistent with the previously reported estimates for Caucasians: ~40–50% for the γ_2 allele and 50–60% for the γ_1 allele (16). Among the cases, the distribution of γ_1 homozygotes, heterozygotes, and γ_2 homozygotes was 32.9, 51.4, and 15.7%, respectively. The distribution was similar for the controls: 34.0, 48.3, and 17.7%. Both distributions were in Hardy-Weinberg equilibrium (cases: χ^2 (*df*, 1) = 1.87, P = 0.17; controls: χ^2 (*df*, 1) = 0.07, P = 0.79).

It has been suggested that gastric ADH3 activity differs among the three genotypes: activity is highest among γ_1 homozygotes, followed by heterozygotes and γ_2 homozygotes (24). However, in some epidemiological studies, the heterozygotes (intermediate oxidizers) and γ_2 homozygotes (slow oxidizers) have been combined in the analysis (20–22). In this study, RRs were computed for the slow and intermediate groups both separately and combined. Regardless of whether the intermediate and slow groups were collapsed, no overall association between *ADH3* and breast cancer risk was found. The adjusted RR for the combined slow and intermediate oxidizers with the fast oxidizers was 1.0 (95% CI = 0.7–1.3). Compared with the fast oxidizers, the adjusted RRs were 0.9 (95% CI = 0.6–1.3) for slow oxidizers and 1.1 (95% CI = 0.8–1.4) for the intermediate oxidizers. When stratified on menopausal status, there still was no association between *ADH3* genotype and breast cancer risk (Table 1). Because there is prior biological and epidemiological evidence to suggest that ADH3 activity is distinct for each genotype, heterozygotes were not combined with either homozygous group in subsequent analyses.

On the basis of categories of alcohol consumption (nondrinkers, ≤10, >10 g/day), no association between current daily alcohol consumption and risk of breast cancer was observed among the overall study population (RR = 1.1 for >10 g/day compared with none; 95% CI = 0.7–1.6; *P*, test for trend = 0.94). On the basis of the estimated RRs and 95% CIs comparing all combinations of alcohol consumption and *ADH3* genotype to the nondrinking fast oxidizers, there was no clear evidence of effect modification by *ADH3* genotype [Table 2; χ^2 (*df*, 1) =2.03; P = 0.15].

Among postmenopausal women (321 cases and 474 controls), a weak, nonsignificant association was observed between alcohol consumption and breast cancer risk (RR = 1.3 for >10 g/day compared with none; 95% CI = 0.9–2.1; *P*, test for trend = 0.41; Table 3). There was no indication of an interaction between alcohol and *ADH3* genotype for breast cancer risk [χ^2 (*df*, 1) = 0.58; P = 0.45].

Among the 182 premenopausal women (88 cases and 94 controls), the association between alcohol consumption and breast cancer risk was estimated very imprecisely because of the small sample size [RR = 1.5 for ≤10 g/day compared with none (95% CI = 0.7–3.1); and RR = 0.5 for >10 g/day compared with none (95% CI = 0.2–1.4)]. Furthermore, there was no evidence of an interaction between alcohol and *ADH3* genotype [χ^2 (*df*, 1) = 0.65l P = 0.42].

A weak, nonsignificant association between alcohol consumption during the ages of 18–22 and breast cancer risk was observed (RR = 1.2 for >3 drinks/week compared with none; 95% CI = 0.7–1.9; *P*, test for trend = 0.30). This relationship was not modified by *ADH3* genotype [χ^2 (*df*, 1) = 0.21; P = 0.65].

In addition, we assessed the correlations between current daily alcohol consumption and plasma hormone levels

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Table 1 *ADH3* genotype and breast cancer risk

ADH3 genotype	Cases, *n* (%)	Controls, *n* (%)	RR (95% CI) Matched[a,b]	RR (95% CI) Multivariate[c,d]
All women				
Fast	153 (33)	211 (34)	1.0 (Ref)[e]	1.0 (Ref)
Intermediate	239 (51)	300 (48)	1.2 (0.9–1.5)	1.1 (0.8–1.4)
Slow	73 (16)	110 (18)	1.0 (0.7–1.5)	0.9 (0.6–1.3)
Premenopausal				
Fast	30 (33)	35 (36)	1.0 (Ref)	1.0 (Ref)
Intermediate	47 (52)	48 (50)	1.2 (0.6–2.2)	1.1 (0.5–2.5)
Slow	14 (15)	14 (14)	1.1 (0.5–2.8)	1.4 (0.5–4.0)
Postmenopausal				
Fast	106 (32)	157 (33)	1.0 (Ref)	1.0 (Ref)
Intermediate	168 (51)	236 (49)	1.1 (0.8–1.5)	0.9 (0.6–1.3)
Slow	54 (17)	88 (18)	1.0 (0.6–1.5)	0.7 (0.5–1.2)

[a] Matched on year of birth, menopausal status, postmenopausal hormone use, fasting status, time and date of blood draw.
[b] Unconditional logistic regression controlling for the matching factors was utilized for premenopausal women because of small sample size.
[c] In addition to matching factors, controlled for parity, age of first birth, age of menarche, body mass index, family history, alcohol consumption, benign breast disease, weight gain since age 18, age of menopause, and duration of postmenopausal hormone use.
[d] Unconditional logistic regression controlling for the matching factors, parity, age of first birth, age of menarche, body mass index, family history, alcohol consumption, benign breast disease, and weight gain since age 18 was utilized for premenopausal women because of small sample size.
[e] Ref, within reference values.

Table 2 RRs and 95% CIs for breast cancer risk stratified by *ADH3* genotype and daily alcohol consumption prior to diagnosis

ADH3 genotype	Alcohol consumption			*P*, test for trend[a]
	None	≤10 g/day	≥10 g/day	
Combined				
Cases, *n* (%)[b]	138 (30)	213 (47)	104 (23)	
Controls, *n* (%)[b]	195 (32)	268 (44)	149 (24)	
Matched RR[c]	1.0 (Ref)	1.1 (0.8–1.5)	0.9 (0.6–1.3)	0.35
Multivariate RR[d]	1.0 (Ref)	1.2 (0.9–1.6)	1.1 (0.7–1.6)	0.94
Fast				
Cases, *n* (%)	55 (12)	63 (14)	31 (7)	
Controls, *n* (%)	61 (10)	98 (16)	47 (8)	
Matched RR[c]	1.0 (Ref)	0.8 (0.5–1.3)	0.7 (0.4–1.3)	0.49
Multivariate RR[d]	1.0 (Ref)	0.8 (0.5–1.3)	0.8 (0.4–1.5)	0.83
Intermediate				
Cases, *n* (%)	61 (13)	124 (27)	50 (11)	
Controls, *n* (%)	97 (16)	127 (21)	72 (12)	
Matched RR[c]	0.8 (0.5–1.3)	1.2 (0.7–1.8)	0.8 (0.5–1.3)	0.60
Multivariate RR[d]	0.7 (0.4–1.2)	1.1 (0.7–1.8)	0.8 (0.4–1.4)	0.91
Slow				
Cases, *n* (%)	22 (5)	26 (6)	23 (5)	
Controls, *n* (%)	37 (6)	43 (7)	30 (5)	
Matched RR[c]	0.7 (0.4–1.4)	0.7 (0.4–1.4)	1.0 (0.5–1.9)	0.58
Multivariate RR[d]	0.6 (0.3–1.2)	0.6 (0.3–1.2)	1.1 (0.5–2.4)	0.21
LRT,[e] $P = 0.35$				
LRT,[f] $P = 0.15$				

[a] Unconditional logistic regression controlling for the matching factors was utilized for determining trend within each genotype because of small sample size.
[b] Numbers of cases and controls do not total 465 and 621 because of missing data on alcohol intake.
[c] Matched on year of birth, menopausal status, postmenopausal hormone use, fasting status, time and date of blood draw.
[d] In addition to matching factors, controlled for parity, age of first birth, age of menarche, body mass index, family history, alcohol consumption, benign breast disease, weight gain since age 18, age of menopause, and duration of postmenopausal hormone use.
[e] Likelihood ratio test for the interaction between genotype and alcohol consumption adjusted for the matching factors.
[f] Likelihood ratio test for the interaction between genotype and alcohol consumption adjusted for the matching factors and previously described risk factors.

among postmenopausal controls who were not taking postmenopausal hormones within the 3 months before blood draw (Table 4). Weak correlations were observed with estrone sulfate ($r = 0.14$; $P = 0.02$), bioavailable estradiol ($r = 0.14$; $P = 0.02$), percentage of bioavailable estradiol ($r = 0.14$; $P = 0.01$), testosterone ($r = -0.11$; $P = 0.06$), and SHBG ($r = -0.13$; $P = 0.03$). When stratified by *ADH3* genotype, the correlations for estrone sulfate, estradiol, free estradiol, and DHEAS were statistically significant for the fast oxidizers and more positive compared with the slow and intermediate oxidizers. However, a significant trend in the relationship between alcohol consumption and plasma hormone level based on oxidative capacity (*ADH3* genotype) was observed only for DHEAS (*P*, test for trend = 0.01). In addition, a statistically significant trend was observed for SHBG (*P*, test for trend = 0.02), and a modest trend was observed for testosterone (*P*, test for trend = 0.07). For SHBG and testosterone, stronger inverse associations between alcohol consumption and plasma hormone levels were observed for slow oxidizers compared with fast oxidizers.

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Table 3 RRs and 95% CIs for breast cancer risk stratified by *ADH3* genotype and daily alcohol consumption prior to diagnosis among postmenopausal women

ADH3 genotype	Alcohol consumption			*P*, test for trend[a]
	None	≤10 g/day	>10 g/day	
Combined				
Cases, *n* (%)[b]	89 (28)	146 (46)	86 (27)	
Controls, *n* (%)[b]	150 (32)	210 (44)	114 (24)	
Matched RR[c]	1.0 (Ref)[d]	1.2 (0.8–1.7)	1.2 (0.8–1.8)	0.62
Multivariate RR[e]	1.0 (Ref)	1.3 (0.9–1.9)	1.3 (0.9–2.1)	0.41
Fast				
Cases, *n* (%)	34 (11)	45 (14)	25 (8)	
Controls, *n* (%)	48 (10)	73 (15)	33 (7)	
Matched RR[c]	1.0 (Ref)	1.0 (0.5–1.7)	1.1 (0.5–2.2)	0.81
Multivariate RR[e]	1.0 (Ref)	1.0 (0.5–1.9)	1.2 (0.5–2.8)	0.60
Intermediate				
Cases, *n* (%)	40 (13)	84 (26)	41 (13)	
Controls, *n* (%)	73 (15)	102 (22)	57 (12)	
Matched RR[c]	0.9 (0.5–1.7)	1.3 (0.7–2.2)	1.1 (0.6–2.0)	0.98
Multivariate RR[e]	0.7 (0.4–1.5)	1.2 (0.6–2.2)	0.9 (0.5–1.9)	0.71
Slow				
Cases, *n* (%)	15 (5)	17 (5)	20 (6)	
Controls, *n* (%)	29 (6)	35 (7)	24 (5)	
Matched RR[c]	0.8 (0.4–1.9)	0.7 (0.3–1.5)	1.3 (0.6–2.7)	0.50
Multivariate RR[e]	0.6 (0.2–1.5)	0.6 (0.3–1.4)	1.3 (0.5–3.0)	0.17
LRT,[f] *P* = 0.56				
LRT,[g] *P* = 0.45				

[a] Unconditional logistic regression controlling for the matching factors was utilized for determining trend within each genotype because of small sample size.
[b] Numbers of cases and controls do not total 328 and 481 because of missing data on alcohol intake.
[c] Matched on year of birth, menopausal status, postmenopausal hormone use, fasting status, time and date of blood draw.
[d] Ref, within reference values.
[e] In addition to matching factors, controlled for parity, age of first birth, age of menarche, body mass index, family history, alcohol consumption, benign breast disease, weight gain since age 18, age of menopause, and duration of postmenopausal hormone use.
[f] Likelihood ratio test for the interaction between genotype and alcohol consumption adjusted for the matching factors.
[g] Likelihood ratio test for the interaction between genotype and alcohol consumption adjusted for the matching factors and previously described risk factors.

Discussion

Pharmacokinetic differences in ethanol metabolism are a plausible determinant of susceptibility to the many diseases associated with alcohol. Among the class I ADH enzymes, essential for ethanol metabolism, three *ADH2* alleles (β_1, β_2, β_3) and two *ADH3* alleles (γ_1, γ_2) have been identified and well characterized (16, 17). Although they differ by only one amino acid, the *ADH2* alleles have been shown to vary by 20-fold in the rate of ethanol oxidation (16). More than 90% of Caucasians have the slowest oxidizing *ADH2* allele (β_1), thus limiting statistical power to investigate potential association(s) between variant *ADH2* alleles and disease among Caucasians. The *ADH3* alleles have also been shown to have distinct kinetic properties, but not to the same extent as the *ADH2* alleles (16). The 2.5-fold variation in V_{max} between $\gamma_1\gamma_1$ and $\gamma_2\gamma_2$ enzymes is thought to contribute to differences in the rate of ethanol oxidation; however, the *ADH3* polymorphism has not been shown to have any clear short-term effects on blood alcohol levels in human feeding studies (16, 25). The prevalence of the variant *ADH3* allele (γ_2) is high in Caucasian populations (40–50%) and lower in African-Americans (15%) and Asians (5%; Ref. 16). Epidemiological studies have associated the *ADH3* polymorphism with the risk of alcoholism and alcoholic liver cirrhosis, suggesting a functional role for variation in *ADH3* (26, 27).

In a case-control study with 134 premenopausal and 181 postmenopausal cases, Freudenheim *et al.* (22) observed an increased risk of breast cancer among premenopausal women for the fast oxidizing genotype, particularly among moderate to heavy drinkers. In our prospective study, we observed no increase in risk of breast cancer associated with the *ADH3* genotype, regardless of alcohol consumption or menopausal status. The discrepancy could be attributed to different levels of average alcohol consumption among the two study populations or small sample sizes. Freudenheim *et al.* (22) had 260 premenopausal women (134 cases and 126 controls), and our study had 182 premenopausal women (88 cases and 94 controls).

One difficulty in the interpretation of our nested case-control study was the lack of a significant positive relationship of alcohol consumption with breast cancer risk, which was observed previously in the overall cohort (1). In the pooled analysis of prospective cohort studies, the multivariate RR was 1.09 (95% CI = 1.04–1.13) for a 10 g/day increment in alcohol consumption (5). When alcohol consumption is treated as a continuous variable, the multivariate RR for a 10 g/day increment of alcohol consumption in our nested case-control study is close to this estimate (RR = 1.08; 95% CI = 0.96–1.22). However, even with 465 cases, our study had inadequate power to detect this modest association.

In addition, we did not observe an association between past alcohol consumption (ages 18–22) and overall breast cancer risk. The majority of epidemiological studies have focused only on current alcohol consumption in relation to breast cancer risk (1–5). There is some evidence to suggest that current alcohol consumption is more relevant to breast cancer risk than consumption in the distant past (28).

A hypothesized mechanism for the relationship between alcohol consumption and breast cancer is an effect of alcohol on steroid hormone levels. To date, there have been eight published studies evaluating the association between alcohol intake and urine or plasma estrogen levels among European or Caucasian postmenopausal women (13). Estrone sulfate, the most abundant estrogen in postmenopausal women, has

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Table 4 Pearson correlations between current alcohol intake and plasma hormone levels[a]

Hormone	r (P)				P, test for trend
	Combined	Fast	Intermediate	Slow	
Estrone sulfate	0.14 (0.02) n = 278	0.26 (0.02) n = 83	0.08 (0.34) n = 138	0.19 (0.15) n = 57	0.84
Estrone	0.03 (0.57) n = 297	0.15 (0.18) n = 84	−0.03 (0.74) n = 152	0.09 (0.49) n = 61	0.88
Estradiol	0.07 (0.23) n = 296	0.23 (0.03) n = 83	−0.01 (0.93) n = 151	−0.01 (0.96) n = 62	0.21
Free estradiol	0.09 (0.11) n = 291	0.23 (0.04) n = 83	0.02 (0.80) n = 148	0.07 (0.60) n = 60	0.35
% free estradiol	0.06 (0.33) n = 295	0.02 (0.85) n = 84	0.08 (0.35) n = 151	0.14 (0.29) n = 60	0.59
Bioavailable estradiol	0.14 (0.02) n = 293	0.21 (0.06) n = 83	0.08 (0.35) n = 151	0.22 (0.09) n = 59	0.97
% bioavailable estradiol	0.14 (0.01) n = 298	0.17 (0.12) n = 84	0.13 (0.12) n = 153	0.12 (0.35) n = 61	0.79
Androstenedione	−0.02 (0.75) n = 289	0.11 (0.34) n = 82	−0.10 (0.25) n = 146	−0.04 (0.77) n = 61	0.39
Testosterone	−0.11 (0.06) n = 291	0.08 (0.50) n = 82	−0.19 (0.02) n = 148	−0.19 (0.13) n = 61	0.07
DHEA	0.00 (0.98) n = 266	0.17 (0.14) n = 76	−0.08 (0.37) n = 136	−0.02 (0.90) n = 54	0.23
DHEAS	0.06 (0.30) n = 292	0.28 (0.01) n = 80	0.08 (0.32) n = 151	−0.23 (0.08) n = 61	0.01
SHBG	−0.13 (0.03) n = 289	0.04 (0.75) n = 83	−0.17 (0.03) n = 149	−0.22 (0.10) n = 57	0.02

[a] Adjusted for smoking, body mass index, age, and laboratory batch.

been hypothesized to be a major source of estradiol in both malignant and normal breast tissue (29, 30). Hankinson *et al.* (12) investigated endogenous plasma hormone levels among 217 healthy, postmenopausal women, a subset of the NHS cohort oversampled for women with moderate alcohol consumption, and found a statistically significant correlation between alcohol consumption and estrone sulfate levels ($r = 0.17$; $P = 0.02$). A similar positive correlation was observed among the largely independent subset of women in this study. In addition, we observed weak positive correlations between alcohol consumption and bioavailable estradiol and percentage of bioavailable estradiol. When stratified by *ADH3* genotype, there was no evidence of a trend in the relationship between alcohol consumption and hormone level dependent on oxidative capacity (*ADH3* genotype) for any of these hormones. These data are compatible with the absence of an interaction between alcohol consumption, *ADH3* genotype, and breast cancer risk in this study population.

Modest inverse associations between alcohol consumption and hormone levels were observed with testosterone and SHBG. Madigan *et al.* (31) also observed a weak inverse association between alcohol consumption and SHBG among postmenopausal women whose alcohol consumption was ≥20 g/week. Obesity, a risk factor for breast cancer in postmenopausal women, is strongly associated with decreased SHBG (31, 32). Increased tissue availability of estrogens as a result of decreased SHBG is a potential mechanism for the relationship between obesity, as well as alcohol consumption and risk of postmenopausal breast cancer (8).

Statistically significant trends in the relationship between alcohol consumption and hormone level dependent on *ADH3* genotype were observed for DHEAS and SHBG. For DHEAS, a modest positive correlation was observed among rapid oxidizers, and a modest inverse correlation was observed among slow oxidizers. SHBG was modestly inversely correlated with alcohol consumption among the slow and intermediate oxidizers only. Some researchers have suggested that SHBG serves as a vehicle for receptor binding and cell transport, so protein-bound estradiol may be an active component (33). Testosterone had a similar relationship; inverse correlations were observed among the slow and intermediate oxidizers only. Heavy alcohol consumption reduces testosterone levels (34–36). Human studies have shown that male alcoholics who abstain from drinking experience increases in testosterone levels (36). These data suggest that among rapid oxidizers, fast clearance of alcohol from the bloodstream may mitigate the depression of androgen and SHBG levels associated with alcohol intake. However, studies in men and in women including a larger proportion of heavier drinkers are clearly needed.

In conclusion, these data suggest that variation at the *ADH3* locus modestly influences the response of some hormones and SHBG to alcohol consumption, but this variation is neither independently associated with breast cancer nor a modifier of the association between alcohol and breast cancer risk among light to moderate drinkers. Similar to the data from the overall cohort and consistent with other epidemiological studies, we observed a modest association with increasing alcohol consumption and breast cancer risk in postmenopausal women (1–5). This relationship was not significantly modified by *ADH3* genotype. No association between alcohol and breast cancer was observed among premenopausal women; however, the number of premenopausal women in this study was small. The prospective design, relatively large number of incident cases, and high follow-up rate of our study strengthen the validity of our results. Although large studies with a higher proportion of heavier alcohol consumers would be necessary to exclude a modest interaction of alcohol, *ADH3* genotype, and breast cancer risk, our study suggests that any such interaction is likely to be weak or nonexistent.

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