

Alcohol metabolism, alcohol intake, and breast cancer risk: a sister-set analysis using the Breast Cancer Family Registry

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Abstract Moderate alcohol intake has been consistently associated with a modest (30–50%) increase in breast cancer risk, but it remains unclear if certain individuals have higher susceptibility to the harmful effects of alcohol intake. Individuals differ in their ability to metabolize alcohol through genetic differences in alcohol dehydrogenase (*ADH*), the enzyme that catalyzes the oxidation of approximately 80% of ethanol to acetaldehyde, a known carcinogen. Using data from the Breast Cancer Family Registry ($n = 811$ sister sets), we examined whether sisters with breast cancer differ with respect to alcohol consumption and alcohol metabolism (measured by polymorphisms in *ADH1B* and *ADH1C*) compared to their sisters without breast cancer. Neither alcohol drinking nor alcohol metabolizing *ADH1B* and *ADH1C* genotypes were associated with breast cancer risk. However, only 19%

and 42% of sisters were discordant by *ADH1B* and *ADH1C*, respectively, and even fewer were discordant by both genotype and alcohol intake, making it difficult to detect differences if they existed.

Keywords Breast cancer · Alcohol · Alcohol dehydrogenase

Introduction

Alcohol intake, if causal, may be one of the few modifiable risk factors for breast cancer prevention identified to date. Despite the increasing epidemiologic evidence that alcohol intake is associated with an elevated risk of breast cancer, the overall magnitude of the association has been small, with relative risks ranging from 1.3–1.5 in relation to intake of 1–2 drinks

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per day [1–12]. It is more difficult to rule out confounding and bias as alternative explanations for associations of low magnitude, even if the findings have been consistently replicated [13]. Studies that examine biological mechanisms can move forward the understanding of the underlying etiology, because they can help rule out biases present in studies that rely solely on self-report of alcohol consumption as the main explanation for the association.

Alcohol may influence cancer risk through a number of mechanisms, including 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, 14–19]. In addition to these potential mechanisms, alcohol dehydrogenase metabolizes approximately 80% of ethanol into acetaldehyde, a known carcinogen [1]. Acetaldehyde induces sister chromatid exchange, mutations, and chromosomal aberrations in cell cultures and in human lymphocytes [17, 20] 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 sites that are not necessarily in direct contact with ethanol (e.g., breast) [16]. Alcohol dehydrogenase can also increase cancer risk by producing reactive oxygen species [21]. Recent laboratory evidence suggests that alcohol dehydrogenase, which is highly expressed in normal mammary tissue, may be functioning as a tumor suppressor [22]. Approximately 96–98% of the activity of alcohol dehydrogenase (*ADH*) in the body is in the liver, but it is expressed and regulated in a number of tissues, including breast tissue [21, 23]. Polymorphisms in both *ADH1B* and *ADH1C* have been examined with respect to breast cancer risk. Presence of the *ADH1B*2* or *ADH1C*1* allele increases oxidation and metabolism of alcohol [24–26]. The nomenclature for *ADH* has changed recently from *ADH₂* and *ADH₃* to *ADH1B* and *ADH1C*, respectively [25].

Three studies have investigated the association between *ADH1C* genotype, alcohol intake and breast cancer risk [27–29]; two supported a stronger association with breast cancer risk from alcohol consumption among fast metabolizers (characterized as *ADH1C*1/1* genotype) [27, 28]. Two studies have examined the association between *ADH1B* genotype and breast cancer risk with inconsistent findings [30, 31]. These studies compared breast cancer cases to unrelated controls. To examine whether alcohol consumption and its potential modification by genotype could explain breast cancer risk within families, we undertook a study

of alcohol intake, alcohol metabolism (as measured by *ADH1B* and *ADH1C* genotype), and breast cancer risk using data from the Breast Cancer Family Registry.

Methods

Study population

In 1995, the NCI funded six international sites establishing the Breast Cancer Family Registry (Breast CFR) (<http://epi.grants.cancer.gov/CFR/>), a resource for genetic studies of breast cancer [32]. Briefly, six participating sites from the USA, Canada, and Australia ascertained families either from population-based cancer registries (producing population-based families: San Francisco Bay Area, California; Ontario, Canada, and Melbourne and Sydney Australia) or from clinical and community settings (producing clinic-based families: New York, NY; Philadelphia, PA; and Salt Lake City, Utah). All sites who had available DNA and questionnaire data for sister sets (full sisters only) where at least one sister was affected with breast cancer and one sister was unaffected with breast cancer were eligible to participate in the current study. Each family could contribute multiple affected and/or unaffected sisters. Altogether there were 811 family sets (201 from New York, 304 from Ontario and 283 from California, and 23 from Philadelphia and Utah combined). Of these sets, 75% were non-Hispanic white, 21% Hispanic, 3% African American, and 1% of other race/ethnicities.

All sites used common questionnaires on family history, epidemiologic risk factors, and dietary intake. The family history questionnaire was completed by the proband (initial person contacted in the family) and obtained information on vital status, date of birth, date of death, and date of cancer diagnosis for all first-degree relatives, and more distant relatives with a personal history of cancer. The risk factor questionnaire was completed by participating probands and relatives and sought information on demographics, personal history of cancer, breast and ovarian surgeries, radiation exposure, smoking and alcohol consumption, menstrual and pregnancy history, breast feeding history, hormone use, weight, height, and physical activity. The standard alcohol questions asked those who had consumed at least one drink per week for six months or longer to give the age when they started consuming alcohol, if and at what age they stopped, and how much they usually drank in a week by type of alcohol (beer, wine, and liquor). For the present analysis, information for the latter question was summed to give the total number of drinks per week usually consumed.

Genotyping

At all sites, blood samples were collected from probands and from adult first-degree relatives, including sisters. DNA has been extracted using common procedures. DNA was genotyped using either TaqMan (*ADHIB*, rs#1229984) or template-directed primer extension with detection of incorporated nucleotides by fluorescence polarization (*ADHIC*, rs#698) [27, 33] in a 96 microwell based format. All analyses were performed blinded to case-control status. For *ADHIB*, TaqMan primers and probes were synthesized by Applied Biosystems (Foster City, CA) and samples run on an Applied Biosystems 7500 thermal cycler. For *ADHIC*, 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° for 5 min 30 s was followed by 34 cycles of 94° for 30 s, 60° for 45 s and 72° for 1 min, followed by 4 min at 72°. Primers and dNTPs were digested with 1 unit of shrimp alkaline phosphatase (1 u/ μ l, Roche) after addition of 1 μ l of 10 \times buffer and 1 unit E.Coli exonuclease I (10 u/ μ l, United States Biochemical, Cleveland, OH) and 7.9 μ l of water for 45 min at 37° followed by heating at 95° 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° for 2 min followed by 30 cycles of 95° for 15 s and 55° for 30 s. Plates were read on a Perkin Elmer Victor instrument. Reliability based on 201 repeat samples, was 97% and 98% for *ADHIB* and *ADHIC*, respectively.

Statistical methods

We first compared differences between genotypes and breast cancer risk factors using the Chi-square test for categorical variables, and the Analysis of Variance test for continuous variables [34]. We analyzed the data using conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the main effects of genotype on breast cancer risk [35]. All

models adjusted for reference age as a continuous variable. Reference age was defined as age at diagnosis for the cases and age at questionnaire completion for the unaffected sisters. We examined confounding by body mass index (BMI) in categories (<25 kg/m², \geq 25 kg/m²), age at menarche, and parity. All analyses were done by family sets so that the following factors were controlled for by design rather than in the statistical analysis: race/ethnicity, study site, and family history of breast cancer. Variables were kept in the final multivariate model if they altered the parameter estimates for the exposure of interest by at least 10% [36]. For the largest clinic-based site (New York) we were able to assess whether truncating exposures and confounders for the unaffected sister based on the age of diagnosis for the affected sister altered the effect estimates. Overall there were no material differences in results, so we defined exposure and confounder information by reference age for all sites as described above.

Effect modification by genotype was first examined through the 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 [35]. We 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 [36]. We separately stratified these models by menopausal status. Women who reported having a period or given birth within a year of the reference age were considered premenopausal and women who had not had a period within this time or reported having both ovaries removed were considered postmenopausal. Women who had a hysterectomy without oophorectomy or who were using hormone replacement therapy without having stopped menstruating were considered of unknown menopausal status unless their reference age was \geq 55 years in which case they were considered postmenopausal. We ran the overall models for all five sites. Two sites, however, had too few sister sets ($n = 23$) to yield stable OR estimates, so we examined the three larger sites only.

Results

Table 1 summarizes the associations between genotype and selected demographic information and alcohol consumption among the unaffected sisters. Women who were homozygous for the fast metabolizing alleles for each gene (*ADHIB**2 and *ADHIC**1) were less likely to drink alcohol than women who were homozygous for the slow metabolizing allele (*ADHIB**1 and

Table 1 Characteristics of unaffected sisters by alcohol metabolism genotype in the Breast Cancer Family Registry

Variable	Value	<i>ADH1B</i> Genotype			<i>P</i> value
		<i>ADH1B</i> *1/1 <i>slow</i> # (%)	<i>ADH1B</i> *1/2 <i>intermediate</i> # (%)	<i>ADH1B</i> *2/2 <i>fast</i> # (%)	
Menopausal status	Premenopausal	570 (63.8)	124 (75.6)	25 (60.9)	0.01
	Postmenopausal	323 (36.2)	40 (34.4)	18 (39.1)	
Reference age	Mean, years	48.7	47	47.8	0.16
	σ years	10.5	10.2	10.5	
Age at menarche	Mean, years	12.8	12.8	13.2	0.32
	σ years	1.6	1.9	1.7	
Parity	Nulliparous	168	32	11	0.69
	parous	722	132	35	
BMI	Mean	25.8	25.3	24.6	0.23
	σ	5.7	5.9	3.5	
Total alcohol consumption	Nondrinkers	420 (47.0)	108 (65.9)	38 (82.6)	<0.0001
	Drinkers	473 (53.0)	56 (34.1)	8 (17.4)	
	Mean, drinks (among drinkers)	7.0	4.4	4.7	
	σ drinks	8.1	3.4	3.6	
Race/ethnicity	Nondrinkers	420 (47.0)	108 (65.9)	38 (82.6)	<0.0001
	0–7 drinks per week	307 (34.4)	48 (29.3)	7 (15.2)	
	≥ 7 drinks per week	166 (18.6)	8 (4.9)	1 (2.2)	
	White	711 (80.0)	91 (55.5)	6 (13.0)	
Race/ethnicity	African American	27 (3.0)	3 (1.8)	0	<0.001
	Hispanic	150 (16.8)	68 (41.5)	39 (84.8)	
	Other	5 (0.6)	2 (1.2)	1 (2.2)	
Variable	Value	<i>ADH1C</i> Genotype			<i>p</i> value
Menopausal status	Premenopausal	306 (64.4)	340 (66.4)	76 (65.5)	0.81
	Postmenopausal	169 (35.6)	172 (33.6)	40 (34.5)	
Reference age	Mean, years	47.8	48.9	49.1	0.22
	σ years	10.3	10.4	11.4	
Age at menarche	Mean, years	12.8	12.9	12.7	0.43
	σ years	1.7	1.6	1.5	
Parity	Nulliparous	87 (18.4)	99 (19.4)	25 (21.6)	0.73
	Parous	386 (81.6)	412 (80.6)	91 (78.5)	
BMI	Mean	25.8	25.6	25.9	0.88
	σ	5.6	5.7	5.6	
Total alcohol consumption	Nondrinkers	263 (55.3)	248 (48.4)	55 (47.4)	0.06
	Drinkers	212 (44.6)	264 (51.6)	61 (52.6)	
	Mean, drinks (among drinkers)	6.3	7.2	6.3	
	σ drinks	8.5	7.6	4.6	
Race	Nondrinkers	263 (55.3)	248 (48.4)	55 (47.4)	0.13
	0–7 drinks per week	149 (31.4)	171 (33.4)	42 (36.2)	
	≥ 7 drinks per week	63 (13.3)	93 (18.2)	19 (16.4)	
	White	305 (64.2)	408 (79.7)	95 (81.9)	
Race	African American	24 (5.1)	6 (1.2)	0	<0.001
	Hispanic	141 (29.7)	96 (18.8)	20 (17.2)	
	Other	5 (1.0)	2 (0.4)	1 (0.9)	

*ADH1B**2). Unaffected sisters with the variant allele for *ADH1B**2 are more likely to be nonwhite and those with the variant allele *ADH1C**2 are more likely to be white.

Neither alcohol consumption alone (OR = 0.9, 95% CI = 0.6–1.1 for ≥ 7 drinks per week; OR = 0.9, 95% CI = 0.7–1.1 for < 7 drinks per week relative to nondrinkers), *ADH1C* genotype (OR = 1.0, 95% CI = 0.7–1.5 for *ADH1C**2/2; OR = 1.0, 95% CI = 0.8–1.3; for *ADH1C**1/2, compared with *ADH1C**1/1), nor

ADH1B genotype (OR = 0.9, 95% CI = 0.4–2.1 for *ADH1B**2/2; OR = 1.2, 95% CI = 0.8–1.7 for *ADH1B**1/2, compared with *ADH1B**1/1) were associated with breast cancer risk (Table 2).

Table 3 reports the findings for *ADH1C* genotype, alcohol intake, and breast cancer risk for all sister sets and separately by menopausal status. Breast cancer risk was assessed relative to the more common referent group of homozygous wild-type fast metabolizers (*ADH1C**1/1). Overall, there were no statistically

Table 2 Alcohol consumption, *ADHIC* and *ADHIB* genotypes and breast cancer risk

Variable		Age-adjusted OR*	95% CI*
Alcohol consumption	Nondrinker	1.0	
	<7 drinks per week	0.91	0.73–1.14
	≥7 drinks per week	0.85	0.63–1.14
<i>ADHIC</i> Genotype	<i>ADHIC</i> *1/1 (<i>fast</i>)	1.0	
	<i>ADHIC</i> *1/2 (<i>intermediate</i>)	1.04	0.81–1.33
	<i>ADHIC</i> *2/2 (<i>slow</i>)	1.04	0.71–1.52
<i>ADHIB</i> Genotype	<i>ADHIB</i> *1/1 (<i>slow</i>)	1.0	
	<i>ADHIB</i> *1/2 (<i>intermediate</i>)	1.15	0.80–1.66
	<i>ADHIB</i> *2/2 (<i>fast</i>)	0.91	0.40–2.11

* Odds ratio (OR) and 95% confidence interval (CI), adjusted for reference age

Table 3 Alcohol consumption, *ADHIC* genotype and breast cancer risk, by menopausal status

	Age-adjusted OR * (95% CI)	All sister sets (<i>N</i> = 788) OR ** (95% CI)	Premenopausal Sister Sets (<i>N</i> = 523) OR** (95% CI)	Postmenopausal Sister Sets (<i>N</i> = 265) OR** (95% CI)
<i>ADHIC</i> *1/1, nondrinker	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
<i>ADHIC</i> *1/2, nondrinker	1.10 (0.79–1.53)	1.10 (0.78–1.55)	0.99 (0.60–1.63)	0.69 (0.28–1.68)
<i>ADHIC</i> *2/2, nondrinker	1.11 (0.64–1.91)	1.18 (0.67–2.05)	0.62 (0.24–1.62)	1.75 (0.53–5.82)
<i>ADHIC</i> *1/1, <7 drinks/week	0.84 (0.59–1.18)	0.84 (0.59–1.20)	0.75 (0.45–1.25)	1.32 (0.52–3.37)
<i>ADHIC</i> *1/2, <7 drinks/week	0.98 (0.68–1.41)	1.03 (0.70–1.51)	0.74 (0.42–1.32)	1.18 (0.45–3.10)
<i>ADHIC</i> *2/2, <7 drinks/week	1.12 (0.65–1.91)	1.17 (0.68–2.03)	1.09 (0.50–2.37)	1.61 (0.43–6.08)
<i>ADHIC</i> *1/1, ≥7 drinks/week	0.93 (0.57–1.50)	0.91 (0.55–1.51)	0.97 (0.44–2.15)	1.84 (0.64–5.26)
<i>ADHIC</i> *1/2, ≥7 drinks/week	0.88 (0.56–1.39)	0.87 (0.55–1.40)	0.61 (0.26–1.40)	1.32 (0.46–3.82)
<i>ADHIC</i> *2/2, ≥7 drinks/week	0.61 (0.27–1.39)	0.64 (0.28–1.46)	0.36 (0.11–1.16)	1.25 (0.18–8.63)

* Odds ratio (OR) and 95% confidence interval (CI), adjusted for reference age

** Adjusted for reference age, body mass index, age at menarche, and parity

significant associations by genotype and alcohol consumption. Patterns by drinking level can be compared by examining the ratio of the odds ratios for a given genotype category. There are no clear patterns in breast cancer risk by alcohol consumption, overall or by menopausal status. Patterns by genotype can be compared by examining the ratio of the odds ratios for a given alcohol level. Although fast metabolizers have a 42% higher odds ratio ($0.91/0.64 = 1.42$) than slow metabolizers among heavier drinkers (≥ 7 drinks per week), there is an opposite pattern ($0.84/1.17 = 0.72$) among lighter drinkers (< 7 drinks per week). Separate analyses for the three largest study centers were not materially different from the overall estimates (data not shown).

Table 4 reports the findings for *ADHIB* genotype, alcohol intake, and breast cancer risk for all sister sets. As before, all point estimates are relative to a common referent group of nondrinkers who are homozygous wild-type slow metabolizers (*ADHIB**1/1). Although there is an elevation among fast metabolizers

(*ADHIB**2/2) of approximately 50%, this elevation is not statistically significant and is seen only among the nondrinkers. These results are not further stratified by menopausal status because of the smaller number of discordant sets available for these analyses. Again, no clear patterns emerged and there were no statistically significant associations by genotype and alcohol consumption with respect to breast cancer risk. As noted for *ADHIC*, separate analyses by study site were not materially different from the overall estimates (data not shown). There were too few discordant sister sets to examine interactions between both *ADHIC* and *ADHIB* genotypes.

Discussion

We investigated the role of alcohol metabolism, alcohol consumption and breast cancer risk by examining alcohol consumption by genotype status among sisters

Table 4 Alcohol consumption, *ADH1B* genotype and breast cancer risk

	Age-adjusted* OR (95% CI)	Multivariate-adjusted** OR (95% CI)
<i>ADH1B</i> *1/1 and <i>ADH1B</i> *1/2, nondrinker	1.0 (Ref)	1.0 (Ref)
<i>ADH1B</i> *2/2, nondrinker	1.54 (0.99–2.40)	1.53 (0.98–2.40)
<i>ADH1B</i> *1/1 and <i>ADH1B</i> *1/2, <7 drinks/day	1.09 (0.83–1.42)	1.15 (0.87–1.51)
<i>ADH1B</i> *2/2, <7 drinks/day	0.70 (0.38–1.28)	0.73 (0.40–1.35)
<i>ADH1B</i> *1/1 and <i>ADH1B</i> *1/2, ≥7 drinks/day	0.90 (0.65–1.26)	0.95 (0.68–1.34)
<i>ADH1B</i> *1/1, ≥7 drinks/day	0.51 (0.14–1.84)	0.52 (0.15–1.86)

* Odds ratio (OR) and 95% confidence interval (CI) adjusted for reference age

** Adjusted for reference age and body mass index

discordant for breast cancer. Overall, we found no association between alcohol intake, *ADH1C*, and *ADH1B* genotype and breast cancer risk among sisters discordant for breast cancer. The lack of a main effect for *ADH1C* agrees with recently published data from the Breast Cancer Association Consortium (BCAC) which found no overall association in 7,805 cases and 7,320 controls, although the association with alcohol intake was not evaluated [37]. Evidence from other studies investigating either *ADH1C* [27–29] or *ADH1B* [30, 31], alcohol intake, and breast cancer risk has been mixed, all of which have compared breast cancer cases to unrelated controls. In this study, we examined full sisters discordant by breast cancer status to see if alcohol, and its potential modification by genotype, could contribute to differences in breast cancer risk within families. This approach eliminates any potential confounding by race/ethnicity due to population stratification. The lack of association with alcohol consumption and *ADH1B* and *ADH1C* genotypes in this study may be explained by methodological limitations, including nondifferential measurement error of self-reported alcohol consumption and/or genotype, although a high rate of reproducibility was demonstrated for the latter, and a lack of statistical power because of high concordance of genotype and alcohol consumption.

The frequency of the high risk *ADH1C**1 allele was 66% among the unaffected sisters which is similar to the frequency (58%) reported previously in European Whites [28]. The *ADH1C* allele and genotype frequency were similar across all three published studies [27–29] and the unaffected sisters in this study. The frequency of the high risk *ADH1B**2 allele was 11% which is also similar to the other two published studies published on *ADH1B* and breast cancer risk [30, 31]. Thus, allele and genotype frequency differences between this and other studies cannot explain the lack of association in this study population. Further, as in

other studies we found that women with the fast metabolizer variant allele for *ADH1C* and *ADH1B* were less likely to consume alcohol [27, 31]. This also argues against our measure of genotype as a reason explaining the lack of association.

It is likely that there was some nondifferential measurement error in alcohol intake. Overall, we observed no effect between alcohol consumption and breast cancer risk in these data. The questions on alcohol consumption used in this study are similar to those used in many other epidemiologic studies that have detected modest associations in breast cancer risk from alcohol consumption. Thus, the lack of association is more likely due to the concordance of sisters both on genotype and on alcohol consumption. Of the 132 sister sets that were discordant on *ADH1B* genotype, only 45 were also discordant on alcohol consumption as classified by the categories used in our analyses. Of the 308 sister sets that were discordant on *ADH1C* genotype, only 141 were also discordant on alcohol consumption. We considered potential confounding by other known risk factors for breast cancer and adjustment for these confounders did not materially impact our null findings.

Overall, this study does not support an association between alcohol intake, alcohol metabolism and breast cancer risk. While sibling-based analyses offer some advantages over other studies in terms of reducing the potential bias from population stratification, for risk factors of modest magnitude and where sisters may be similar in behavior such as alcohol consumption, sibling-based analyses may be underpowered to detect differences

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