Published in final edited form as:
Alcohol Clin Exp Res. 2007 March ; 31(3): 467–476. doi:10.1111/j.1530-0277.2006.00334.x.

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

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Abstract

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

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

Results—Among drinkers, the median intake of total alcohol was 13 grams per week (10^{th} to 90^{th} percentiles; 4.5 – 135.9) in cases and 18 grams per week (10^{th} to 90^{th} percentiles; 4.5–104.1) in controls. Women who drank alcohol tended to be at an increased risk of developing breast cancer compared to those who did not drink (O.R. =1.40, 95% CI 0.97, 2.03), particularly those who were pre-menopausal at the time of breast cancer diagnosis (OR = 2.69, 95% CI: 1.00, 7.26). Of the known functional alleles, breast cancer risk was not significantly increased among carriers of at least one *ADH1C*1* or *ADH1B*2* allele, when compared to those heterozygous or homozygous for either the *ADH1C*2* or *ADH1B*1* allele. However, breast cancer risk tended to be lower among women who inherited the *ADH1B*896G* allele (O.R. = 0.62, 95%CI 0.37,_ 1.04). Haplotype frequencies were not significantly different between cases and controls.

Conclusion—Low levels of alcohol are associated with a modest increase in breast cancer risk that is not altered by known functional allelic variants of the *ADH1B* and *1C* gene. The protective association conferred by the *ADH1B*896G* allele needs further evaluation.

Keywords

Alcohol dehydrogenase; genotypes; breast cancer

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Background

A number of epidemiological studies have demonstrated that regular alcohol intake may be associated with an increase in the incidence of breast cancer in women {Hamajima,N. 2002; Smith-Warner,S.A. 1998; Tjonneland,A. 2003; Willett,W.C. 1987;} with some evidence that there is a dose-response relationship independent of beverage type {Singletary,K.W. 2001;}. A pooled analysis of six prospective studies in Europe, Canada and the United States, reported a 9% increase in the risk of breast cancer for every 10 gram (0.75-1 drink) increase in alcohol intake per day {Smith-Warner,S.A. 1998;}. However, a consistent increase in the risk of breast cancer has not been reported at lower consumption levels {Kropp,S. 2001; Petri,A.L. 2004; Smith-Warner,S.A. 1998;}. In animal models ethanol intake has been demonstrated to cause mammary tumors {Singletary,K.W. 1991;}.

Despite evidence of a positive association between alcohol intake and breast cancer among humans and animals, the underlying biological mechanisms have yet to be clearly defined. In humans, 80% of ethanol is primarily oxidized to acetaldehyde by alcohol dehydrogenase (ADH) in the liver {Bosron,W.F. 1986;}. Acetaldehyde has been shown to directly cause DNA damage by: the formation of adducts; single and double strand breaks; cross-links and protein cross-links; and the inhibition of DNA repair {Grafstrom,R.C. 1994; Kuykendall,J.R. 1994; Ristow,H. 1995; Vaca,C.E. 1995;}. Chronic alcohol consumption can induce the cytochrome P450 enzymes, in particular CYP2E1, which assists in the conversion of alcohol to acetaldehyde {Gonzalez,F.J. 2005;}. CYP2E1 is also involved in the metabolism of various pro-carcinogens to carcinogens {Gonzalez,F.J. 2005;}. Further, both CYP2E1 and xanthine oxidoreductase (XOR), an enzyme involved in the metabolism of acetaldehyde to acetate, generate reactive oxygen species that have been implicated in carcinogenesis {McManaman,J.L. 1999; Wright,R.M. 1999;}). In MCF-7 breast cancer cell lines, alcohol at moderate doses down-regulates mRNA expression and protein levels of BRCA1, a known breast cancer tumor suppressor gene, while stimulating estrogen receptor expression {Fan,S. 2000;}. Moderate alcohol intake has also been associated with increased levels of circulating estrogens and DHEAS, and decreased levels of sex hormone binding globulin (SHBG) in pre and postmenopausal women {Dorgan,J.F. 1994; Garcia-Closas,M. 2002; Hankinson,S.E. 1995; Hines,L.M. 2000; Reichman,M.E. 1993;} Sjerksma et al 2004,

There are at least five different classes of human ADH isoenzymes based on differences at the molecular level {Bosron,W.F. 1986; Smith,M. 1973;}. Class I ADH polypeptide subunits hybridize to form homo and heterodimers that are encoded by three specific gene loci, *ADH1A* (alpha), *ADH1B*(beta) and *ADH1C* (gamma), previously known as *ADH1, ADH2* and *ADH3*, respectively. These loci are in close proximity to one another. Functional differences in the kinetic and catalytic properties of the gamma subunit with respect to alcohol metabolism *in vivo*, led to the identification of two single nucleotide polymorphisms (SNPs) in the *ADH1C* gene--Valine349Isoleucine at exon 8; and Arginine 271 Glutamine at exon 6 {Hoog,J.O. 1986;}. Similarly a functional SNP in the *ADH1B* gene (previously known as *ADH2*) has been identified as a result of alterations in the beta subunit-- Histidine 47Arginine at exon 3 {Hurley,T.D. 1990; Matsuo,Y. 1989;}.

One prospective study and three case-control studies have examined the association between the *ADH1C*1* allele, alcohol intake and the risk of breast cancer with conflicting results {Coutelle,C. 2004; Freudenheim,J.L. 1999; Hines,L.M. 2000; Terry,M.B. 2005;}. Two published studies, a case series, and a case-control study, have reported a protective association between alcohol drinkers who were also carriers of the *ADH1B*2* variant and the development of breast cancer {Lilla,C. 2005; Sturmer,T. 2002;}. Using prospectively gathered data, our aims were to extend these recent analyses by conducting a nested case-control study to examine the association of alcohol consumption, 5 single nucleotide polymorphisms of the *ADH1C* and

ADH1B genes and the risk for breast cancer. These polymorphisms include three known functional variants of the *ADH1C* and *ADH1B* genes and two that are common in Caucasians but not associated with an amino acid change.

METHODS

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

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

Laboratory Assays and Genotyping

Plasma, buffy coat, and red blood cells were separated and stored at −70 degrees °C within 24 hours of collection. The alkaline lysis method was used to extract DNA from peripheral buffy coat {Klintschar,M. 2000;}. DNA concentration was set at 100 μg/ml. The *ADH1CEx8-56A>G* – rs 698, *ADH1CEx6-14G>A* -rs1693482, *ADH1C IVS6 +10G>A* - rs 1789912, and the *ADH1B Ex3-+23 A >G – rs 1229984*, *ADH1BIVSI +896A>G* - rs 1353621 were assessed using the Taqman® or 5'nuclease assay (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). Previously 217 samples, 106 controls and 111 cases were genotyped for the *ADH1C+56A>G* polymorphism using a modified version of the PCR/RFLP method of Groppi et al. {Groppi,A. 1990;}. There was 98% concordance between the 53 samples that had been analyzed by PCR and Taqman. A prior PCR/RFLP result was used for those 93 cases

and 70 controls that were unable to be genotyped by Taqman for the *ADH1C1+56A>G* polymorphism.

Statistical Analysis

Differences in the distribution of demographic, lifestyle and breast cancer risk factors were compared between cases and controls using chi-squared tests for categorical variables and t-tests for continuous variables. Odds ratios (OR) and 95% Confidence Intervals (CI) for the association between these factors and breast cancer were also calculated using conditional logistic regression. Wine, beer, and liquor intake were converted to grams/week based on information from the USDA national nutrient database (a 12-ounce can of beer is equal to 13 grams of ethanol, 1 medium glass of table wine has 9.6 grams of ethanol and 1 shot of liquor has 14 grams of ethanol) {USDA National Nutrient Database 2005;}. Total alcohol intake was calculated based on the sum in grams per week of wine, beer, and liquor intake for each individual. The median, 10^{th} and 90^{th} percentiles for total alcohol intake were calculated for cases and controls. Given the narrow distribution of drinkers, information on wine, beer and liquor was condensed to 2 categories: nondrinkers and drinkers.

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

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

Hardy Weinberg equilibrium was assessed for each genotype based on the frequency of the alleles in control groups using chi-square tests. Pearson correlation coefficients were calculated between each pair of genotypes. Known functional genotypes *ADH1C*2* and *ADH1B*1* were designated as slow alleles and *ADH1C*1* and *ADH1B*2* as fast alleles based on *in vitro* data {Bosron,W.F. 1986; Hurley,T.D. 1990;}. In assessing the association between genotypes and breast cancer, the reference group was defined as women with no fast alleles. Women homozygous and heterozygous for the fast alleles were assessed separately and then also combined into one category. For the other two SNPs, *ADH1C+10G>A* and *ADH1B +896A>G*, the most prevalent homozygous alleles were used as the reference group. To assess

the combined effect of *ADH* genotype and alcohol on breast cancer risk new variables were created with the reference group being nondrinkers homozygous for the slow allele. Effect modification by genotype, menopausal status, and BMI of the estimated ORs was assessed by the statistical significance of the product term in the logistic regression model.

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

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

RESULTS

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

Fifty-two percent of cases and 58% of controls did not drink. Among those women who drank alcohol the median consumption was 13.0 grams per week for cases (10^{th} to 90^{th} percentiles; 4.5 –135.9) and 18 grams per week (10^{th} to 90^{th} percentiles; 4.5–104.1) for controls. Among controls alcohol intake varied by education, but not by age at baseline or BMI. Women with a 12^{th} grade education or better were more likely to drink alcohol than those with less (32% versus 18%). Women who drank alcohol were at an increased risk of breast cancer compared to those who did not (O.R. = 1.40, 95% CI 0.97, 2.03) (table 2). When stratified by menopausal stage at breast cancer diagnosis, the odds of developing breast cancer was 2.69 (95% CI 1.00, 7.26) in women $\leq$ 51 years of age who drank alcohol, relative to non-drinkers, and 1.25 (95% CI 0.84, 1.87) among older female drinkers > 51 years compared to non-drinkers (table 2). The interaction was not statistically significant (p = 0.16). Similar results were obtained when the cut point of $\leq$ 55 years was used (data not shown).No significant associations were observed between alcohol intake and estrogen or progesterone hormone receptor status (table 2) or grade of tumor (data not shown). When stratified by education, a significant association between

total alcohol intake and breast cancer risk was only observed among women drinkers with ≥ 12th grade education compared to non-drinkers (O.R. = 1.49, 0.99, 2.24).

The association between different types of alcohol and breast cancer risk was also examined. Women who drank wine were 1.6 times more likely to develop breast cancer than non-wine drinkers (O.R. = 1.60, 95%CI 1.01, 2.54). However, no association was observed for women who drank beer (O.R. = 0.95, 95%CI 0.56, 1.63) or liquor (O.R. =1.10, 95%CI 0.65, 1.86).

The genotype distribution among the control subjects reflect frequencies previously reported for the SNPs (http://snp500cancer.nci.nih.gov) and all were in Hardy-Weinberg equilibrium. Fifteen percent of cases and eleven percent of controls were missing information on all 5 genotypes. A Pearson correlation coefficient of 0.8 was observed between *ADH1C+56A>G* and *ADH1C+14G>A*. Carriers of at least one *ADH1C *1(+56A)* allele were not at significantly higher risk of developing breast cancer than women homozygous for the *ADH1C*2 (+56G)* allele in a multivariate analysis adjusted for matching factors (O.R. = 1.16, 95%CI 0.77, 1.76). Results of a similar magnitude were observed for carriers of at least one *ADH1C*1(+14A)* allele (O.R. = 1.23, 95%CI 0.73, 2.07) or at least one *ADH1B*2* (*+23G)* allele (O.R. = 1.55, 95%CI 0.68, 3.56) (table 3). However, women with at least one *ADH1B+896G* allele had a reduced risk of developing breast cancer when compared to women with the ADH1B+896A allele (table 3). The presence of an *ADH1C+10A* allele did not confer any additional breast cancer risk (table 3).

Power was limited to assess gene-gene interactions. Exploratory analyses revealed no significant associations. Five haplotypes were identified among the five SNPs. Overall, the difference between cases and controls was not statistically significant for either the global test or individual haplotypes.

Table 4 reports on the association between *ADH* genotype status, alcohol intake and the risk of breast cancer. A non-statistically significant increase in breast cancer risk was consistently observed among carriers of at least one *ADH1C*1* or *ADH1B*2* allele who drank alcohol when compared to women homozygous for the *ADH1C*1, ADH1B*2* allele (table 4). The interaction between genotype and alcohol intake with respect to breast cancer risk for each association was not statistically significant. Women who inherited at least one ADH1B+896G allele and were nondrinkers were at decreased risk of developing breast cancer compared to drinkers homozygous for the ADH1B+896A alleles, although the interaction did not meet criteria for statistical significance (table 4). The association between the *ADH1C+10G>A* genotype and breast cancer risk did not vary by alcohol intake (table 4).

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

DISCUSSION

In this prospective study, we observed a small but statistically significant increase in the risk of breast cancer only among pre-menopausal women who would be considered light to moderate drinkers. The presence of functional variants of the *ADH1C* or *ADH1B* gene, known to increase ADH activity *in-vitro*, did not modify this association. However, the presence of at least one *ADH1B+896G* allele was observed to significantly reduce breast cancer risk when compared to women homozygous for the *ADH1B +896A* allele.

Three other prospective studies have reported increased breast cancer risk of a similar magnitude among light to moderate drinkers, two of which included pre-menopausal women {Friedenreich,C.M. 1993; Holmberg,L. 1994; van den Brandt,P.A. 1995;}. In one study, a dose response was observed only among pre-menopausal women (p trend = 0.07). Similar results were also reported in a large case-control study where information of lifetime alcohol intake was collected {Freudenheim,J.L. 1999;}. The biological mechanism behind a possible difference in breast cancer risk from alcohol consumption based on menopausal status is unclear. Age related differences in ADH and CYP2E1enzyme activity in breast tissue is a possible explanation since such changes in enzyme activity have been observed in gastric tissue and blood {Bebia,Z. 2004; Moreno,A. 1994; Pozzato,G. 1995;}.

The evaluation of the functional variants in the *ADH1C* gene, alcohol intake and breast cancer risk was based on sound biological rationale from *in vitro* studies that reported differences in ADH enzyme activity arising from modifications in the gamma and beta polypeptide subunits. *In vitro*, the gamma-1 or gamma-2 polypeptide subunits encoded by the *ADH1C*1* allele metabolize alcohol to acetaldehyde two and half times faster than the gamma *ADH1C*2* allele {Bosron,W.F. 1986;}. Further these enzymes have been detected in breast epithelial cells where 85% of breast cancer originate {Jelski,W. 2006; Triano,E.A. 2003;}. In three case-control studies, two that measured lifetime intake {Freudenheim,J.L. 1999; Terry,M.B. 2005;}, the *ADH1C*1* allele has been shown to significantly modify the association of alcohol and breast cancer particularly in pre-menopausal women {Coutelle,C. 2004; Freudenheim,J.L. 1999; Terry,M.B. 2005;}. In all three studies, the risk of breast cancer was at least 2 fold greater among women homozygous for the *ADH1C*1* allele who drank alcohol, compared to nondrinkers. These results were not reproduced in a prospective study of 465 incident breast cancer cases and 621 controls {Hines,L.M. 2000;}. Using nondrinkers as the reference group, they observed a small increase in breast cancer risk among women who drank alcohol greater than or equal to 10 grams per day (O.R. = 1.1, 95% CI 0.7, 1.6) that was unchanged by *ADH1C* genotype {Hines,L.M. 2000;}. The lack of association seen in our study and that by Hines et al. may reflect the relatively low level of alcohol intake reported by women in these studies. Epidemiological studies in other cancers such as head and neck suggest that functional variants of ADH may only modify cancer risk among heavy drinkers and not among light drinkers {Harty,L.C. 1997; Olshan,A.F. 2001;} Schwartz et al. 2001). An alternate explanation for the modest breast cancer risk observed at low levels of alcohol intake may be due to reported elevations in circulating endogenous hormones such as estradiol and DHEAS {Dorgan,J.F. 1994; Hines,L.M. 2000;}. The strong correlation observed and the lack of synergistic effect between the two functional polymorphisms in the *ADH1C* gene is consistent with recent re-sequencing (http://egp.gs.washington.edu/data/adh1c/) that supports the likelihood that these two genotypes are in linkage disequilibrium {Edman,K. 1992;}.

Few studies have examined the association between the functional *ADH1B*2* variant and cancer in Caucasians because of its low prevalence. *In vitro*, the beta-1 polypeptide subunit, a product of the *ADH1B*2* allelic variant, oxidizes ethanol 100 times faster than products of the *ADH1B*1* variant {Hurley,T.D. 1990;}. In a case-control study of German women, a reduction in breast cancer risk was reported in carriers of the *ADH1B*2* variant who on average consumed 12 or more grams of alcohol per day {Lilla,C. 2005;}. A protective association was also reported in a case-only study of 274 women with invasive breast cancer {Sturmer,T. 2002;}. These results were not replicated in our study but we did confirm the low prevalence of the *ADH1B*2* variant among Caucasians women {Brennan,P. 2004; Lilla,C. 2005;}. In Asians, the presence of the ADH1B*2 variant indirectly limits their alcohol consumption due to toxic side effects such as flushing produced by high levels of acetaldehyde {Borras,E. 2000;} Seitz et al 2001).

There are a number of possible explanations for the observed protective association between carriers of the *ADH1B+896G* genotype, a non-functional SNP located at intron 1, and breast cancer risk. The *ADH1B+896G* genotype may be in linkage disequilibrium with another known or yet to be identified functional SNP of the *ADH1B* gene or other genes in close proximity. Another possibility, although less likely, is that the intron 1 has a protective function of its own (ref).

Strengths of our study include the prospective collection of information on alcohol intake prior to the diagnosis of breast cancer (minimizing bias due to differential reporting by cases and controls), long term follow-up (up to 13 years) and the population-based study sample. In addition, the associations between other potential risk factors and breast cancer were comparable to published studies, suggesting good internal validity. Further, the similar results obtained from our imputed datasets suggest that significant bias was not introduced by the missing data. Limitations of our study include the large number of non-drinkers, limited sample size to analyze gene-gene interactions and alcohol intake data from a single time point.

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

Acknowledgments

We would like to acknowledge and thank all the participants of CLUE 2 and also Judith A. Hoffman Bolton, and Alyce Burke at the George W. Comstock Center for Public Health Research and Prevention. The analyses for this study were supported by a grant from the Susan G. Komen Cancer Foundation. Data collection and genotyping for this study was supported by grants from the National Cancer Institute (5U0a1CA/ES62988), the National Institute of Environmental Health Sciences (DHHS Grant ES060520 and the NIEHS Intramural Program. Dr. Visvanathan is a recipient of an American Society of Clinical Oncology (ASCO) Career Development Award and a KO7 Preventive Oncology Academic Award (Ca111948) from the National Cancer Institute.

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Table 1

Descriptive characteristics of breast cancer cases and controls, Washington County, MD

	Cases N =321 %	Controls N =321 %	OR[1](95% CI[2])
Age (yrs[3]) at baseline			
Mean (SD[4])	56.8 (12.4)	56.6 (12.3)	1.16 (0.98,1.37)
Race			
White	99	99	N/A
Black	1	1	
Other	<1		
BMI[5] at baseline (kg/m^2)			
< 25	42	50	1.0
25 – 29.9	34	31	1.39 (0.96,1.99)
≥ 30	24	19	1.60 (1.04,2.45)
			P trend = 0.02
Smoking			
Never	62	65	1.0
Former	25	20	1.27 (0.87,1.85)
Current	13	15	0.84 (0.52,1.36)
			P trend = 0.91
Education (grade)			
<12	24	29	1.0
=12	42	41	1.23 (0.82,1.84)
>12	34	30	1.39 (0.91,2.11)
>12	34	30	P trend = 0.13
Marital Status			
Never Married	5	5	1.0
Married now	73	68	1.09 (0.53,2.27)
Other	22	27	0.84 (0.39,1.79)
Missing		<1	P trend = 0.27
Ever pregnant			
No	12	8	1.0
Yes	68	68	0.61 (0.33,1.11)
Missing	20	24	
Age at first birth (yrs[3])			
Nulliparous	12	8	1.0
<20	18	17	0.61 (0.30,1.24)
20–24	32	31	0.62 (0.32,1.20)
25–29	15	14	0.71 (0.34,1.49)
≥ 30	3	5	0.41 (0.13,1.29)
Missing	20	25	P trend = 0.26
Months breast feeding			
None	43	40	1.0
1–6	9	10	0.87 (0.42,1.77)
>6	15	18	0.79 (0.45,1.41)
Missing	33	32	P trend = 0.43
Age at menarche (yrs[3])			
<12	13	15	1.0
12–13	48	39	1.80 (1.02,3.16)
>13	19	22	1.17 (0.63,2.16)
Missing	20	24	P trend = 1
Oral contraceptive use			
Never	74	75	1.0
Former	25	21	1.26 (0.80,2.00)
Current	1	3	0.57 (0.17,1.95)
Missing		1	P trend = 0.3
Other hormone use			
Never	79	78	1.0
Former	9	12	0.79 (0.46,1.35)
Current estrogen and/or progesterone use	11	8	1.30 (0.71,2.37)
Missing	1	2	P trend = 0.4
Maternal Family history (1st & 2nd degree relatives)			
No	63	71	1.0
Yes	20	9	2.32 (1.35,3.97)
Missing	17	20	
Menopausal status at baseline	26	29	1.0
Pre-menopausal	71	70	2.00 (0.37,10.9)
Post-menopausal	3	1	
Missing			

	Cases N =321 %	Controls N =321 %	OR[1](95% CI[2])

[1] OR = odds ratios that were calculated using conditional logistic regression.

[2] CI = confidence interval.

[3] yrs = years,

[4] SD= standard deviation.

[5] BMI = body mass index (kg/m^2) calculated from self reported height and weight measurements.

Table 2

Association between alcohol intake and breast cancer risk in Washington County, Maryland

Alcohol Intake	Cases N = 321	Median (grams/week) (10^{th}, 90^{th} ,pctile[1])	Controls N= 321	Median (grams/week) (10^{th}, 90^{th} ,pctile[1])	Adj. O.R.[2], 95% CI[3]
Total					
Non-drinkers	167	0	187	0	1.00 (ref)
Drinkers	95	13.0 (4.5, 135.9)	76	18.0 (4.5, 104.1)	1.40 (0.97,2.03)
Missing	59		58		
Pre-menopausal at BC[4] diagnosis					
Non-drinkers	26	0	35	0	1.00 (ref.)
Drinkers	15	6.5 (4.5, 106.8)	9	17.5 (4.5, 52.8)	2.69 (1.00,7.26)
Missing	9		8		
Post-menopausal at BC[4] diagnosis					
Non-drinkers	141	0	152	0	1.00 (ref.)
Drinkers	80	13.0 (4.5, 139.1)	67	18.5 (4.5, 166.3)	1.25 (0.84,1.87)
Missing	50		50		
					P interaction = 0.16
Estrogen receptor[5] negative					
Non-drinkers	25	0	30	0	1.00 (ref.)
Drinkers	19	11.0 (4.5, 105.8)	14	12.8 (4.5, 102.5)	1.84 (0.75,4.51)
Missing					
Estrogen receptor positive					
Non-drinkers	112	0	129	0	1.00 (ref.)
Drinkers	64	14.8 (4.5, 142.3)	48	23.5 (4.5, 174.1)	1.47 (0.93, 2.31)
Missing					
					P interaction = 0.75

[1] pctile = percentile.

[2] O.R. = Odds ratios, adjusted for matching factors (baseline menopausal status and age).

[3] CI = Confidence Interval.

[4] BC = breast cancer; women ≤ 51 were categorized as pre-menopausal and women > 51 as post-menopausal in women in whom menopausal status at diagnosis was unknown; 51 cases and controls missing information on estrogen receptor status and therefore the cases and controls do not add up to 321.

Table 3

Associations between *ADH* genotypes and breast cancer risk in Washington County, MD

Genotypes	Controls N=321	Controls N=321Adjusted OR[1] (95% CI[2])
Functional		
DH1C (Ex8-56A>G)		
2,2	50	601.00 (ref)
1,2	133	1371.14 (0.73,1.78)
1,1	120	1151.18 (0.75,1.86)
1,2/1,1	253	2521.16 (0.77,1.76)
Missing	18	9
		P trend = 0.50
ADH1C (Ex6-14G>A)		
2,2	29	391.00 (ref)
1,2	100	1051.24 (0.71,2.17)
1,1	98	991.21 (0.69,2.13)
1,2/1,1	198	2041.23 (0.73,2.07)
Missing	94	78
		P trend = 0.62
ADH1B (Ex3− +23 A >G)		
1,1	246	2701.00 (ref)
1,2	14	101.43 (0.62,3.34)
2,2	1	0N/A
1,2/2,2	15	101.55 (0.68,3.56)
Missing	60	41
		P trend = 0.24
Other		
ADH1C (IVS6 +10G>A)		
G,G	101	1011.00 (ref)
A,G	103	1001.03 (0.69,1.53)
A,A	48	350.79 (0.47,1.34)
A,G/A,A	151	1350.96 (0.66,1.38)
Missing	69	85
		P trend = 0.49
ADH1B (IVSI +896A>G)		
A,A	96	1061.00 (ref)
A,G	117	1080.87 (0.59,1.28)
G,G	52	330.62 (0.37,1.04)
A,G/G,G	169	1410.79 (0.55,1.14)
Missing	56	74
		P trend = 0.08

[1] O.R. = Odds ratio, adjusted for matching factors (baseline menopausal status and age).

[2] CI = confidence interval. The rs numbers used by the NCI SNP 500 database (http://snp500cancer.nci.nih.gov/home) are as follows; *ADH1CEx8-56A>G – rs 698, ADH1CEx6-14G>A* - rs 1693482, *ADH1B (Ex3- +23 A >G – rs 1229984, ADH1CIVS6 +10G>A* - rs 1789912, *ADH1BIVSI +896A>G* - rs 1353621.

Table 4

Association between *ADH* genotype status, alcohol intake and the risk of breast cancer, Washington County, MD.

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

[1] O.R. = Odds ratio, adjusted for matching factors (baseline menopausal status and age).

[2] C1 = confidence interval, r identifier used by the NCI SNP 500 data base http://snp500cancer.nci.nih.gov/home).