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ORIGINAL PAPER

Alcohol dehydrogenase 3 and risk of esophageal and gastric adenocarcinomas

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Abstract
Objectives Alcohol increases esophageal squamous carcinoma risk but has been less consistently associated with esophageal adenocarcinoma. Alcohol dehydrogenase catalyzes the oxidation of approximately 80% of ethanol to acetaldehyde, a carcinogen. The alcohol dehydrogenase gene has several polymorphisms which may lead to faster conversion of ethanol to acetaldehyde, which may increase cancer risk.
Methods We undertook a study to examine whether a common polymorphism in the alcohol dehydrogenase 3 gene was associated with a higher risk of esophageal adenocarcinoma using data and biological samples collected for the Esophageal and Gastric Cancer Study ($n = 114$ esophageal and gastric cardia adenocarcinoma, $n = 60$ non-cardia gastric carcinoma, $n = 23$ cases of esophageal squamous cell carcinoma and 160 controls).
Results Individuals homozygous for ADH_3^{1-1} had a higher risk of each tumor type compared to individuals who had ADH_3^{2-2} or ADH_3^{1-2} genotype (OR = 1.7, 95% CI = 1.0–2.9 for esophageal and gastric cardia adenocarcinomas; OR = 1.7, 95% CI = 0.7–4.3 for esophageal squamous cell carcinoma; and OR = 2.8, 95% CI = 1.5–5.1 for non-cardia gastric cancer). The elevation in risk from homozygosity of the ADH_3^1 allele was seen in drinkers and

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nondrinkers, although the risk estimate was only significant for drinkers, particularly of liquor.

Conclusion These data suggest *ADH3* genotype may be associated with risk of esophageal and gastric cardia adenocarcinomas.

Keywords Alcohol · Esophageal adenocarcinoma · Alcohol dehydrogenase

Introduction

The etiologic role of alcohol intake in esophageal squamous cell carcinoma is well established (reviewed in [1–4]). In contrast, little consistency has been found for esophageal adenocarcinoma, the rapidly increasing histologic subtype. While some earlier studies reported an increased risk in esophageal adenocarcinoma from alcohol consumption [5–9], three large studies found no elevated risk among drinkers [10–12]. As esophageal adenocarcinoma is now the most common subtype of esophageal cancer in the US and other western countries [13], it is important to clarify the potential role, if any, of modifiable risk factors such as alcohol consumption.

One mechanism of action for alcohol in carcinogenesis may be through its carcinogenic and mutagenic metabolites. Ethanol is metabolized to acetaldehyde within esophageal mucosa. Acetaldehyde induces sister chromatid exchange, mutations, and chromosomal aberrations in cell cultures and in human lymphocytes [1, 14, 15]. The metabolism of ethanol to acetaldehyde and then to acetic acid is determined by several enzyme systems which vary from person to person [1]. Of special interest is alcohol dehydrogenase, which catalyzes the oxidation of approximately 80% of ethanol to acetaldehyde. The alcohol dehydrogenase gene has several polymorphisms: ADH_2 polymorphisms have been primarily found among Asians, whereas ADH_3 polymorphisms are commonly seen among white, black, and Asian populations [16, 17]. Individuals homozygous for the ADH_3^1 allele have a 2–3 faster rate of conversion of ethanol to acetaldehyde than those individuals homozygous for the ADH_3^2 allele [18–20]. Individuals with ADH_3^{1-1}, ADH_3^{1-2}, and ADH_3^{2-2} are classified as fast, intermediate, and slow metabolizers, respectively. The nomenclature for ADH_3 has recently been changed to *ADH1C*, but for purposes of comparison with the previously published literature we will use the older nomenclature (ADH_3) [21].

We undertook a study to examine whether fast metabolizers of alcohol, defined by their alcohol dehydrogenase 3 (ADH_3^{1-1}) genotype, have a higher risk of esophageal adenocarcinoma using data and biological samples collected for the Esophageal and Gastric Adenocarcinoma (EGA) study. Most adenocarcinomas occur in the lower third of the esophagus and are similar to tumors of the gastric cardia with respect to many epidemiologic risk factors, so we grouped both esophageal and gastric cardia adenocarcinomas together (n = 114). For comparison, we also examined non-cardia gastric adenocarcinoma (n = 60), a small subset of esophageal squamous cell carcinoma (n = 23), and population-based controls (n = 160). The parent project did not observe an increased association between alcohol and esophageal adenocarcinoma or subsites of gastric adenocarcinoma [10]. However, we hypothesized that even in cases where no overall risks with alcohol intake are seen, adverse effects of alcohol may be evident when subjects are stratified by *alcohol dehydrogenase 3* genotype.

Materials and methods

Study population

The EGA study is a multi-site (the state of Connecticut, a 15-county area of New Jersey, and a three-county area of western Washington state) population-based case–control study [10]. Eligible cases were all English-speaking men or women between the ages of 30–79 newly diagnosed with invasive adenocarcinomas of the esophagus or gastric cardia ages 30–79 years identified in these location for the following time periods: 1 February 1993 through 31 January 1995 (Connecticut), 1 April 1994 through 30 November 1994 (New Jersey), and 1 March 1993 through 2 February 1995 in Washington. Cases of esophageal and gastric cardia adenocarcinoma were the target cases for this study. In addition, a sample of esophageal squamous cancer cases and non-cardia gastric cancer cases were selected for comparison, and were frequency-matched to the target cases by 5-year age groups and geographic location. They were additionally frequency-matched by sex and race in New Jersey, and by sex in Connecticut and Washington State. All case group classifications were confirmed by study pathologists based on reviews of slides, surgical notes, and medical records using uniform criteria. A population-based control group was selected using random digit dialing methods for subjects under 65 years of age, and Health Care Finance Administration rosters for subjects 65 years and over. Controls were frequency matched to the target cases using the same criteria that was used for the comparison cases. Of the eligible cases and controls, 80.6% of the target cases (esophageal adenocarcinoma (n = 293) and gastric cardia adenocarcinomas (n = 261)), 74.1% of the comparison cases (esophageal squamous (n = 221) and other non-cardia gastric cancer (n = 368)), and 73.7% of the controls (n = 695) were interviewed using in-person structured questionnaires; for approximately 30% of the cases, next of kin were interviewed because the case had died prior to interview or was too sick to participate.

Subjects from Washington and nine out of 15 participating counties in New Jersey were asked to provide 30-ml blood samples. Subjects from Connecticut were not asked to donate a blood sample. Samples were obtained from 114 cases with esophageal or gastric cardia adenocarcinoma; 60 with non-cardia gastric adenocarcinoma, 23 with squamous carcinomas cases, and 160 controls.

Genotyping

DNA isolated from blood cells was genotyped using template-directed primer extension with detection of incorporated nucleotides by fluorescence polarization in a 96 microwell-based format essentially as previously described [22]. All analyses were performed blinded to case–control status. Master DNA 96-well plates containing 10 ng/μl were used to make replica plates containing 25 ng DNA/well. For PCR amplification, the primers (forward 5′-CCC AAA CTT GTG GCT GAC TT-3′, reverse 5′-TCA CAC TTA CTT ATA TGA CAG GCA G-3′) gave a 493 bp product. Conditions for amplification were 0.2 μl (8 pmol/μl) forward and reverse primers, 0.4 μl 25 mM $MgCl_2$, 1 μl 10× PCR buffer, 0.1 μl (5 u/ml) Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN), 0.25 μl (10 mM) dNTPs (Roche), and 5.35 μl 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/μl, Roche) after addition of 1 μl of 10× buffer and 1 unit *E. coli* exonuclease I (10 u/μl, United States Biochemical, Cleveland, OH) and 7.9 μl 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 μl of reaction mixture was added 0.05 μl Acycloprimer enzyme, 1 μl G/A Terminator mix, 2 μl 10× reaction buffer, 0.5 μl extension primer (10 pmol/μl), and 9.45 μl 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.

Covariate assessment

During the in-person interview, respondents were queried about their alcohol intake for each type (beer, wine, hard liquor) separately [10]. Subjects who answered yes to drinking at least 12 alcoholic beverages of any kind in their lifetime were asked over 35 questions on alcohol consumption including age started and stopped (if applicable) drinking each type of beverage, periods of abstinence, total years drinking beverage, total years not drinking beverage, usual intake of beverage, usual unit of intake, and frequency of intake.

The EGA study interviewers also collected extensive information on a number of potential risk factors for esophageal and gastric cardia cancers including age, sex, race, income, years of education, tobacco use, body size, medication use, other beverage consumption (including coffee and tea intake), and a full dietary assessment. Tobacco consumption in terms of duration, intensity, and start and stop patterns, was queried separately for cigarettes, cigars, pipes, chewing tobacco, and snuff.

Statistical methods

We first compared differences between genotypes and esophageal cancer risk factors using the Chi-square test for categorical variables, and the Analysis of Variance test for continuous variables [23]. Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the main effects of genotype (ADH_3^{1-1} and ADH_3^{1-2} relative to ADH_3^{2-2}) on esophageal cancer risk [24]. Based on estimates for these three groups, we conducted further analyses combining ADH_3^{1-2} and ADH_3^{2-2} because of the similarity in effect estimates for these two groups. We also conducted further analyses examining *ADH* genotype as an ordered categorical variable assuming a dose-allele response and stratifying this order variable by alcohol consumption.

All models included the frequency-matching factors of age, gender, and geographic site. We also examined confounding by the following factors: race, BMI, education, smoking status, total caloric intake, and reflux disease. We compared the change in estimate for the exposure coefficient between statistical models with and without the potential confounder. Variables were kept in the final model if they altered the parameter estimates on the exposure by at least 10% [25].

Effect modification by genotype was examined through use of stratified analysis, running separate models for each subgroup, as well as by comparing the log-likelihood statistic for models that included a multiplicative interaction term in the logistic regression model to those without [24]. We evaluated additive interaction by using indicator terms for those with the genotype only, exposure only, and those with both the genotype and exposure of interest [25]. We also presented models which separately stratified by alcohol exposure status. We only examined effect modification for the cases of esophageal and gastric cardia adenocarcinoma relative to controls because the other tumors had too few cases for meaningful analyses.

Table 1 Allele and genotype frequency for ADH_3, among cases of esophageal and gastric cancers by tumor type in Connecticut, New Jersey, and western Washington State, 1993–1995

ADH3 genotype	Cases	Controls	Odds ratio[a] (95% CI)
Esophageal and gastric cardia adenocarcinoma cases (n = 114)			
Allele frequency (%)			
ADH_3^1	62.3%	53.8%	
ADH_3^2	37.7%	46.2%	
		χ^2 test $p = 0.22$	
Genotype			
ADH_3^{2-2}	17	33	1.0
ADH_3^{1-2}	52	82	1.2 (0.6–2.5)
ADH_3^{1-1}	45	45	2.0 (1.0–4.2)
$ADH_3^{1-2} + ADH_3^{2-2}$	69	115	1.0
ADH_3^{1-1}	45	45	1.7 (1.0–2.9)
Esophageal squamous cell carcinoma (n = 23)			
Allele frequency (%)			
ADH_3^1	60.9 %	53.8 %	
ADH_3^2	39.1 %	46.2 %	
		χ^2 test $p = 0.31$	
Genotype			
ADH_3^{2-2}	4	33	1.0
ADH_3^{1-2}	10	82	1.0 (0.3–3.3)
ADH_3^{1-1}	9	45	1.7 (0.5–5.9)
$ADH_3^{1-2} + ADH_3^{2-2}$	14	115	1.0
ADH_3 $^{1-1}$	9	45	1.7 (0.7–4.3)
Non-cardia gastric cancer (n = 60)			
Allele frequency (%)			
ADH_3^1	70.0%	53.8%	
ADH_3^2	30.0%	46.2%	
		χ^2 test $p = 0.02$	
Genotype			
ADH_3^{2-2}	7	33	1.0
ADH_3^{1-2}	22	82	1.3 (0.5–3.3)
ADH_3^{1-1}	31	45	3.3 (1.3–8.5)
$ADH_3^{1-2} + ADH_3^{2-2}$	29	115	1.0
ADH_3^{1-1}	31	45	2.8 (1.5–5.1)

[a] ORs are adjusted for age, gender, and geographic site

Results

Allele and genotype frequencies for *ADH3* are reported in Table 1. The odds ratio for esophageal and gastric cardia adenocarcinoma was increased 2-fold for individuals with ADH_3^{1-1} relative to ADH_3^{2-2} genotype (OR = 2.0, 95% CI = 1.0−4.2). Increased risk for ADH_3^{1-1} genotype was similar for esophageal adenocarcinoma (OR = 2.2, 95% CI = 0.8−6.0) and gastric cardia adenocarcinoma (OR = 1.8, 95% CI = 0.7–4.3). ADH_3^{1-1} genotype was also associated with esophageal squamous cell carcinoma (OR = 1.7, 95% CI = 0.5–5.9) and for non-cardia gastric adenocarcinomas (OR = 3.3, 95% CI = 1.3–8.5) relative to ADH_3^{2-2} genotype. The genotypes among controls were in Hardy–Weinberg equilibrium (chi-square value = 0.96). Table 1 also presents the odds ratios for individuals with ADH_3^{1-1} genotype compared with individuals with ADH_3^{1-2} or ADH_3^{2-2}, as the latter two groups were more similar with respect to cancer risk.

Table 2 summarizes associations between genotype and various descriptive factors including age, geographic site, gender, race, body mass index (BMI), total caloric intake, smoking status, and alcohol consumption. Non-white subjects were more likely to be ADH_3^{1-1} genotype, there were no individuals with ADH_3^{2-2} among this group. None of the other characteristics and risk factors differed materially by genotype status. Alcohol consumption was not different between those who had genotyping data available and those that did not (*p*-value comparing ever/never by type between those with genotype and those without for beer $p = 0.13$, for wine $p = 0.89$, and for liquor $p = 0.90$).

Table 2 Characteristics of different alcohol dehydrogenase 3 (*ADH3*) status among controls

Characteristics	ADH_3^{1-1} (n = 45)	ADH_3^{1-2} (n = 82)	ADH_3^{2-2} (n = 33)	p-value
Age (years) ($\mu \pm$ SD)	61.76 ± 11.13	63.51 ± 9.69	63.00 ± 12.88	0.68
Site				
Washington	15 (33.33)	34 (41.46)	12 (36.36)	0.65
New Jersey	30 (66.67)	48 (58.54)	21 (63.64)	
Gender				
Men	35 (77.78)	71 (86.59)	28 (84.85)	0.43
Women	10 (22.22)	11 (13.41)	5 (15.15)	
Race				
White	38 (84.44)	80 (97.56)	33 (100.00)	0.02[a]
Black	3 (6.67)	1 (1.22)	0 (0.00)	
Others	4 (8.89)	1 (1.22)	0(0.00)	
BMI (kg/m^2)	24.27 ± 2.79	25.31 ± 3.34	26.00 ± 3.20	0.05
Total caloric intake (kilokcal/day) (M ± SD)	1.87 ± 0.60	1.88 ± 0.69	1.89 ± 0.71	0.99
Smoking status				
Current smokers	12 (26.67)	23 (28.05)	7 (21.21)	0.10
Ex-smokers	17 (37.78)	31 (37.80)	21 (63.64)	
Non-smokers	16 (35.56)	28 (34.15)	5 (15.15)	
Alcohol				
Never	12 (26.67)	11 (13.41)	7 (21.21)	0.17
Ever	33 (73.33)	71 (86.59)	26 (78.79)	
Beer				
Never	20 (44.44)	28 (34.15)	15 (45.45)	0.38
Ever	25 (55.56)	54 (65.85)	18 (54.55)	
Wine				
Never	31 (68.89)	44 (53.66)	20 (60.61)	0.24
Ever	14 (31.11)	38 (46.34)	13 (39.39)	
Liquor				
Never	22 (48.89)	34 (41.46)	17 (51.52)	0.54
Ever	23 (51.11)	48 (58.54)	16 (48.48)	

[a] Fisher's exact test

Table 3 reports the genotype associations for esophageal and gastric cardia adenocarcinomas according to alcohol intake. Panel A summarizes models among all individuals grouping the individuals with ADH_3^{1-2} or ADH_3^{2-2} genotype together. Individuals with ADH_3^{1-1} genotype had an elevated risk of 60–70% of esophageal and gastric cardia adenocarcinoma relative to those with ADH_3^{1-2} or ADH_3^{2-2} genotype irrespective of whether they drank beer or wine. However, individuals with ADH_3^{1-1} genotype who drank liquor had an increased risk (OR = 3.1, 95% CI = 1.3–7.3) that was higher than the additive effects from genotype (OR = 1.6, 95% CI = 0.7–4.0) and liquor drinking (OR = 1.1, 95% CI = 0.6–2.3). There were no statistically significant multiplicative interactions (beer p = 0.46, wine p = 0.44, liquor p = 0.39).

Panel B summarizes the genotype associations with esophageal and gastric cardia adenocarcinoma risk when stratified by drinking status. In these models, we treat *ADH3* genotype as an ordered categorical variable to examine whether there is a dose-allele effect. When restricted to drinkers only, individuals with ADH_3^{1-2} had a 2-fold (OR = 2.1, 95% CI = 1.3–3.3) and individuals with ADH_3^{1-1} had over a 4-fold (OR = 4.3, 95% CI = 1.7–11.2) increase in risk compared to those with ADH_3^{2-2} genotype. Similar patterns were observed by type of alcohol (beer, wine, liquor). However, nondrinkers who had ADH_3^{1-1} genotype also had an elevated, though not statistically significant, increase in risk (OR = 2.0, 95% CI = 0.2–18.4) and tests for multiplicative interactions with alcohol were not statistically significant (beer p = 0.56, wine p = 0.52, liquor p = 0.61, any alcohol p = 0.22). The overall inference did not change did not materially change when we restricted the analyses to white subjects, but the point estimates were slightly higher for all comparisons (see Column 6 in Table 3).

We further explored genotype and exposure interactions by intensity and duration of alcohol consumption. These

Table 3 Adjusted[a] ORs and 95% CI for esophageal and gastric cardia adenocarcinoma in relation to alcohol consumption

	Genotype	Cases EA + GCA	Controls	OR (95% CI)[a]	Among whites only OR (95% CI)[a]
Panel A: Exposure and genotype associations relative to common referent					
Beer					
Never	ADH_3^{2-2} + ADH_3^{1-2}	26	43	1.0	1.0
	ADH_3^{1-1}	16	20	1.7 (0.7–4.2)	1.9 (0.7–4.8)
Ever	ADH_3^{2-2} + ADH_3^{1-2}	43	72	0.7 (0.3–1.5)	0.7 (0.3–1.4)
	ADH_3^{1-1}	29	25	1.8 (0.8–4.3)	2.1 (0.9–5.1)
Wine					
Never	ADH_3^{2-2} + ADH_3^{1-2}	48	64	1.0	1.0
	ADH_3^{1-1}	29	31	1.8 (0.9–3.9)	2.3 (1.0–5.0)
Ever	ADH_3^{2-2} + ADH_3^{1-2}	21	51	0.6 (0.3–1.2)	0.7 (0.3–1.4)
	ADH_3^{1-1}	16	14	1.8 (0.7–4.4)	2.0 (0.8–5.1)
Liquor					
Never	ADH_3^{2-2} + ADH_3^{1-2}	28	51	1.0	1.0
	ADH_3^{1-1}	16	22	1.6 (0.7–4.0)	2.0 (0.8–5.0)
Ever	ADH_3^{2-2} + ADH_3^{1-2}	41	64	1.1 (0.6–2.3)	1.3 (0.6–2.8)
	ADH_3^{1-1}	29	23	3.1 (1.3–7.3)	4.1 (1.7–10.1)
Panel B: Ordinal genotype associations stratified by drinking status[b]					
Nondrinkers	ADH_3^{2-2}	5	7	1.0	1.0
	ADH_3^{1-2}	13	11	1.4 (0.5–4.3)	1.7 (0.6–5.4)
	ADH_3^{1-1}	8	12	2.0 (0.2–18.4)	3.0 (0.3–27.3)
Drinkers	ADH_3^{2-2}	12	26	1.0	1.0
	ADH_3^{1-2}	39	71	2.1 (1.3–3.3)	2.2 (1.4–3.5)
	ADH_3^{1-1}	37	33	4.3 (1.7–11.2)	4.8 (1.8–12.2)
Beer drinkers	ADH_3^{2-2}	10	18	1.0	1.0
	ADH_3^{1-2}	33	54	2.0 (1.2–3.5)	2.2 (1.3–4.0)
	ADH_3^{1-1}	29	25	4.1 (1.3–12.3)	5.0 (1.6–15.2)
Wine drinkers	ADH_3^{2-2}	6	13	1.0	1.0
	ADH_3^{1-2}	15	38	2.1 (1.0–4.3)	2.3 (1.1–4.9)
	ADH_3^{1-1}	16	14	4.2 (1.0–18.6)	5.1 (1.1–22.9)
Liquor drinkers	ADH_3^{2-2}	10	16	1.0	1.0
	ADH_3^{1-2}	31	48	1.7 (1.0–2.9)	2.0 (1.1–3.5)
	ADH_3^{1-1}	29	23	3.0 (1.0–8.6)	3.9 (1.3–11.7)

[a] ORs are adjusted for age, geographic site, gender, race, BMI, education, smoking status, total caloric intake, reflux disease and other type of alcohol consumption.

[b] Estimated by treating *ADH3* genotype as an ordered categorical variable.

analyses were limited by sample size constraints, but generally supported the overall conclusion from Table 3 that point estimates for individuals with ADH_3^{1-1} had a positive association with esophageal and gastric adenocarcinoma risk irrespective of drinking status (data not shown).

Discussion

Our study, while small, is the first to examine, the role of alcohol dehydrogase genotype and risk of esophageal adenocarcinoma. The few studies addressing whether the association between alcohol dehydrogenase genotype and esophageal cancer were conducted using only esophageal squamous carcinomas [26–28]. As in most previous investigations of esophageal and gastric adenocarcinoma, our parent study did not identify a carcinogenic effect from drinking alcohol [10]. However, we hypothesized that even in cases where no overall risks with alcohol intake are seen, adverse effects of alcohol may be evident when subjects are stratified by *alcohol dehydrogenase 3* genotype.

We observed a 2-fold increase in risk of esophageal and gastric cardia adenocarcinoma risk among individuals homozygous for the fast metabolizing *ADH3* genotype compared to those with the slow metabolizing genotype. Overall, the increase in effect size was seen in drinkers and nondrinkers, but the results were only statistically significant among categories of drinkers. For example, liquor drinkers with the fast metabolizing *ADH3* genotype had a 3-fold increase in esophageal and gastric cardia adenocarcinoma risk relative to nondrinkers with intermediate or

slow metabolizing *ADH3* genotype. When treating the *ADH3* genotype as an ordinal categorical variable, thereby assuming a dose-allele response, the increase in risk was 2-fold among intermediate metabolizers and over 4-fold among fast metabolizers compared to slow metabolizers. Exploratory analyses by more detailed measures of alcohol consumption also supported the overall conclusion that genotype was associated with outcome irrespective of exposure level, though results were statistically significant among liquor drinkers. We did not examine gene environment interactions for esophageal squamous cell and non-cardia gastric cancers because of sample size constraints.

While there was some suggestion that subgroups of drinkers had statistically significant associations between *ADH3* genotype and esophageal and gastric adenocarcinomas, the point estimates for genotype and cancer associations were similar between drinkers and nondrinkers and the tests for statistical interaction were insignificant. Until replicated using samples from a much larger study, these data are more consistent with concluding an association for genotype irrespective of exposure. It has been suggested that examining the main effects of genotype may even proxy for exposures that may be differentially recalled, in this case alcohol [29, 30]. We found no association, however, between alcohol consumption and *ADH3* genotype in this study so it is unlikely that association between genotype and cancer risk in the nondrinkers is from misclassification of drinking status with drinkers in the nondrinking category.

Alternative explanations for our findings should be considered. Even though the percentage of subjects with available blood samples was low, it is unlikely that selection bias can explain our findings as we have no reason to believe that genotype status is related to survival or affected participation in the study. Further, there were no differences in reported beer, wine, liquor, or drinking intensity between those who had blood samples available for genotyping and those who did not. For recall bias to be a likely explanation for our findings, the reporting of alcohol intake would have to vary by genotype, which is unlikely to have occurred. We were also able to assess confounding by a number of variables included in the main study questionnaire. For an unmeasured confounder to explain the results we found, it would have to be differentially distributed across genotype-exposure strata.

Overall, our study supports an association between *alcohol dehydrogenase 3* genotype and risk of esophageal and gastric adenocarcinoma. The size of effect estimates between *ADH3* genotype and esophageal and gastric adenocarcinoma were similar between drinkers and nondrinkers but were statistically significant only among drinkers. Larger studies are needed to understand the role, if any, alcohol-metabolizing genes may play in shaping risk of esophageal and gastric adenocarcinomas.

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