

Alcohol Dehydrogenase 3 Genotype and Risk of Oral Cavity and Pharyngeal Cancers

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Background: The consumption of alcoholic beverages is a strong risk factor for cancers of the oral cavity and pharynx (oral cancers). Alcohol dehydrogenase type 3 (ADH₃) metabolizes ethanol to acetaldehyde, a carcinogen. We evaluated whether individuals homozygous for the fast-metabolizing ADH₃¹ allele (ADH₃¹⁻¹) have a greater risk of developing oral cancer in the presence of alcoholic beverage consumption than those with the slow-metabolizing ADH₃² allele (ADH₃¹⁻² and ADH₃²⁻²). **Methods:** As part of a population-based study of oral cancer conducted in Puerto Rico, the ADH₃ genotypes of 137 patients with histologically confirmed oral cancer and of 146 control subjects (i.e., individuals with no history of oral cancer) were determined by molecular genetic analysis of oral epithelial cell samples. Risks were estimated by use of multiple logistic regression analyses. **Results:** Compared with nondrinkers with the ADH₃¹⁻¹ genotype, consumers of at least 57 alcoholic drinks per week with the ADH₃¹⁻¹, ADH₃¹⁻², and ADH₃²⁻² genotypes had 40.1-fold (95% confidence interval [CI] = 5.4–296.0), 7.0-fold (95% CI = 1.4–35.0), and 4.4-fold (95% CI = 0.6–33.0) increased risks of oral cancer, respectively; the risk associated with the ADH₃¹⁻¹ genotype, compared with the ADH₃¹⁻² and ADH₃²⁻² genotypes combined, was 5.3 (95% CI = 1.0–28.8) among such drinkers. Considering all levels of alcohol consumption, the risk of oral cancer per additional alcoholic drink per week increased 3.6% (95% CI = 1.9%–5.4%) for subjects with the ADH₃¹⁻¹ genotype and 2.0% (95% CI = 0.9%–3.0%) for subjects with the ADH₃¹⁻² or ADH₃²⁻² genotype (two-sided *P* = .04). **Conclusions:** The ADH₃¹⁻¹ genotype appears to substantially increase the risk of ethanol-related oral cancer, thus providing further evidence for the carcinogenicity of acetaldehyde. [J Natl Cancer Inst 1997; 89:1698–1705]

The incidence of oral cavity and pharyngeal ("oral") cancers among Puerto Rican men is among the highest in the Western hemisphere (1). As in most Western populations (2–8), alcohol drinking and tobacco smoking are the major risk factors for oral cancer in Puerto Rico (9). Heavy use of alcoholic beverages or tobacco substantially increases the risk of oral cancer (ninefold and fourfold, respectively, in one large U.S. study), with risks surpassing 35-fold among those with both behaviors (2).

The mechanism by which alcoholic beverages cause oral cancer have not been conclusively identified. Ethanol and its metabolites, specifically acetaldehyde, might be directly responsible, although free-radical damage from these compounds (10)

or contaminants of alcoholic beverages (e.g., *N*-nitrosamines and urethane) also may play a role. In short-term cell culture assays, including those that use human cells, acetaldehyde (but not ethanol) causes mutations and other DNA damage (11–16). Acetaldehyde forms adducts with DNA *in vitro* and *in vivo* (17,18), initiates the transformation of rat kidney cells (19), and inhibits DNA repair (13,20). Although not commonly tested, acetaldehyde appears to be a nasal tract carcinogen when it is inhaled by laboratory rodents (21). Taken together, these findings indicate that acetaldehyde might be the carcinogenic agent associated with alcohol drinking.

Ethanol is primarily (80%) oxidized to acetaldehyde by alcohol dehydrogenase (ADH), but it is also oxidized to a lesser extent by cytochrome P45011E1 (22). There are several ADH subtypes, some of which have genetic variants with altered kinetic properties (23). ADH type 3 (ADH₃) is polymorphic in Caucasians, and enzymes encoded by the ADH₃¹ allele metabolize ethanol to acetaldehyde 2.5 times faster than those encoded by the ADH₃² allele (23). Most ethanol oxidation occurs in the liver, although such activity is also present in the oral cavity and other digestive tract tissues (24–29).

In this study, we evaluate whether the ADH₃ genetic polymorphism modifies the risk of alcohol-related oral cancer.

Subjects and Methods

Study Subjects

These data are part of a population-based, case-control study conducted in Puerto Rico to investigate the risk factors for oral cancer. Through the Puerto Rico Central Cancer Registry and island pathology laboratories, we ascertained 487 histologically confirmed incident oral cavity and pharyngeal cancer cases of nonglandular epithelial origin exclusive of the nasopharynx, lip, and salivary glands—International Classification of Diseases for Oncology (ICD-O-II) topography rubrics C01–C06, C09–C10, and C12–C14 and ICD-O-II morphology rubrics 8010–8081 and 8090–8130 (30)—diagnosed between December 1, 1992,

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and February 28, 1995, in Puerto Rican residents aged 21–79 years. Population-based control subjects with no history of oral cancer ($n = 629$) were selected by two methods: 1) a multistage area probability sample for those aged 65 years or less ($n = 346$) and 2) a random sample of Medicare enrollees from tapes supplied by the Health Care Financing Administration (Baltimore, MD) for those over the age of 65 ($n = 283$). To achieve an age–sex distribution similar to that of the case subjects, we selected control subjects by using probabilities based on the age–sex profile of oral cancer patients enrolled in the Puerto Rico Central Cancer Registry from 1989 through 1990. The interview response rates were 71% for case subjects and 83% for control subjects.

Based on their residence and date of interview, 283 case subjects were eligible to provide oral epithelial cell specimens for genetic studies; these case subjects included all those residing in the San Juan metropolitan area ($n = 140$) and those residing elsewhere on the island who were interviewed after June 28, 1994 ($n = 143$). Subjects residing outside the San Juan metropolitan area who were interviewed on or before June 28, 1994, were not eligible for donation of oral epithelial cell specimens. The final study group consisted of 137 case subjects (48% of those eligible). Seventy-four (26%) case subjects died or were too ill to participate, 22 (8%) refused, 24 (8%) could not be located, seven (2%) were not contacted at their physician's request, eight (3%) did not complete the study protocol for other reasons, and 11 (4%) could not be assigned an ADH₃ genotype after repeated assay attempts. Eighty-nine (65%) cancers arose in the oral cavity, 47 (34%) were located in the pharynx, and one (0.7%) encompassed both anatomic sites. All but one case subject with a transitional cell carcinoma were diagnosed with squamous cell carcinomas.

A total of 258 control subjects were eligible for donation of oral epithelial cell specimens on the basis of their residence and date of interview; control subjects included all those residing in the San Juan metropolitan area ($n = 185$) and those residing elsewhere on the island who were interviewed after June 28, 1994 ($n = 73$). Subjects residing outside the San Juan metropolitan area who were interviewed on or before June 28, 1994, were not eligible for donation of oral epithelial cell specimens. Interviews with control subjects aged 65 years or less were completed prior to June 28, 1994. The final study group included 146 control subjects (57% of those eligible). Sixty-three (24%) potential control subjects refused to participate, 35 (14%) could not be located, 10 (4%) were deceased or too ill to participate, one (0.4%) did not complete the study protocol for other reasons, and three (1%) could not be assigned an ADH₃ genotype after repeated assay attempts.

All subjects gave written informed consent to participate in the study. Subjects who donated oral epithelial cell specimens completed an additional consent process. The study protocol was approved by the institutional review boards at the National Cancer Institute and the University of Puerto Rico.

Interview Data

Trained interviewers used a structured questionnaire to collect information from the participants regarding demographic factors, usual adult diet, and tobacco and alcohol use 1 year before the interview. We computed the average number of alcoholic drinks consumed per week by summing the contribution from each type of alcohol; one drink was considered equivalent to 1.5 ounces of liquor, 4 ounces of wine, or 12 ounces of beer. For 12 subjects (five case subjects and seven control subjects), intake of one ($n = 10$) or two ($n = 2$) beverage types was unknown and did not contribute to the total. Subjects who consumed fewer than 12 alcoholic drinks in their lifetimes were considered to be nondrinkers. Subjects (five case subjects) with calculated intakes exceeding 200 alcoholic drinks per week were assigned a value of 200 because of concern about the validity of the calculated values. A referent group of nondrinkers and three groups containing approximately equal numbers of drinkers were defined for categorical analyses (0, >0–14, 15–56, and ≥ 57 drinks per week), with cutpoints made at multiples of seven to facilitate conversion to drinks per day. We computed the number of alcoholic beverages consumed during the subject's lifetime by multiplying the average number of drinks consumed per week by 52 (the number of weeks per year) and by the number of years during which consumption occurred. Calculations were performed separately for beer, wine, and liquor and summed to obtain the total lifetime number of alcoholic beverages.

Collection of Oral Epithelial Cell Specimens

We collected specimens of oral epithelial cells by making at least 15 strokes on the oral mucosae with a soft-bristled cytobrush (Medical Packaging Corp., Camarillo, CA), rinsing the mouth with 10 mL sterile water, expectorating the

rinsed, and mixing it with 10 mL 2 \times Standard Transport Medium (STM) (Digene Diagnostics, Inc., Silver Spring, MD). Areas of tumor or of prior surgery were avoided. Specimens were stored at -70°C , usually within 24 hours of collection.

DNA Extraction

Oral rinse specimens were thawed at room temperature, agitated for 1.5 hours at 70°C to inactivate potential pathogens, incubated with 160 U ribonuclease for 30 minutes at 65°C , treated overnight with 80 U proteinase K at 50°C , and then heated to 95°C for 30 minutes. DNA was precipitated overnight at -20°C by use of sodium acetate (0.1 \times volume of sample) and isopropanol (1.5 \times volume of sample plus sodium acetate) supplemented with glycogen (final concentration = 20 $\mu\text{g}/\text{mL}$). Samples were spun for 30 minutes in a Beckman L5–50B swinging bucket ultracentrifuge (Beckman Scientific Instruments, Fullerton, CA) at 10 000 rpm at 4°C . DNA was washed with 70% ethanol, resuspended in TE buffer (i.e., 10 mM Tris–HCl and 1 mM EDTA) at a concentration of 0.05 $\mu\text{g}/\mu\text{L}$, and stored at 4°C before use within 1 week.

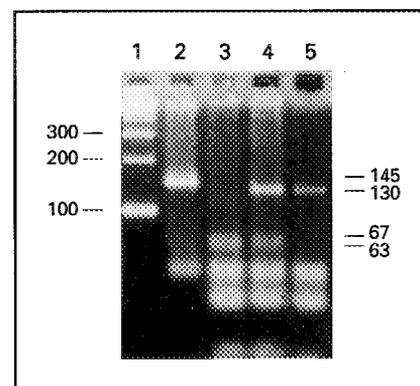
Determination of ADH₃ Genotype

For prevention of amplification of the closely related ADH₁ and ADH₂ genes, samples were initially digested with the *Nla* III restriction enzyme (New England Biolabs, Inc., Beverly, MA) according to the manufacturer's instructions. A 145-base-pair (bp) section of the ADH₃ gene was amplified by polymerase chain reaction (PCR) (31,32) by use of 0.15 μg genomic DNA with primers 321 (5'-GCTTTAAGAGTAAATATTCTGTC) and 351 (5'-AATCTACTCTTTCCGAAGC) of Groppi et al. (33); a thermal cycler (The Perkin-Elmer Corp., Norwalk, CT) was used. Fifty microliters of the reaction mixture, containing 0.15 μg genomic DNA in 10 mM Tris–HCl (pH 8.0), 3 mM MgCl₂, 50 mM KCl, 0.4 μM of each primer, 300 μM of each deoxyribonucleotide triphosphate (Pharmacia Biotech, Inc., Piscataway, NJ), and 2.5 U *Taq* polymerase (The Perkin-Elmer Corp.) and overlaid with mineral oil, underwent an initial denaturing step (1 minute at 94°C); 35 cycles of denaturing (30 seconds at 94°C), annealing (30 seconds at 55°C), and extension (30 seconds at 72°C); and a final extension step (7 minutes at 72°C).

ADH₃ genotypes were determined by treatment of 20 μL of the PCR product with 5 U of *Ssp* I restriction enzyme (New England Biolabs, Inc.) in 5.6 mM MgCl₂, 32 mM KCl, and 72 mM Tris–HCl (pH 8.25). Samples were incubated at 37°C for 24 hours, treated with an additional 5 U of *Ssp* I, and incubated for another 24 hours. Fifteen microliters of digestion products was run on a 4% Metaphor agarose gel (FMC Corp., Rockland, ME) at 120 V, stained with ethidium bromide, and photographed with Polaroid type 667 film while under UV light. The ADH₃¹ allele was represented by 67-, 63-, and 15-bp fragments, and the ADH₃² allele appeared as 130- and 15-bp fragments. Fig. 1 illustrates the three possible genotypes, i.e., ADH₃¹, ADH₃¹⁻², and ADH₃²⁻².

Genotyping was performed (by investigators who were blinded to the sub-

Fig. 1. Determination of alcohol dehydrogenase type 3 (ADH₃) genotypes. Polymerase chain reaction (PCR) products were digested with the *Ssp* I restriction enzyme and analyzed by Metaphor agarose gel (4.0%) electrophoresis. A 100-base-pair (bp) marker ladder served as a referent for DNA fragment sizes (lane 1). The three possible genotypes are shown: ADH₃¹⁻¹ (67- and 63-bp fragments, lane 3), ADH₃¹⁻² (130-, 67-, and 63-bp fragments, lane 4), and ADH₃²⁻² (130-bp fragments, lane 5). The undigested PCR product is shown for comparison (145-bp fragments, lane 2). The 15-bp fragments associated with all ADH₃ genotypes cannot be visualized.



jects' case or control status) in batches containing equal numbers of samples from case and control subjects. Positive (samples of known genotype) and negative (PCR reagents without DNA) controls were included in each batch of samples. Two reviewers independently read the results. Genotyping results were 100% concordant for duplicate assays (PCR and *Ssp* I digestion) performed on a 10% random sample of specimens.

Statistical Methods

Differences among distributions of selected variables were evaluated by use of the chi-squared statistic for categorical data and the analysis of variance (ANOVA) statistic for continuous data, as computed by the PC-SAS procedure PROC FREQ (34). Associations between putative risk factors and oral cancer were assessed as the odds ratio (OR) and its 95% confidence interval (CI) through logistic regression analyses by use of the PC-SAS (34) procedure PROC LOGISTIC (35,36). Risk estimates were adjusted for subjects' sex and age (<45, 45-65, and >65 years). Multivariate models included additional terms for subjects' cigarette use (pack-years as a continuous variable), non-cigarette tobacco use (ever/never), fruit and vegetable intake excluding potatoes (servings per week as a continuous variable), and, where indicated, alcohol intake (drinks per week as a continuous variable) or ADH₃ genotype (ADH₃¹⁻¹, ADH₃¹⁻², and ADH₃²⁻²). To test for linear trend, we treated the categorical alcohol variable as a continuous variable in a logistic regression model in which each level was represented by the median value of that category in the control group. In analyses comparing the three ADH₃ genotypes, the ADH₃¹⁻¹ genotype served as the referent category because there were no nondrinker case subjects with the ADH₃²⁻² genotype (the *a priori* low-risk genotype). All tests of statistical significance were two-sided.

Interaction between alcohol intake and ADH₃ genotype was assessed in a logistic regression analysis in which the change in risk associated with alcohol intake among subjects with the ADH₃¹⁻¹ genotype was compared with the changes in risk among subjects with the ADH₃¹⁻² and ADH₃²⁻² genotypes. For all genotypes, the baseline risk was that of nondrinkers. Alcohol intake was defined as a continuous variable based on subjects' weekly intakes (not categorical data) with the use of separate terms for subjects with each of the three ADH₃ genotypes. The fit of the data in the log-linear interaction model was

evaluated by use of the S-PLUS procedure GAM (37), and the fitted values were plotted using the S-PLUS procedure GLM (37).

We calculated the attributable risk of ADH₃, which is defined as the proportion of case subjects attributable to the ADH₃¹⁻¹ genotype, for each of four strata (0, >0-14, 15-56, and ≥57 alcoholic drinks per week) according to formulas 17 and 18 of Benichou (38). We determined the population attributable risk by using the weighted sum approach (38), a method that incorporated potential interaction between alcoholic beverage intake and ADH₃ genotype. Attributable risk estimates were based on ORs associated with the ADH₃¹⁻¹ genotype for oral cancer within strata of alcohol intake, adjusted for subjects' age (≤65 or >65 years), cigarette use (pack-years as a continuous variable), fruit and vegetable intake excluding potatoes (servings per week as a continuous variable), and, in the 0 and >0-14 drinks per week strata, sex. We calculated stratum-specific variance estimates by using the techniques reported in Appendix 1.3 by Benichou (38). The variance of the population attributable risk was calculated from the variances of the stratum-specific attributable risks under the assumption that the number of case subjects in each stratum was conditionally multinomial.

Results

Differences in the age and residence distributions between case and control subjects (Table 1) reflect the sampling strategy for biologic specimens described in the "Subjects and Methods" section. Also, as a consequence of the specimen-sampling strategy, control subjects in the present analysis were more likely than all interviewed control subjects to be at least 65 years old (69.2% versus 45.0%) and to reside in the San Juan metropolitan area (63.7% versus 29.4%). There were no substantial differences in sex, race, and alcohol intake between all interviewed control subjects and the subset of 146 control subjects included in the present analysis.

The risk of oral cancer rose with increased alcohol intake (*P*

Table 1. Subject characteristics and risks of oral cancer, Puerto Rico, 1992-1995*

	Case subjects (n = 137)		Control subjects (n = 146)		OR† (95% CI)	OR‡ (95% CI)
	No.	%	No.	%		
Sex						
Male	123	89.8	112	76.7		
Female	14	10.2	34	23.3		
Age, y						
<45	5	3.7	6	4.1		
45-54	24	17.5	16	11.0		
55-64	40	29.2	24	16.4		
65-74	57	41.6	67	45.9		
75-79	11	8.0	33	22.6		
Race						
White	91	66.4	102	69.9	1.0§	1.0§
Black	15	11.0	10	6.9	1.5 (0.6-3.5)	1.1 (0.4-3.1)
Mestizo	18	13.1	24	16.4	0.8 (0.4-1.6)	0.7 (0.3-1.7)
Other	13	9.5	10	6.9	1.1 (0.5-2.8)	1.2 (0.4-3.3)
Residence						
San Juan metropolitan area	61	44.5	93	63.7		
Other areas in Puerto Rico	76	55.5	53	36.3		
Alcoholic drinks per week						
0	12	8.8	41	28.1	1.0§	1.0§
>0-14	16	11.7	56	38.4	1.3 (0.5-3.5)	1.2 (0.4-3.4)
15-56	46	33.6	36	24.7	6.4 (2.3-18.1)	4.7 (1.6-14.1)
≥57	63	46.0	13	8.9	22.9 (7.4-71.1)	13.2 (3.9-44.0)
					<i>P</i> _{trend} <.001	<i>P</i> _{trend} <.001

*OR = odds ratio; CI = confidence interval.

†Adjusted for sex and age.

‡Adjusted for sex, age, cigarette use, non-cigarette tobacco use, and fruit and vegetable intake; ORs for race also adjusted for alcohol intake.

§Reference category.

for trend <.001); the risks were significantly elevated among those who drank at least 15 alcoholic drinks per week (Table 1). Men were heavier drinkers than women; only one female case subject consumed 15 or more alcoholic drinks per week. The risks of oral cancer associated with alcohol use were similar for the subset of subjects in the present analysis and all interviewed subjects.

The genotype distributions among the control subjects were in agreement with those predicted under the conditions of Hardy-Weinberg equilibrium ($P = .98$). The ADH_3^1 and ADH_3^2 allele frequencies were 0.62 and 0.38, respectively, in control subjects and 0.68 and 0.32, respectively, in case subjects ($P = .16$) (Table 2). The genotype and allele frequencies were similar in men and women. Among case subjects, the distribution of ADH_3 genotypes did not differ significantly between those who participated in the study within 108 days after diagnosis (median time interval) and those who participated after 108 days after diagnosis ($P = .91$). Age at diagnosis of oral cancer was not related to subjects' ADH_3 genotype (ADH_3^{1-1} , 61.7 years; ADH_3^{1-2} , 64.9 years; ADH_3^{2-2} , 60.7 years; $P = .14$).

The age- and sex-adjusted risk of oral cancer was lower among individuals with the ADH_3^{1-2} genotype (OR = 0.7; 95% CI = 0.4–1.2) or ADH_3^{2-2} genotype (OR = 0.7; 95% CI = 0.3–1.5) compared with those with the ADH_3^{1-1} genotype (referent). Risk estimates were similar when they were adjusted further for cigarette use, non-cigarette tobacco use, alcohol intake, and fruit and vegetable intake (Table 3). The multivariate adjusted risk of oral cancer was higher (OR = 1.3; 95% CI = 0.8–2.4) among subjects with the ADH_3^{1-1} genotype than among the combined group of subjects with the ADH_3^{1-2} or ADH_3^{2-2} genotypes.

In Table 3, the multivariate adjusted risks of oral cancer are shown by ADH_3 genotype and alcohol intake. Among nondrinkers and consumers of up to 56 alcoholic drinks per week, the risks were similar among subjects with the ADH_3^{1-1} , ADH_3^{1-2} , and ADH_3^{2-2} genotypes. Among subjects who drank at least 57 alcoholic beverages per week, however, the risks were increased 40.1-fold (95% CI = 5.4–296.0) for those with the ADH_3^{1-1} genotype, 7.0-fold (95% CI = 1.4–35.0) for those with the ADH_3^{1-2} genotype, and 4.4-fold (95% CI = 0.6–33.3) for those with the ADH_3^{2-2} genotype, compared with nondrinkers with the ADH_3^{1-1} genotype. Thus, for consumers of at least 57 drinks per week, the multivariate adjusted risk was 5.3-fold (95% CI = 1.0–28.8) higher among persons with the ADH_3^{1-1} genotype than among the combined group of subjects with the ADH_3^{1-2} or

the ADH_3^{2-2} genotype and was not appreciably changed by adjustment for the amount of alcohol consumed as a continuous variable. Further adjustment for annual household income, education, body mass index, and a history of alcohol-related cancer (i.e., cancers of the oral cavity, pharynx, larynx, esophagus, and liver) in at least one parent or sibling did not materially change the risk estimates in Table 3.

There were no significant departures from a log-linear relationship between alcohol intake (as a continuous variable) and the multivariate adjusted risk of oral cancer for subjects with each ADH_3 genotype (ADH_3^{1-1} , $P = .14$; ADH_3^{1-2} , $P = .09$; ADH_3^{2-2} , $P = .08$). With each additional alcoholic drink consumed per week, the risk of oral cancer increased by 3.6% (95% CI = 1.9%–5.4%) among subjects with the ADH_3^{1-1} genotype, 1.9% (95% CI = 0.8%–3.1%) among subjects with the ADH_3^{1-2} genotype, and 2.0% (95% CI = 0.3%–3.7%) among subjects with the ADH_3^{2-2} genotype (Table 4 and Fig. 2). A significantly higher rate of increase in the risk of oral cancer per additional alcoholic drink per week was associated with the ADH_3^{1-1} genotype (3.6%; 95% CI = 1.9%–5.4%) than with the ADH_3^{1-2} and ADH_3^{2-2} genotypes combined (2.0%; 95% CI = 0.9%–3.0%) ($P = .04$). Differences in risk associated with alcohol intake among subjects with the different ADH_3 genotypes were more pronounced for oral cavity cancers (ADH_3^{1-1} , 3.7%; ADH_3^{1-2} , 1.8%; ADH_3^{2-2} , 1.0%) than for pharyngeal cancers (ADH_3^{1-1} , 3.9%; ADH_3^{1-2} , 2.4%; ADH_3^{2-2} , 3.1%). Risk estimates were essentially unchanged when alcohol intakes exceeding 200 drinks per week were not recoded to 200.

The multivariate adjusted population attributable risk of oral cancer due to the ADH_3^{1-1} genotype was 17.9% (95% CI = –3.2%–39.0%). Among consumers of at least 57 alcoholic beverages per week, the attributable risk due to the ADH_3^{1-1} genotype was 45.2% (95% CI = 25.0%–67.2%).

Alcohol intake (drinks per week) did not vary significantly by ADH_3 genotype among control subjects ($P = .66$) or case subjects ($P = .19$). Among those who drank at least 57 alcoholic drinks per week, the mean weekly alcohol intakes were 66.5, 94.2, and 90.0 drinks for control subjects with the ADH_3^{1-1} , ADH_3^{1-2} , and ADH_3^{2-2} genotypes ($P = .40$), respectively, and 130.9, 106.2, and 131.6 drinks for case subjects with the respective ADH_3 genotypes ($P = .37$). The number of alcoholic beverages consumed during the subjects' lifetimes (a measure reflecting duration and intensity of drinking) was also not associated with ADH_3 genotype among control subjects ($P = .78$) or case subjects ($P = .33$).

Table 2. Distributions of alcohol dehydrogenase type 3 (ADH_3) genotypes and allele frequencies, Puerto Rico, 1992–1995*

	ADH ₃ genotype frequencies, No. (%)			ADH ₃ allele frequencies, proportion	
	ADH ₃ ¹⁻¹	ADH ₃ ¹⁻²	ADH ₃ ²⁻²	ADH ₃ ¹	ADH ₃ ²
Control subjects (n = 146)	56 (38.4)	70 (47.9)	20 (13.7)	0.62	0.38
Males (n = 112)	42 (37.5)	56 (50.0)	14 (12.5)	0.62	0.38
Females (n = 34)	14 (41.2)	14 (41.2)	6 (17.6)	0.62	0.38
Case subjects (n = 137)	66 (48.2)	54 (39.4)	17 (12.4)	0.68	0.32
Males (n = 123)	61 (49.6)	45 (36.6)	17 (13.8)	0.68	0.32
Females (n = 14)	5 (35.7)	9 (64.3)	0 (0.0)	0.68	0.32

*The genotype distributions for male versus female subjects were not significantly different among control subjects ($P = .60$, two-sided chi-squared test) or among case subjects ($P = .09$, two-sided chi-squared test).

Table 3. Joint effects of alcohol dehydrogenase type 3 (ADH₃) genotype and weekly alcohol intake on risk of oral cancer, Puerto Rico, 1992–1995: odds ratios and 95% confidence intervals*

Genotype	Odds ratio (95% confidence interval)†				Total‡
	0 drink/wk	>0–14 drinks/wk	15–56 drinks/wk	≥57 drinks/wk	
ADH ₃ ¹⁻¹	1.0§ [5, 15]	1.2 (0.3–5.2) [8, 23]	3.5 (0.8–15.8) [18, 16]	40.1 (5.4–296.0) [35, 2]	1.0 [66, 56]
ADH ₃ ¹⁻²	0.9 (0.2–3.7) [7, 21]	0.9 (0.2–4.2) [7, 26]	4.1 (0.9–18.7) [18, 15]	7.0 (1.4–35.0) [22, 8]	0.7 (0.4–1.3) [54, 70]
ADH ₃ ²⁻²	— [0, 5]	1.0 (0.1–12.2) [1, 7]	6.3 (1.1–36.8) [10, 5]	4.4 (0.6–33.3) [6, 3]	0.6 (0.3–1.6) [17, 20]
Total	1.0 [12, 41]	1.2 (0.4–3.3) [16, 56]	4.7 (1.6–14.4) [46, 36]	13.1 (3.9–44.2) [63, 13]	

*Adjusted for sex, age, cigarette use, non-cigarette tobacco use, and fruit and vegetable intake.

†Numbers of case and control subjects are shown in brackets for each set of estimated values.

‡Odds ratios also adjusted for alcohol intake.

§Reference category.

||Odds ratios also adjusted for ADH₃ genotype.

Discussion

The results of our case-control study in Puerto Rico suggest that an individual's ADH₃ genotype influences the risk of alcohol-related oral cancer, particularly among consumers of 57 or more alcoholic drinks per week. A statistically significant higher rate of increase in the risk of oral cancer per additional alcoholic drink per week was found among persons with the ADH₃¹⁻¹ genotype than among the combined group of subjects with the ADH₃¹⁻² or the ADH₃²⁻² genotype. Consistent with a mechanism involving ethanol metabolism, oral cancer risk did not vary by ADH₃ genotype among nondrinkers. The risks associated with the ADH₃¹⁻¹ genotype were greater for tumors arising in the oral cavity than for tumors occurring in the pharynx.

Our findings are consistent with those from a recent study (39) of Caucasian alcoholic men in France (39 case subjects and 37 control subjects) in whom the ADH₃¹⁻¹ genotype was associated with a 2.6-fold (95% CI = 0.7–10.0) higher risk of oropharyngeal cancer and a 6.1-fold (95% CI = 1.3–28.6) higher risk of laryngeal cancer. The alcoholic beverage intakes of the

French subjects were roughly equivalent to the highest exposure group (≥57 drinks per week) in our study.

Regardless of their ADH₃ genotype, individuals who consumed more than 14 alcoholic drinks per week were at a higher risk of developing oral cancer than were nondrinkers, which emphasizes the importance of limiting alcohol intake in the prevention of oral cancer (8). We estimated that 17.9% of oral cancer cases in the population and nearly half (47.2%) of the oral cancers that develop in drinkers of at least 57 alcoholic beverages per week are attributable to the ADH₃¹⁻¹ genotype.

According to the most recent (1983–1987) Surveillance, Epidemiology, and End Results (SEER) Program¹ data available that include Puerto Rico, the annual incidence of oral cancer per 100 000 men was 9.8 for mainland U.S. whites, 16.4 for U.S. blacks, and 18.9 for Puerto Ricans (rates adjusted with the use of the World Health Organization's standard world population) (1). The ADH₃ allele frequencies in our study (ADH₃¹ = 0.62 and ADH₃² = 0.38) are intermediate between those for U.S. whites (0.55 and 0.45) and U.S. blacks (0.85 and 0.15) (23,40); thus, they do not fully explain the variations in oral cancer incidence among these populations. Differences in alcohol and tobacco use appear to be important determinants of the racial and ethnic patterns of oral cancer (41). The self-reported race of subjects in our study was not associated with oral cancer or with ADH₃ genotype, although Puerto Ricans are not easily classified into racial categories. It may be possible to investigate and control for race or ethnic factors in other populations.

The ADH₃¹⁻¹ genotype is associated with a more rapid metabolism of ethanol to acetaldehyde than the other ADH₃ genotypes. ADH activity in colon and stomach tissues is highest among individuals with the ADH₃¹⁻¹ genotype, intermediate among those with the ADH₃¹⁻² genotype, and lowest among those with the ADH₃²⁻² genotype (27,28). *In vitro* kinetic studies [reviewed in (23)] indicate that enzymes encoded by the ADH₃¹ allele can metabolize ethanol 2.5 times faster than enzymes encoded by the ADH₃² allele; *in vivo* studies and investigations of mixed enzymes comprised of ADH₁, ADH₂, and ADH₃ subunits have not been performed. Thus, our finding of a higher risk of oral cancer among individuals thought to metabolize ethanol more quickly (i.e., those with the ADH₃¹⁻¹ geno-

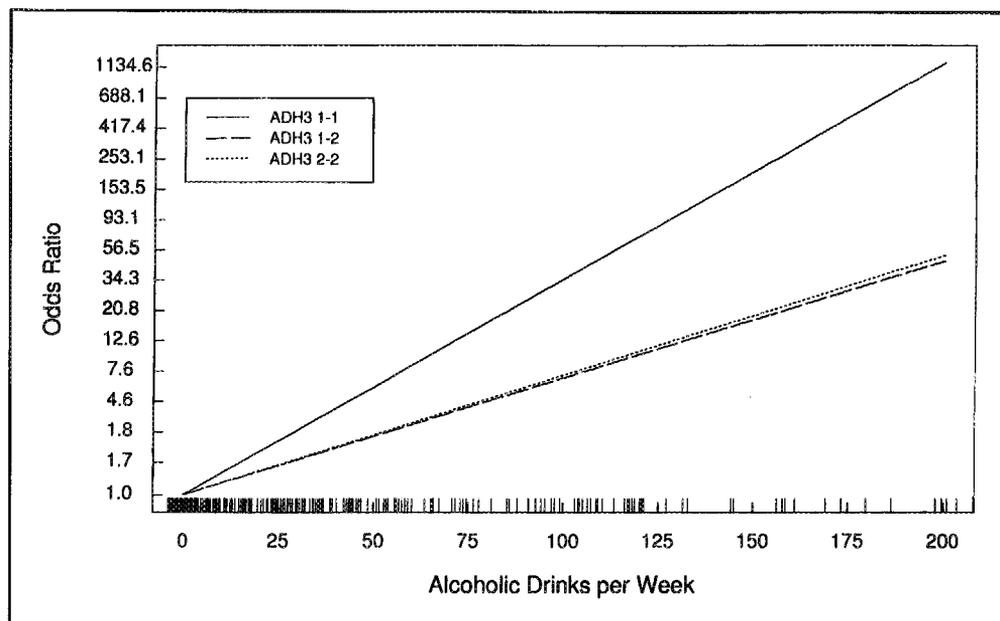
Table 4. Percent change in oral cancer risk for each additional drink per week by alcohol dehydrogenase type 3 (ADH₃) genotype, Puerto Rico, 1992–1995

ADH ₃ genotype	Change in risk per drink per week (95% confidence interval)*	P†
All oral cancer		
ADH ₃ ¹⁻¹	3.6% (1.9%–5.4%)	
ADH ₃ ¹⁻²	1.9% (0.8%–3.1%)	.05
ADH ₃ ²⁻²	2.0% (0.3%–3.7%)	.13
Cancer of oral cavity		
ADH ₃ ¹⁻¹	3.7% (1.9%–5.6%)	
ADH ₃ ¹⁻²	1.8% (0.6%–3.0%)	.04
ADH ₃ ²⁻²	1.0% (–0.7%–2.7%)	.02
Cancer of pharynx		
ADH ₃ ¹⁻¹	3.9% (1.7%–6.2%)	
ADH ₃ ¹⁻²	2.4% (1.0%–3.8%)	.15
ADH ₃ ²⁻²	3.1% (0.9%–5.4%)	.54

*Adjusted for sex, age, cigarette use, non-cigarette tobacco use, and fruit and vegetable intake.

†Two-sided P for interaction; reference category = ADH₃¹⁻¹.

Fig. 2. Risks (i.e., odds ratios) of oral cancer associated with alcohol intake by alcohol dehydrogenase type 3 (ADH₃) genotype, with values fitted from a logistic regression model with separate terms for alcohol intake (continuous variable) for subjects with the ADH₃¹⁻¹, ADH₃¹⁻², and ADH₃²⁻² genotypes. Risks were adjusted for subjects' sex, age, cigarette use, non-cigarette tobacco use, and fruit and vegetable intake. The risk of oral cancer increased by 3.6%, 1.9%, and 2.0% per alcoholic drink per week among subjects with the ADH₃¹⁻¹, ADH₃¹⁻², and ADH₃²⁻² genotypes, respectively. Ticks along the horizontal axis indicate the distribution of study subjects.



type) implicates acetaldehyde in alcohol-related oral carcinogenesis. Our results are consistent with experimental evidence of the carcinogenic, mutagenic, and other DNA-damaging effects of acetaldehyde (11–20).

Further evidence that variation in ethanol metabolism may influence the risk of oral cancer derives from a hospital-based case-control study in which male Japanese drinkers with a form of aldehyde dehydrogenase type 2 (ALDH₂) and substantially impaired ability to oxidize acetaldehyde to acetate had a sevenfold to 12-fold increased risk of esophageal carcinoma (42). The mutant ALDH₂ allele was also present in three of three Japanese alcoholics with esophageal cancer and concurrent cancers of the gingiva or epilarynx-hypopharynx (43). The ALDH₂ allele is found only in Asian populations (44) and could not be evaluated in our study.

Local conversion of ethanol to acetaldehyde by ADH in the oral cavity and pharynx may underlie the strong association observed between alcoholic beverage use and cancers at these sites in our study and in other studies (2–7). However, acetaldehyde levels may also increase systemically from hepatic ethanol metabolism and interact with the topical effects of ethanol or other agents. While ADH activity has been detected in tissues of the oral cavity, enzymes encoded by the ADH₃ gene have not (26,28). Further work is needed to determine the relationship between ADH₃ genotype, ADH activity, and ethanol and acetaldehyde levels in tissues of the oral cavity *in vivo*.

We found no evidence that the association between the ADH₃ genotype and the risk of oral cancer is due to an influence of the ADH₃ locus on alcohol intake. This result is consistent with studies in Caucasian populations indicating no relationship between alcoholism and ADH₃ genotype (45–48). However, among Chinese, alcoholism has been associated with the ADH₃² allele (49).

Among the strengths of this study was the use of a population-based sampling design. However, because only 48% of eligible case subjects and 57% of eligible control subjects participated, self-selection or survival-related biases may have influenced the results. Participants in the present analysis (bio-

specimen donors) were representative of all interviewed subjects (71% case subject and 83% control subject participation) with respect to sex, race, and alcohol intake, and the observed differences in the age and residence distributions are consistent with the biospecimen-sampling strategy. However, the comparability of questionnaire participants and nonparticipants is unknown. Data were not available to directly evaluate patient characteristics such as tumor grade, tumor stage, and survival. However, the lack of association between subjects' ADH₃ genotype and time from diagnosis to study participation provides indirect evidence against survival-related bias due to overrepresentation of particular ADH₃ genotypes among nonparticipating case subjects with short oral cancer survival times.

Although unlikely, misclassification is another possible limitation of this study. Oral cancer diagnoses were histologically confirmed. Alcohol intake was determined from responses to 39 questionnaire items, and nearly all responses were internally consistent. It is possible that case subjects underreported their alcohol consumption, as has been observed previously (50–53). However, a study (50) found that such recall bias only slightly attenuated the ORs for alcohol-related breast cancer and did not change the overall findings. In addition, we adjusted for several potentially confounding factors, including tobacco use and diet. Misclassification of ADH₃ genotypes was reduced by numerous quality-control features in the assay, including removal of the closely related ADH₁ and ADH₂ genes as potential templates in the PCR. The proteins encoded by the ADH₃¹ and ADH₃² alleles differ by two amino acid residues at positions 271 and 349. The *Ssp* I restriction site at position 349 studied herein serves as a surrogate marker for the position 271 polymorphism, which is responsible for the altered enzyme kinetics (54). In rare individuals, however, only one of the changes may be present, and the DNA sequence coding for residue 349 would not accurately indicate the DNA sequence at residue 271.

The role of chance must be considered in interpreting our findings. The analysis of heavy drinkers (at least 57 alcoholic drinks per week) was based on a total of 63 case subjects and 13 control subjects. However, while the 95% CIs around point es-

timates of risk were wide, a consistent interaction between ADH₃ genotype and alcohol intake was found when alcohol was analyzed as either a categorical or a continuous variable.

The findings of our study should be considered preliminary, pending replication in other study populations. Future studies should also evaluate the polymorphic ADH₂ and cytochrome P450IIIE1 genes, which encode other proteins that metabolize ethanol (23,55). Genotypes at these loci were not investigated in our study, because the variant alleles occur at frequencies of less than 0.10 in Caucasian populations (23,56,57). It will also be important to determine if our findings extend to other anatomic sites, such as the esophagus, larynx, liver, breast, and possibly large intestine, for which relationships with alcohol consumption have been reported.

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Notes

¹*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

We thank Mr. John Heinrich and Ms. Shirley Friend (Westat, Inc., Rockville, MD) for study management and training in collection of oral epithelial cell specimens, respectively; Mr. Roy Van Dusen (Information Management Systems, Rockville, MD) for computer support; Drs. Patricia Hartge and Mitchell Gail (Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD) for statistical advice; Dr. Curtis Harris (Division of Basic Sciences, NCI) for insightful discussions of laboratory issues; Dr. Lucinda Carr (Indiana University, Indianapolis) for provision of positive control samples for the ADH₂ genotyping assay; Ms. Elise Bowman (Division of Basic Sciences, NCI), Dr. Joel Palefsky (University of California at San Francisco), and Dr. Yvonne Reid (American Type Culture Collection, Rockville, MD) for advice and assistance with DNA extraction; and Dr. Montserrat Garcia-Closas (Division of Cancer Epidemiology and Genetics, NCI) for implementation of the S-PLUS programs.

Manuscript received May 15, 1997; revised August 13, 1997; accepted September 11, 1997.