Alcohol Dehydrogenase 3 Genotype as a Risk Factor for Upper Aerodigestive Tract Cancers

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Objective: To assess alcohol dehydrogenase 3 (ADH3) polymorphism at position Ile349Val as indicator of risk factor for upper aerodigestive tract (UADT) cancer to verify its association with UADT cancer in nonalcoholic or nonsmoking individuals.

Design: Cross-sectional study.

Setting: Primary care or referral center.

Patients: The study group consisted of 141 consecutive patients with newly diagnosed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx admitted for surgical treatment. The comparison group consisted of 94 inpatients without cancer from the A. C. Camargo or other São Paulo (Brazil) hospital and 40 healthy individuals.

Intervention: All participants were interviewed and data were collected using a structured questionnaire. After written informed consent was obtained, 20 mL of blood was collected in heparinized tubes.

Main Outcome Measures: Odds ratio for ADH3 genotypes using logistic regression models.

Results: After adjustment for sex, age, tobacco use, and history of cancer in first-degree family relatives, a significantly higher odds ratio for UADT cancer was observed among individuals with AA genotype and low cumulative alcohol consumption (≤100 kg of ethanol) (odds ratio = 3.8 [95% confidence interval, 1.5-9.7]). A 4-fold increase in odds ratio for UADT cancer among individuals with AA genotype and low tobacco consumption (≤25 pack-years) was also found in the adjusted model.

Conclusions: These results suggest that genotype AA may be a risk factor for UADT cancer, especially in individuals with low alcohol or tobacco consumption. However, further epidemiological case-control or cohort studies, preferably prospective, are needed to establish the exact role of ADH3 polymorphism and its association with the development of UADT cancers.

Arch Otolaryngol Head Neck Surg. 2004;130:78-82

The incidence rates of head and neck cancer in southern Brazil are among the highest in the world in both men and women, and the rates of squamous cell carcinoma (SCC) of oral, pharyngeal, and laryngeal sites are rising among men in many countries.

Upper aerodigestive tract (UADT) cancers are a heterogeneous group of neoplasms with similar etiology. Although several epidemiologic investigations have shown tobacco and alcohol to be risk factors in the development of SCC of the head and neck, such cancers develop in only a small proportion of individuals who use tobacco and alcohol, and some patients who have these cancers have had no apparent exposure to tobacco and alcohol. This suggests that host susceptibility may play an important role in SCC of the head and neck.

Several studies have shown an increased risk for UADT cancers among heavy drinkers or nonsmoking alcohol consumers compared with abstainers. Chronic alcohol consumption is correlated with carcinogenesis through a variety of mechanisms, and the basal and epithelial cells of UADT cancers may also be damaged by high ethanol concentrations on the exposed mucosae, leading to higher carcinogenic and procarcinogenic concentrations.

The influence of alcohol consumption on the risk of cancer may be due to mucosal alcohol dehydrogenase (ADH) or to oropharyngeal bacteria that convert ethanol to acetaldehyde, a possible UADT carcinogen. There are polymorphic variants of ADH, and ADH3, which is known to produce enzymes with distinct kinetic properties, can cause variation in the capacity of individuals to metabolize alco-
Studies have shown controversial results regarding ADH3 polymorphism as a possible risk factor for SCC of the head and neck. Given that tobacco and alcohol consumption are the most important risk factors for UADT cancers, ADH3 polymorphism at position Ile349Val was assessed as a risk factor for UADT cancers in nonalcoholic, alcoholic, non-smoking, and smoking study subjects.

From 1993 to 2001, 141 consecutive patients newly diagnosed with SCC of the oral cavity, oropharynx, hypopharynx, or larynx and admitted for surgical treatment at the Department of Head and Neck Surgery and Otorhinolaryngology of the Hospital do Cancer A. C. Camargo, São Paulo, Brazil, were enrolled in this study. The sites of these unpreviously treated tumors were the oral cavity (n=63 [44.7%]), oropharynx (n=41 [29.1%]), hypopharynx (n=8 [5.7%]), and larynx (n=29 [20.6%]).

From 1993 to 2001, 2 unmatched groups of cancer-free individuals were selected as comparison groups: 40 healthy volunteers, and 94 inpatients from the A. C. Camargo or other São Paulo hospital who had diseases other than cancer (3 patients had infectious diseases; 22 had benign neoplasms; 4 had endocrine diseases; 20 had diseases of the cardiovascular system; 1 had asthma; 31 had diseases of the digestive system; there was only 1 case of hepatic cirrhosis; 4 had diseases of the genitourinary tract; 1 had osteomuscular disease; 2 had congenital disorders; 2 had ill-defined diagnostic conditions; and 4 had received traumas).

A trained nurse interviewed all participants using a structured questionnaire to obtain detailed information on socioeconomic and demographic characteristics and on lifetime tobacco and alcoholic beverage consumption.

The study protocol was approved by the institutional ethics committee. After participants had given written informed consent, 20 mL of blood was collected from them in heparin-containing tubes. A leukocyte pellet was extracted from each blood sample with the Wizard Genomic DNA Purification Kit (Promega, Madison, Wis) to obtain genomic DNA. Polymorphism analysis was performed after amplification by polymerase chain reaction (PCR).

The PCR amplification was performed using the primers 5’-CTT-TAA-GAG-TAA-AGA-ATC-TGT-CC-3’ (sense) and 5’-ACC-TGG-TTC-CTG-GAA-AGA-G-3’ (antisense) for genotyping ADH3 polymorphism at codon 349, which generated 140–base pair fragments. These fragments were amplified in 25 µL of reaction mixture containing about 100 ng of genomic DNA, 6.25 pmol of each primer, 1× PCR buffer (20mM Tris hydrochloric acid [pH 9.0], 50mM potassium chloride), 3mM magnesium chloride, 0.2mM of each of the deoxyribonucleotides adenosine triphosphate, thymidine triphosphate, guanine triphosphate, and cytosine triphosphate, and 1 U of TaqDNA polymerase (Amplitaq Gold; Applied Biosystems, Foster City, Calif). The reactions were performed by incubating the reaction mixture at 94°C for 10 minutes, followed by 35 cycles at 94°C for 45 seconds and at 65°C for 1 minute, then at 72°C for 1 minute, with a final elongation step at 72°C for 7 minutes.

Polymorphism analysis was performed with the Wave system (Transgenomic Inc, Omaha, Neb) after the PCR products were denatured at 94°C for 5 minutes and cooled to 65°C, with a temperature change of 1°C per minute. The samples were then kept at 4°C until 3 µL was injected in the column, and the PCR products were separated over 8 minutes (flow rate, 0.9 mL/min) through a linear acetonitrile gradient automatically determined by the size and G-C content of the fragment as described by Underhill et al.15 The optimal melting temperature for PCR fragments analysis was 63°C.

Every sample in which only 1 peak was identified was further analyzed by preparing equimolar mixtures with PCR products from known wild-type samples to potentially generate heteroduplex DNA. After denaturation, renamelling, and reinjection, the appearance of heterozygous elution profiles after addition of the wild-type sample suggested the presence of a homozygous mutant genotype.

Only 10% of samples with heterozygous and homozygous profiles were examined by direct sequencing to confirm the presence of the polymorphism because it is known that the same polymorphism gives identical chromatographic tracing among individuals, and common polymorphisms can be identified by pattern recognition alone.16

The sequence analysis was performed using the Dynamic ET Terminator Cycle Sequencing kit (Amersham Biosciences, Piscataway, NJ).

Genotype analysis was performed for 148 subjects with cancer and 137 subjects without cancer, but the ADH3 genotype could not be determined for 7 subjects with cancer and 3 without.

For univariate data analysis, the associations of categorical data were determined using the frequency χ² tests and 2 × 2 tables; when the expected frequency was less than 5, the Fisher exact test was used. The Hardy-Weinberg equilibrium analysis (p² + 2pq + q², where q = 1 – p) was performed to estimate the expected genotype distribution (AA, GA, GG), which was compared with observed genotype frequencies using a χ² test. Unconditional logistic regression with 95% confidence interval (CI) was performed to obtain the estimated odds ratio (OR) between genotype and UADT cancer.17 Owing to the small number of subjects with the homozygous G allele, those who had GG genotype were grouped with those who had an heterozygous allele (GA or GG). A multivariate logistic regression model was used, controlling for the confounding effects of age, sex, lifetime tobacco and alcohol consumption, and cancer history in first-degree relative (no/yes answer).

Lifetime consumption of alcohol was collected for 4 types of alcoholic beverages: beer, wine, hard liquor, and cachaca, a spirit distilled from sugar cane. Cumulative alcohol exposure was expressed in kilograms of ethanol calculated from the consumption of individual beverages over the patient’s entire life.18 As an expression of cumulative exposure, the consumed amount of ethanol in kilograms was combined with drinking frequency and years of drinking into a synthetic index. A dose of ethanol consumed corresponded to 5% for beer, 10% for wine, 50% for hard liquor, or 50% for cachaca.18 Cumulative exposure to tobacco smoking was expressed in pack-years, defined as the cumulative exposure equivalent to packs of cigarettes smoked per day multiplied by the number of years the patient smoked. For the computation of pack-years of tobacco consumption we assumed the following equivalence: 1 pack equals 20 industrial cigarettes, 4 hand-rolled cigarettes of black tobacco, 4 cigars, or 5 pipefuls of pipe tobacco.19 Medians or quartiles were considered cutoffs for continuous variables. All statistical analyses were performed using the statistical software package Stata, release 7.0 (StataCorp, College Station, Tex).

RESULTS

This investigation was based on 141 subjects (110 male and 31 female) with UADT cancer and 134 subjects (91 male and 43 female) without cancer. Those without cancer (median age, 58 years; range, 22-90 years) were likely...
to be younger than those with cancer (median age, 53 years; range, 17-90 years). The main characteristics of the participants, such as demographics, lifestyle variables, and ADH3 genotype distribution, are presented in Table 1.

The distribution of the ADH3 genotypes AA, GA, and GG was similar in the group with cancer (51.8%, 41.8%, and 6.4%, respectively) and in the group without cancer (48.5%, 44.0%, and 7.5%) (P = .84). The genotype distribution in the group without cancer was consistent with the Hardy-Weinberg equilibrium (P = .84). Table 2 shows the distribution of genotypes and demographic and lifestyle variables. No statistically significant P value was obtained by χ² test regarding the effects of the study variables on genotypes GA and GG on the one hand, and AA on the other.

Univariate and multivariate logistic regression analyses of genotypes provided nonsignificant results, and there were no significant associations between genotype and cancer site (oral cavity, pharynx, or larynx) (data not shown).

In stratified analysis, the logistic regression model, adjusted for potential confounders (age, sex, family history of cancer, and tobacco and alcohol consumption), showed a high risk of UADT cancer for subjects with homozygous AA genotype and low cumulative alcohol consumption (≤100 kg of ethanol) (OR, 3.8; 95% CI, 1.5-9.7). However, for subjects with a cumulative alcohol consumption greater than 100 kg of ethanol, AA genotype was nonsignificant (OR, 0.5) (Table 3). Also, there was a significant 4-fold OR increase in subjects with AA genotype and low tobacco consumption (<25 pack-years); and a negative OR was found for heavy smokers (≥25 pack-years), but a nonsignificant OR of 0.4 (95% CI, 0.2-1.0) was found for heavy smokers with AA genotype (Table 3).

**COMMENT**

Alcohol dehydrogenase 3 polymorphism at Ile349Val as a risk indicator for UADT cancers was assessed to verify its association with cancer in individuals with low levels of alcohol or tobacco consumption.

Analysis stratified by alcohol and tobacco consumption showed ADH3 polymorphism as an added risk for individuals with the AA genotype and low tobacco smoking or alcohol intake, but nonstratified logistic regression analysis showed no significant added risk. Bouchard et al verified the role of ADH3 genotype in patients with UADT cancer. The authors examined the adenine to guanine change (Ile349Val amino acid substitution), but observed no association between genotype and UADT cancer; however, they showed strong interaction between ADH3 and high alcohol consumption on the one hand and oral cavity/pharyngeal cancers on the other. In another recent study, Olshan et al examined differ-
ences between the Ile (ADH3*1) and Val (ADH3*2) codons at amino acid residue 349 in patients with head and neck cancer but found no significant associations. Also, they found no joint effects or interaction between the ADH3 genotype and alcohol consumption. By contrast, the results of a study recently conducted by Zavras et al.19 on the population of Athens, Greece, suggest an interaction of genotype and environment in oral cancer.

Because of our small sample size, we could not verify interaction between genotype and phenotype in this study.

Our study proposed to verify the association between alcohol or tobacco consumption and ADH3 polymorphism at position Ile349Val as an indicator of risk for UADT cancers. We observed that 37 subjects in our group with cancer (26%) and 96 subjects in our group (61%) without cancer reported a low consumption of alcoholic beverages, and a similar distribution was observed for low tobacco consumption. Head and neck cancer is rare in nonsmokers and alcohol abstainers, and only a few studies have included a sufficient number of patients with cancer.20,21 Chronic ethanol consumption has been associated with malnutrition and with inadequate diets deprived of known cancer-protective elements.9 Because only tobacco and alcohol consumption were evaluated in this study, we were not be able to control for fruit and vegetable consumption, which has been shown to be a protective factor for UADT cancers.18,22

Approximately 70% of our participants without cancer were selected from the same hospital as those with cancer or from a neighborhood hospital, and 30% were healthy volunteers. However, the observed ADH3 genotype distribution and that expected from the Hardy-Weinberg equilibrium model suggested no selection bias (P>0.05, χ² test). In spite of the small sample size and the fact that groups were not matched by sex, age, and cancer family history, stratified analysis showed significant added risk of UADT cancers in individuals with ADH3 AA genotype. Furthermore, our results showed a significant 4-fold risk increase for UADT cancers in individuals with ADH3 AA genotype and low consumption of alcohol or tobacco, but, contrary to our expectations, our results also showed heavy consumption of alcohol and smoking tobacco to be protective; nonsignificant ORs, however, were obtained.

The various xenobiotic-metabolizing enzymes found in the mucosa of the upper aerodigestive tract are involved in activating the metabolism of carcinogens or procarcinogens.7 Phase I enzyme oxidative alcohol dehydrogenase is responsible for carcinogen activation, there are many isofoms of ADH, and other genes are also involved. Although there are phase II detoxification enzymes, which generally inactivate the genotoxic compounds, aldehyde dehydrogenase is involved in the carcinogen phase II pathway in ethanol metabolism.8 The ADH3 gene (a class I ADH gene) plays a major role in ethanol oxidation,10 and the results of this study place genotype AA of ADH3 polymorphism as a candidate gene for head and neck cancer risk in individuals with low alcohol or tobacco consumption.

Although increased risks were detected in this study, data could have been confounded by the differences between groups for adjusted variables. Study power and risk significance may have been more convincing if the study design had been case-control. Therefore, the results need to be confirmed in further studies large enough to provide statistical power, preferably matched (by age, sex, and family history of cancer) case-control or prospective epidemiological cohort study, to establish the exact role of ADH3 polymorphism. Multiple gene interaction, genotype-environment interaction, and the systemic and topic effects of the presence or absence of tobacco and alcohol should be considered, and their association with the development of UADT cancers.

Table 3. Odds Ratios for Cancer of the Upper Aerodigestive Track According to ADH3 Genotypes and Lifetime Alcohol and Tobacco Consumption

<table>
<thead>
<tr>
<th>Stratified Models</th>
<th>Adjusted Odds Ratio (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol consumption ≤100 kg of ethanol</td>
<td>AA vs GG + GA 3.77 (1.5-9.7)</td>
<td>.006</td>
</tr>
<tr>
<td>Alcohol consumption &gt;100 kg of ethanol</td>
<td>AA vs GG + GA 0.52 (0.2-1.2)</td>
<td>.12</td>
</tr>
<tr>
<td>Tobacco consumption &gt;25 pack-years</td>
<td>Alcohol consumption &gt;100 kg of ethanol AA vs GG + GA 4.27 (1.7-10.8)</td>
<td>.002</td>
</tr>
<tr>
<td>Tobacco consumption &gt;25 pack-years</td>
<td>Alcohol consumption &gt;100 kg of ethanol AA vs GG + GA 0.43 (0.2-1.0)</td>
<td>.05</td>
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</tbody>
</table>

*Odds ratios were obtained by logistic regression model adjusted by sex, cancer in first-degree relative, and age (≤55 and >55 years).

Submitted for publication April 18, 2003; accepted September 2, 2003.

This study was supported in part by a grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo (Centros de Pesquisa, Inovação e Diufusão grant 98/14335-2).

This study was presented in poster form at the annual meeting of the American Head and Neck Society; May 2, 2003; Nashville, Tenn.

We thank Julia M. F. Toyota for her fieldwork assistance and Valeria Paixão for her laboratory work.

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