Alcohol drinking is a major risk factor for head and neck cancer (HNC). This risk may be modified by alcohol dehydrogenase (ADH) genes, particularly ADH1B and ADH1C, that oxidise ethanol to its carcinogenic metabolite, acetaldehyde. A meta-analysis was conducted to assess the association between ADH1B and ADH1C and HNC risk. Twenty-nine studies from 28 articles identified from a literature search were included. Summary odds ratios (meta-ORs) were generated using random effect models. A reduced risk for HNC was associated with carrying the ADH1B*2 and ADH1C*1 alleles that confer faster metabolism of ethanol to acetaldehyde [meta-OR ADH1B, 0.50; 95% confidence interval (CI): 0.37–0.68, 13 studies; meta-OR ADH1C, 0.87; 95% CI: 0.76–0.99, 22 studies]. ADH1B*2 and ADH1C*1 alleles appear to be protective for HNC, possibly due to: (i) decreasing the opportunity for oral microflora to produce acetaldehyde locally from a prolonged systemic circulation of ethanol, (ii) preventing ethanol from acting as a solvent for other carcinogens, and (iii) decreasing the amount of ethanol a person consumes since a consequent peak in systemic acetaldehyde could cause discomfort. These results underscore the importance of ADH1B and ADH1C in the association between alcohol consumption and the risk for HNC.

Introduction

Cancers of the head and neck (oral cavity, pharynx and larynx) are a related group of cancers. It has been estimated that ~400 000 incident cases of cancer of the oral cavity and pharynx and 160 000 incident cases of laryngeal cancer occur every year with ~300 000 people dying of the disease (1). The incidence varies worldwide, with some of the highest incidence areas being Southeast Asia and parts of Europe and South America (2). The aetiology of these cancers is largely due to alcohol and tobacco use, although other established risk factors are areca nut use and infection by the human papillomavirus (for cancers of the oropharynx) (3–5). Low fruit and vegetable intake, occupational exposures, poor oral hygiene and mouth-washes containing alcohol have also been associated with this group of cancers (3,6).

The International Agency for Research on Cancer (IARC) has classified alcohol beverages as ‘carcinogenic to humans’ largely (7–9) based on ‘sufficient’ epidemiological evidence for causality for cancers of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum and female breast. This risk appears to be modified by polymorphisms in genes that metabolise alcohol or acetaldehyde (10,11). Ethanol from alcoholic beverages is oxidised to its primary metabolite, acetaldehyde, largely by the family of alcohol dehydrogenase (ADH) enzymes and also to some extent by CYP2E1 in heavy drinkers (12) (Figure 1). Acetaldehyde is metabolised by the aldehyde dehydrogenase (ALDH) enzymes to the non-toxic acetate, at which point it is rapidly cleared from the body (12) (Figure 1).

Acetaldehyde in alcoholic beverages has also been classified as ‘carcinogenic to humans’ by the IARC (9). Acetaldehyde has been shown to be carcinogenic in animals, highly toxic and mutagenic (8,13). Accumulation of acetaldehyde can cause adverse symptoms such as nausea, facial flushing and increased heart rate (9). Its carcinogenic mechanism may be mediated through interference with DNA synthesis and repair, forming DNA adducts, and binding to proteins to alter their structure and function (8,13,14). The mechanism by which ethanol exerts its carcinogenic effects (independent of acetaldehyde) has yet to be identified. It is hypothesised that ethanol may act as a solvent for tobacco carcinogens or that alcoholic beverages could contain carcinogenic impurities (15). Many studies have shown an enhanced oncogenic effect of known carcinogens when co-administered with ethanol (8).

There is evidence that polymorphisms in the ADH gene, that code for efficient (fast) metabolism of ethanol to acetaldehyde, influence the risk of head and neck cancer (HNC). A previous review and pooled analysis of ADH genes and HNC risk in 2004 by Brennan et al. (11) included data from seven published articles on ADH1C and the only article on ADH1B at the time, although none of the seven ADH1C studies were conducted in Asia. Since then, additional studies have been published on the association between ADH1C (15 studies) and ADH1B (11 studies) and HNC risk, including studies in Asian populations. Therefore, we conducted a systematic review and meta-analysis to examine the association between ADH1B and ADH1C polymorphisms and HNC risk overall, by race/ethnicity, by level of alcohol consumption and by disease sites (oral cavity, pharynx and larynx).

ADH family of enzymes

The ADH enzymes are responsible for the majority of the oxidation of ethanol to acetaldehyde, which is metabolised to acetate by ALDH2 (14). There are seven genes within a 380-kb region on the long arm of chromosome 4 that encode the seven known isozymes of human ADH according to their structural
characteristics (12): ADH1A, ADH1B and ADH1C (Class I isozymes formerly known as ADH1, ADH2, and ADH3, respectively), ADH4 (Class II), ADH5 (Class III), ADH6 (Class V) and ADH7 (Class IV) (16). The Class I ADHs, which metabolise most of the alcohol consumed (17), are dimeric molecules formed by the association of α, β or γ subunits: (ADH1A: αα, αβ or αγ; ADH1B: ββ or βγ; ADH1C: γγ) (18,19).

Almost every tissue expresses ADH. Class I ADHs are expressed abundantly in the liver: ADH1A expression occurs primarily during early days of the foetus, becomes less active during gestation and is weakly expressed during adult life when ADH1B and ADH1C (to a lesser extent) predominate expression (20). Both ADH1B and ADH1C have very low expression in the upper gastrointestinal mucosa (21,22).

ADH4, which is minimally involved in ethanol oxidation (19), is primarily expressed in the liver (23,24) but is also expressed at low levels in the stomach, pancreas and small intestine (23). ADH5 is expressed ubiquitously, even in the upper gastrointestinal mucosa, but oxidises ethanol poorly (25). The function of ADH6, which is expressed in the liver and stomach (26), in ethanol metabolism is unclear though amino acid residues in the substrate-binding pocket suggest a low activity for ethanol metabolism (27). ADH7, the major ADH expressed in the upper gastrointestinal mucosa (oesophagus, gingiva, mouth, and tongue) and the stomach (19,21,28), has a lower affinity but higher capacity for oxidising ethanol compared to ADH1B and ADH1C (19).

The ADH1B

Three alleles have been identified in ADH1B that result from functional polymorphisms at both codons 48 (Arg48His; rs1229984) and 370 (Arg370Cys; rs2066702) (16) (Table I). The ADH1B*1 (β1) wild-type allele is defined by arginine residues at codons 48 (Arg48) and 370 (Arg370) while the ADH1B*2 (β2) variant allele is defined by His48 and Arg370. A third allele (ADH1B*3), defined by the haplotype of Arg48 and Cys370, has been identified but reported infrequently in the literature. It is possible that the ADH1B*3 (β3) allele could also be defined by the double variants His48 and Cys370, although this has not been observed (16,29).

The ADH1B*2 allele codes for a ‘fast’ variant of the ADH1B enzyme. ADH1B*2/*2 homozygote variants metabolise ethanol to acetaldehyde ~40 times faster than ADH1B*1/*1
The ADH1C*1 allele has been observed in southwest American Indians, African Americans and in groups of African origin in the Caribbean (33–37). ADH1B*3 encodes for 15% faster metabolism of ethanol than the *1 allele.

The ADH1C

Two alleles have been identified within the ADH1C gene (Table 1). The ADH1C*1 allele is a haplotype defined by Arg272 and Ile350, whereas the ADH1C*2 allele is the haplotype defined by Gln272 and Val350 (38). According to the data from SNP500Cancer database (39), the Arg272Gln (rs1693482) and Ile350Val (rs698) single-nucleotide polymorphisms (SNPs) showed perfect linkage disequilibrium (LD, r² = 1) among subjects of African/African American, Caucasian, Hispanic or Pacific Rim heritages (Chinese, Japanese, Korean). Furthermore, Hashibe et al. (40) reported an r² > 0.97 between rs1693482 and rs698 among Europeans and Latin Americans. Therefore, either rs1693482 or rs698 can be used to distinguish the ADH1C*1 and ADH1C*2 alleles.

The catalytic activity of ADH1C is primarily affected by an amino acid change in codon 272 (Arg272Gln) while codon 350 (Ile350Val) has not been shown to be important for the enzymatic properties of ADH1C (38). The ADH1C*1/*1 genotype is associated with 2.5 times faster metabolism than the ADH1C*2/*2 genotype (17,38). The frequency of the fast metabolising ADH1C*1 allele has been reported to be 48–69% in Caucasians, 75–90% in Africans and 85–100% in Asians (Supplementary Figure 1, available at Mutagenesis Online) (11,41).

Endogenous and microbial ADH in alcohol metabolism

Alcoholic beverages are slowly absorbed by the stomach upon consumption, but most of the ethanol consumed is rapidly absorbed by the intestines and then undergoes metabolism in the liver before it is released into systemic circulation (Figure 1) (8). The ADH1B and ADH1C enzymes in the liver can thus influence the amount of systemic ethanol that is redistributed throughout the body, including to the mucosa of the upper aerodigestive tract.

Homann et al. (42) have proposed that while the majority of ingested ethanol gets quickly absorbed by the intestine and metabolised by the liver, a small portion causes local damage of the upper gastrointestinal mucosa and is also redistributed via blood to the salivary gland. Once the systemic ethanol has reached the oral mucosa through saliva, it is efficiently oxidised to acetaldehyde by the oral microflora (the oral mucosa has minimal endogenous ADH activity), thereby determining the local salivary acetaldehyde concentration to which the tissues of the upper gastrointestinal tract are exposed (42).

Certain oral bacteria (i.e. Neisseria) have high levels of ADH and low levels of ALDH, which would allow acetaldehyde to accumulate in the oral cavity. Neisseria are also able to tolerate higher ethanol levels compared to other bacterial species; thus, alcohol consumption may suppress the growth of ADH-negative bacteria and give ADH-positive bacteria (such as Neisseria) a growth advantage (43).

Materials and methods

The current systematic review and meta-analysis follows PRISMA guidelines (44).

Literature search

A search for literature published in any language was conducted until March 11, 2011, using the PubMed database with the following search terms: ‘(Head and Neck Neoplasms [Mesh] OR ‘Head and neck’ OR ‘oral’ OR ‘pharynx’ OR ‘larynx’ OR ‘upper aerodigestive tract’) AND (ADH1B OR ADH2 OR ‘alcohol dehydrogenase 1B’ OR ‘alcohol dehydrogenase 2’ OR ADH1C OR ADH1B*1 OR ‘alcohol dehydrogenase IC’ OR ‘alcohol dehydrogenase 3’). A total of 98 articles were found using the above search terms (Figure 2). Articles were excluded if they had the following characteristics: (i) review articles (n = 11), (ii) non-epidemiologic articles (n = 10), (iii) assessed non-cancerous conditions only (n = 13), (iv) reported on cancerous sites other than HNC (oral cavity, pharynx, and larynx) (n = 31), (v) correspondence (n = 1), (vi) evaluated the occurrence of multiple lesions in HNC cases (n = 3) and (vii) did not include the SNPs of interest in ADH1B and ADH1C (n = 1). The reference lists of pertinent publications were also reviewed to capture relevant data sources that may not have been identified with the search criteria. A total of 28 articles with 29 studies were included in the final analysis (Supplementary Table 1, available at Mutagenesis Online) (11,40,45–70).

Data abstraction

All articles were evaluated independently by two reviewers (J.S.C. and N.G.) who extracted data that included authors, year of publication, country of origin, sources of case–control selection, number of cases and controls for the genotypes of ADH1B and ADH1C polymorphisms, variables used for matching and for statistical adjustments, and odds ratios (ORs) with corresponding 95% confidence intervals (CIs). For articles that only presented frequency data, ORs and 95% CIs were calculated using the exact method. When there was a cell with a count of 0.5 was added to all cells to permit calculation of the OR and 95% CIs (continuity correction) (71). For studies of upper aerodigestive cancers (HNC and esophageal cancer), data on esophageal cancer were excluded from the analysis when possible.

The data abstraction for ADH1B focused on one SNP, rs1229984 (Arg48His), that constitutes two alleles of ADH1B, allele *1 = Arg48 and allele *2 = His48. The data abstraction for ADH1C focused on two SNPs, rs1693482 (Arg272Gln) and rs698 (Ile350Val), that constitute the two alleles (haplotypes) of ADH1C (*1 = 272Arg + 350Le; *2 = 272Gln + 350Val). For studies that reported the results of these two SNPs separately instead of the haplotypes, the alleles were defined as *1 = 272Arg + 350Le and *2 = 272Gln + 350Val, recognizing that rs1693482 affects the functional activities of ADH1C (38) and is tightly linked to rs698 (Table 1).

Among the 28 articles, 3 (55,58,62) were excluded from the overall analysis due to overlap with other larger published studies but were included in the subgroup analyses (by organ site or level of alcohol drinking) when the larger studies did not provide sufficient information. Data were abstracted from the original manuscripts for 7 studies (45–50,52) except when more detailed information on alcohol consumption was warranted from an analysis that pooled these studies (11). Individual studies, rather than the single pooled information on alcohol consumption was warranted from an analysis that pooled these studies (11). Inconsistencies between studies were examined using the I² statistic and the Q test (73). Forest plots displayed the ORs of individual studies to allow for visual assessment for the extent of heterogeneity between studies (74).

Statistical analysis

All statistical analyses were performed using STATA (version 11.0; StataCorp, College Station, TX, USA). The ‘metan’ command (72) was used generate summary ORs (meta-ORs) from random effect models using the DerSimonian and Laird method (73). Forest plots displayed the ORs of individual studies to provide visual assessment for the extent of heterogeneity between studies (74). Inconsistencies between studies were examined using the I² statistic; I² of 0–24.9%; 25–49.9% and 50% or more indicated low, moderate and high inconsistency between studies, respectively (75).

Analyses were initially performed using both dominant (≥1 copy of the fast allele versus 2 copies of the slow allele) and recessive inheritance models (2 copies of the fast allele versus ≥1 copy of the slow allele), without assuming any mode of inheritance (by analysing heterozygous and homozygous fast genotypes separately). Only the results of a dominant model are presented because (i) one copy of the fast variant allele is enough to affect enzyme activity (ii) the summary ORs for ADH1C were similar for the *1/*2 heterozygotes (meta-OR = 0.88; 95% CI: 0.79–0.97) and *1/*1 homozygotes (OR = 0.84; 95% CI: 0.72–0.99) and (iii) the proportion of ADH1B*2/*2
homoygotes among Caucasians was small. Site-specific analyses were also performed for the oral cavity, pharynx and larynx.

Association between \textit{ADH1B} or \textit{ADH1C} polymorphisms and risk of HNC by alcohol drinking status was assessed through stratified analyses of high and low consumption groups. Because the measures of alcohol consumption (e.g. number of drinks per week, number of times per week, grams per day, etc) were reported differently across studies, we divided the categories of individual studies at the midpoint into ‘low’ and ‘high’ alcohol consumption groups. Studies among heavy drinkers (45,51,55,59) were placed in the high alcohol consumption group. Non-drinkers were analysed separately.

We performed subanalyses comparing unadjusted estimates with those adjusted for alcohol consumption. Though alcohol consumption and cigarette smoking are two strong risk factors for HNC (3–5), they may not be associated with one’s inherited \textit{ADH} genotypes and therefore would not satisfy the definition of a potential confounder nor warrant adjustment in the analyses of \textit{ADH} genes and HNC risk. Further, \textit{ADH} genotypes may influence one’s drinking behaviour (18,76,77) and could therefore be considered an intermediate in the causal pathway, adjusting for an intermediate would distort the results (78). However, adjusting for alcohol consumption (as an intermediate) may assess whether \textit{ADH} genes can influence HNC risk through mechanisms other than drinking behaviour (78).

Sensitivity analysis was performed by dropping one study at a time to assess whether any single study had a strong influence on the summary OR, using the ‘metaint’ command. Additional sensitivity analyses were performed excluding studies with \textit{ADH1B} or \textit{ADH1C} genotypes that were not in Hardy–Weinberg equilibrium. Studies with hospital-based controls were compared to those with population-based controls. We also stratified the analyses by region to account for population stratification (79) and potential regional differences in alcohol exposure. Publication bias was visually examined using funnel plots (80).

Results

\textbf{Meta-analysis results for ADH1B}

A total of 11 articles with 13 studies were included in the overall meta-analysis for \textit{ADH1B} (Table II and Figure 3; Supplementary Table 1, available at Mutagenesis Online). The meta-OR showed that carrying a *2 allele was associated with a reduced risk of HNC (meta-OR = 0.50, 95% CI: 0.37–0.68). The association appeared stronger among Asians (meta-OR = 0.32, 95% CI: 0.20–0.50) compared to other racial/ethnic groups. Combining data for Caucasians in the European and North American studies (40,53,66,68,70) resulted in a meta-OR of 0.70 (95% CI: 0.45–1.10).

Sensitivity analyses did not detect any single study with a dominant influence on the overall meta-OR (data not shown). Excluding two studies that did not test for Hardy–Weinberg
equilibrium (51,60) did not change the result significantly (meta-OR = 0.55, 95% CI: 0.40–0.75). Analysis by source of controls was not possible because only one study used population controls (53). The funnel plot (Figure 4A) showed symmetry, indicating minimal evidence of publication bias.

Site-specific analyses showed that having at least one copy of the ADH1B*2 allele was associated with a reduced risk only for laryngeal cancer, although the results were based on data from only four studies (53,58,66,70) (Table II). Analyses by level of alcohol consumption showed that the association between ADH1B*1/*2 + *2/*2 genotypes and risk of HNC was stronger among those with higher alcohol consumption (high alcohol: meta-OR = 0.32, 95% CI: 0.20–0.52; low alcohol: meta-OR = 0.60, 95% CI: 0.39–0.92; non-drinkers: meta-OR = 1.41, 95% CI: 0.82–2.42); although a chance finding could not be ruled out since results were based on a small number of studies, especially for non-drinkers.

Meta-analysis results for ADH1C

A total of 20 articles with 22 studies were included in the overall meta-analysis for ADH1C (Table II and Figure 5; Supplementary Table 1, available at Mutagenesis Online). Most of these studies had an OR ≤ 1.0 associated with the ADH1C*1 allele, with only 7 studies showing an OR >1.0 (only 1 of the 7 studies was statistically significant). Carriers of the *1 allele had a reduced risk of HNC (meta-OR = 0.87, 95% CI: 0.76–0.99). The association between ADH1C and HNC appeared stronger among Asians (meta-OR = 0.41, 95% CI: 0.15–1.16) compared to other racial/ethnic groups, although the result for Asians were based on only three studies (61,63,64) and was not statistically significant. Combining data for Caucasians in the studies from Europe and North America (40,45,47,48,50,52,53,59,68) resulted in a meta-OR of 0.88 (95% CI: 0.74–1.05).

Sensitivity analyses did not identify any single study with a strong influence on the overall meta-OR (data not shown).

Excluding four studies whose ADH1C genotypes were out of Hardy–Weinberg equilibrium (45,47,49,63) did not change the overall result significantly (meta-OR = 0.85, 95% CI: 0.73–0.98). The funnel plot (Figure 4B) showed asymmetry with an over-representation of smaller studies with relative risks < 1, indicating some evidence of publication bias; however, excluding four studies with relative risks < 1 and large standard errors (45,61,64,67) did not change the meta-OR substantially (meta-OR = 0.88, 95% CI: 0.77–0.99). Most studies used hospital-based controls and showed an inverse association between ADH1C*1/*2 + *1/*1 genotypes and HNC risk (meta-OR = 0.80, 95% CI: 0.69–0.94; 17 studies).

Site-specific analyses showed that the ADH1C*1/*2 + *1/*1 genotypes were associated with a reduced risk for oral (meta-OR = 0.81, 95% CI: 0.60–1.08) and pharyngeal cancer (meta-OR = 0.70, 95% CI: 0.51–0.98) but not for laryngeal cancer (meta-OR = 1.02, 95% CI: 0.79–1.31). Analyses by level of alcohol consumption showed that the ADH1C*1/*2 + *1/*1 genotypes were associated a slightly lower risk of HNC among drinkers (high alcohol: meta-OR = 0.94, 95% CI: 0.69–1.27; low alcohol: meta-OR = 0.90, 95% CI: 0.72–1.12; non-drinkers: meta-OR = 1.10, 95% CI: 0.73–1.66), though none of these results were statistically significant.

Discussion

A previous pooled analysis of 7 studies on ADH1C and HNC risk (11) hypothesised and showed that the fast variants of the ADH genes increase the risk of HNC, possibly by increasing the level of systemic acetaldehyde. At that time, only one article on the association between ADH1B and HNC risk was available and none of the studies of ADH1C were conducted in Asian populations (51). We conducted a meta-analysis that included additional studies on ADH1C (15 studies) and ADH1B (11 studies) published since the pooled analysis.
including studies in Asian populations. We observed that the slow variants of the ADH genes increase the risk of HNC, contrary to the associations proposed by the previous pooled analysis (11).

We observed an inverse association between HNC risk and carrying at least one copy of the fast metabolising ADH1B*2 (meta-OR, 0.50; 95% CI: 0.37–0.68) and ADH1C*1 alleles (meta-OR, 0.87; 95% CI: 0.76–0.99); the majority of individual studies were consistent and showed an OR < 1.0 (ADH1B: 11 of the 13 studies; ADH1C: 15 of 22 studies). The inverse association appeared to be stronger among Asian populations.

The ADH1B*2 and ADH1C*1 alleles may possibly protect against HNC by decreasing the opportunity for oral microflora to produce acetaldehyde locally due to a prolonged systemic circulation of ethanol. Studies have shown that Japanese alcoholics who carry a fast ADH1B*2 allele had lower levels of ethanol and acetaldehyde in their blood and saliva compared to those with the slow ADH1B*1/*1 genotype (81,82). In individuals with the slow ADH1B*1/*1 genotype, higher blood (systemic) ethanol levels could result after alcohol consumption from the slower oxidation of ethanol to acetaldehyde; this systemic ethanol can be efficiently converted to salivary acetaldehyde by the microorganisms in the oral cavity (42). Furthermore, mucosal cells have little ALDH2 activity (and therefore cannot efficiently detoxify acetaldehyde) possibly leaving the mucosal tissue exposed longer to salivary acetaldehyde and thereby increasing the risk of HNC in ADH1B*1 carriers (42).

In the group of Japanese alcoholics, ADH1C genotypes did not affect the level of blood or salivary ethanol or acetaldehyde, whereas the influence of the ADH1B genotype was strong even after accounting for ADH1C genotypes, suggesting that ADH1B was the main determinant of blood and salivary acetaldehyde level (82). ADH1B may be more influential in determining the acetaldehyde level among Pacific Rim Asians because (i) ADH1B variants have a 40-fold difference in enzyme activities that is more prominent than the 2.5-fold difference in enzyme activities between the ADH1C variants (12), and (ii) ADH1C is much less polymorphic among Pacific Rim Asians (ADH1C*2 allele ranges from 5 to 14%) than Caucasians and therefore less relevant in affecting the risk of HNC compared to ADH1B (ADH1B*1 allele ranges from 15 to 41%) (11).

In Caucasians, ADH1C could have a more influential role in alcohol metabolism than ADH1B because of the higher prevalence of the fast allele: ADH1C*1 ranges from 48 to 69% while ADH1B*2 allele ranges from 1 to 5% (11). In a study conducted among 12 Caucasian subjects, Visapää et al. (55) observed that subjects with the fast metabolising ADH1C*1/*1 genotype had higher salivary acetaldehyde levels after alcohol intake compared to ADH1C*2 carriers. The sample size was small (n = 12) and further study is needed to determine the influence of ADH1C alleles on salivary acetaldehyde levels.

ADH1B and ADH1C may determine exposure to ethanol and acetaldehyde through their influence on drinking behaviour. Several studies have shown an increased risk of alcoholism associated with the ADH1B*1 (18,77) and ADH1C*2 alleles (76,77) that confer slow metabolism of ethanol to acetaldehyde. These persons could possibly drink more since they would not experience the toxic side effects of acetaldehyde. The ADH1B and ADH1C fast alleles could be inversely associated with HNC.

![Fig. 3. Analysis of ADH1B and HNC risk by region, dominant model (*2/*2 + *1/*2 versus *1/*1).](image)

### Table: Analysis of ADH1B and ADH1C genotypes by region

<table>
<thead>
<tr>
<th>Region</th>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>Yokoyama (2001)</td>
<td>0.15 (0.06, 0.36)</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>Yokoyama (2006)</td>
<td>0.49 (0.20, 1.20)</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>Asakage (2007)</td>
<td>0.21 (0.11, 0.41)</td>
<td>7.66</td>
</tr>
<tr>
<td></td>
<td>Solomon (2008)</td>
<td>0.76 (0.26, 2.07)</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>Oze (2009)</td>
<td>0.34 (0.23, 0.51)</td>
<td>10.23</td>
</tr>
<tr>
<td></td>
<td>Subtotal (I-squared = 49.6%, p = 0.094)</td>
<td>0.32 (0.20, 0.50)</td>
<td>35.17</td>
</tr>
<tr>
<td>Europe</td>
<td>Risch (2003)</td>
<td>0.06 (0.41, 1.82)</td>
<td>7.13</td>
</tr>
<tr>
<td></td>
<td>Hashibe (Europe)</td>
<td>0.49 (0.35, 0.72)</td>
<td>10.56</td>
</tr>
<tr>
<td></td>
<td>Hashibe (ARCAOE)</td>
<td>0.56 (0.41, 0.74)</td>
<td>11.10</td>
</tr>
<tr>
<td></td>
<td>Soucek (2010)</td>
<td>1.67 (0.45, 6.25)</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td>Marichalar-Mendia</td>
<td>0.20 (0.05, 0.80)</td>
<td>3.49</td>
</tr>
<tr>
<td></td>
<td>Subtotal (I-squared = 38.1%, p = 0.161)</td>
<td>0.58 (0.41, 0.80)</td>
<td>36.02</td>
</tr>
<tr>
<td>Latin America</td>
<td>Hashibe (L. America)</td>
<td>0.61 (0.46, 0.82)</td>
<td>11.15</td>
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<td></td>
<td>Garcia (2010)</td>
<td>0.42 (0.21, 0.85)</td>
<td>7.51</td>
</tr>
<tr>
<td></td>
<td>Subtotal (I-squared = 0.0%, p = 0.334)</td>
<td>0.58 (0.44, 0.75)</td>
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</tr>
<tr>
<td>North America</td>
<td>Wei (2010)</td>
<td>1.29 (0.86, 1.94)</td>
<td>10.15</td>
</tr>
<tr>
<td></td>
<td>Subtotal (I-squared = .%, p = .)</td>
<td>1.29 (0.86, 1.94)</td>
<td>10.15</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>0.50 (0.37, 0.68)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis.
risk, as observed in our meta-analysis, through their direct role in alcohol metabolism and by protecting against heavy alcohol consumption.

The effect of the \( \text{ADH} \) genes in the risk of HNC may be more pronounced among those with higher alcohol consumption. When we stratified by level of alcohol drinking (non-drinker, low, high), there appeared to be a dose–response relationship, with the most protection being offered to those with fast metabolising \( \text{ADH1B} \) (and \( \text{ADH1C} \) to a lesser extent) variants in the highest drinking category. Although carriers of the fast alleles may be protected against alcoholism, some carriers drink heavily, although perhaps less than carriers of the slow variants. The reduced risk for HNC among heavy drinkers with fast variants may be due to faster metabolism of ethanol that (i) reduces the local conversion of systemic ethanol to acetaldehyde by oral microflora and (ii) reduces the carcinogenic effect of ethanol itself (not through acetaldehyde).

Although these results are intriguing, the interpretation is hampered by the different measures employed by individual studies to report levels of alcohol drinking, which created difficulty for creating categories of alcohol consumption for the gene-environment meta-analysis (83).

The risk estimates for \( \text{ADH1B} \) and \( \text{ADH1C} \) were more similar for the oral cavity than for the other HNC sites perhaps because the oral cavity experiences the highest local exposure from direct contact with alcoholic beverages and also from the acetaldehyde produced by oral microflora. Yokoyama et al. (81) showed that acetaldehyde production was significantly and positively associated with counts of salivary bacteria and yeast and also that microorganism counts and acetaldehyde production decreased after 3 weeks of abstinence from alcohol.

It is plausible that chronic alcoholics and those with habitually high alcohol intake have higher amounts of oral microflora and correspondingly poor oral health. It has also

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**Fig. 4.** Funnel plot of studies assessing (A) \( \text{ADH1B} \) and risk of HNC and (B) \( \text{ADH1C} \) and risk of HNC.
been shown previously that poor oral health is a risk factor for cancers of the head and neck, particularly for cancers of the oral cavity and pharynx, independent of tobacco smoking and alcohol drinking (6). Individuals with missing teeth may have a greater burden of oral flora that is more effective in the production of acetaldehyde, thereby placing these individuals at increased risk of HNC (84) (Figure 1). However, it should be noted that there were a relatively small number of studies for the analyses by subsite and the results should be interpreted with caution.

Cigarette smoking is another source of acetaldehyde (85), which has also been shown to increase salivary acetaldehyde production (86) (Figure 1). Since only four studies presented results stratified by smoking habit (40,54,64,66), it was difficult to disentangle the impact of acetaldehyde from cigarette smoking in this analysis; therefore, smoking was not examined in the current meta-analysis for its interaction with ADH1B or ADH1C. However, three studies (40,54,64) did find a significant interaction between ADH genes and smoking on the risk of HNC, with an increased risk of HNC associated with the slow ADH1B*1 and ADH1C*2 alleles only among current and former smokers (58) or among those who had smoked more heavily (54,64). The increased risk of HNC associated with the slow ADH1B*1 and ADH1C*2 alleles among smokers (including current and former smokers) observed by other studies could be due to the effects of acetaldehyde from cigarette smoke being enhanced among carriers of the slow ADH1B and ADH1C alleles who could have more microbial production of acetaldehyde from alcohol consumption.

Acetaldehyde may not be entirely responsible for the carcinogenic effect of alcohol. Ethanol can act as a solvent for carcinogens from tobacco smoke and can enhance chemically induced carcinogenesis (8) (Figure 1). The fast ADH1B*2 and ADH1C*1 alleles may protect against HNC by reducing the accumulation of ethanol and thereby prevent it from acting as a solvent.

Some suggest that the effect of the ADH1C variants and HNC may be through their LD with the ADH1B variants (61,65); however, Hashibe et al. reported that ADH1B Arg48His (rs1229984) and ADH1C Arg272Gln (rs1693482) were independently associated with risk of upper aerodigestive tract cancers even after accounting for the effect of each other (40). In this study, the two measures of LD were reported, $D'$ and $r^2$ (both ranging from 0 to 1), for the linkage between ADH1B Arg48His (rs1229984) and ADH1C Arg272Gln (rs1693482) were $>0.75$ and $\leq 0.03$, respectively, for study subjects from Latin America and Central and Eastern Europe (40). Similarly, we used data from the SNP500Cancer database (39) to calculate the linkage between ADH1B Arg48His and ADH1C Arg272Gln to obtain $D' = 0.81$ and $r^2 = 0.21$ for subjects of Pacific Rim heritage and an $r^2 = 0.11$ among Caucasians (the $D'$ in Caucasians could not be calculated due to a low frequency of the ADH1B*2 allele) (39).
A value of D’ equal to 1 means complete LD, suggesting no evidence of historical recombination, and D’ = 0 indicates no LD. D’ < 1 suggests that a disruption of complete ancestral LD has occurred: intermediate values have no clear meaning in the relative strength of LD and should not be used to measure the degree of LD (87). In addition, D’ can be highly inflated in small sample sizes, especially for rare alleles (such as ADH1B*2 in Caucasians), producing a high D’ value even when markers are not in LD (87). In contrast to D’, r² can be used to measure the extent of LD: r² is subject to less upward inflation by a small sample size compared to D’ (87, 88) and is the preferable measure for a genetic association study. Therefore, judging from the fact that D’ is not near 1, r² is low between ADH1B Arg48His and ADH1C Arg272Gln, and the known functionality of ADH1C Arg272Gln, it is likely that ADH1C Arg272Gln confers an independent influence on HNC risk and not merely through its LD with ADH1B Arg48His. However, the current meta-analysis could not directly evaluate the independent effect of ADH1B Arg48His and ADH1C Arg272Gln on the risk of HNC, which can only be assessed with more studies in different populations or in a pooled analysis.

In addition, though ALDH2 is not polymorphic among Caucasians, it is polymorphic among Pacific Rim Asians with 30–40% of the populations carrying at least one copy of the *2 null allele (11) (Supplementary Figure 1, available at Mutagenesis Online). Two studies showed that the increased HNC risk associated with the ADH1B*1 slow allele is more prominent in the presence of the ALDH2*2 null allele (51, 64). This may partly explain the stronger association between ADH1B and HNC among Asians observed in our analysis. In addition, while the salivary acetaldehyde in subjects with active ALDH2 is mainly produced by oral microflora (89), subjects who are deficient in ALDH2 may have an additional source of salivary acetaldehyde produced by the oxidation of ethanol in the parotid gland (90). Since the fast metabolising variants of ADH1B and ADH1C and the slow metabolising ALDH2 allele are more common in Pacific Rim Asians, further studies of the effects of ADH1B and ADH1C variants, stratified by ALDH2 genotype need to be conducted in these populations.

Recent studies have shown that other ADH genes, including ADH4 and ADH7, are associated with risk of HNC (40, 64, 66). For example, ADH1B and ADH1C are primarily expressed in the liver and adrenal glands whereas ADH7 is expressed in the mucosa of the upper gastrointestinal tract, including the oral cavity, where it may influence the local production of acetaldehyde (22, 28). ADH7 also efficiently metabolises retinol, a vitamin that may be inhibited by alcohol consumption but is required for the growth and differentiation of epithelial tissues of the head and neck (91). Therefore, a complete study is warranted on the association between HNC and (i) acetaldehyde levels in blood and saliva (ii) retinol intake (iii) the contribution of oral microflora and (iv) genes in the alcohol metabolising pathway, especially ADH7, which is expressed in the upper gastrointestinal mucosa, that can limit the systemic bioavailability of alcohol (19) and also influence local acetaldehyde levels.

Several limitations need to be considered when interpreting the results of the current analysis. Designs were heterogeneous across studies with regard to patient population and control selection (population versus hospital based). To incorporate the potential heterogeneity across studies, we used random-effect models to calculate meta-ORs and performed subanalyses by region and by control selection method. An additional source of heterogeneity may be the difference in the choice of study population, as some studies were conducted among heavy drinkers (45, 51, 55, 59, 69), which may have produced different results from studies conducted in the general population. However, this difference should be viewed in terms of gene–environment interaction, with a stronger association between ADH1B*2 and HNC risk observed among heavy drinkers in our analysis when stratified by drinking status.

There were few studies for certain subanalyses (e.g., subsite analysis for ADH1B, the number of Asian studies for ADH1C), which indicates the importance of additional research to fill these insufficiencies. Previous studies suggest that the association between alcohol consumption and HNC is stronger for cancers of the oral cavity and pharynx compared to laryngeal cancer (92–94). Most studies included in our meta-analysis did not examine the association between ADH genes and HNC subsites (oral cavity, pharynx or larynx); therefore, although we attempted to perform meta-analysis by HNC subsites, the small number of studies may preclude a clear interpretation of the results. Finally, some publication bias was suggested, particularly for ADH1C, but a sensitivity analysis showed that it did not impact the summary results.

We also assessed the strength of cumulative evidence for these genetic associations using the ‘Venice Criteria’ (Supplementary Table 2, available at Mutagenesis Online) (95). For ADH1B and ADH1C, the number of subjects easily exceeded the threshold for ‘large-scale evidence’ and the amount of heterogeneity observed indicated a ‘well-conducted meta-analysis with some methodological limitations or moderate between study inconsistency’. We thought that ‘bias, if at all present, could affect the magnitude, but probably not the presence of the association’ for ADH1B since the results did not significantly differ in our sensitivity analyses. However, for ADH1C, the weak meta-OR (0.87) increases the likelihood that the presence of bias could invalidate the observed association and therefore we thought that there was ‘considerable potential for or demonstrable bias that can affect even the presence or absence of the association’. Taken together our results provide ‘moderate evidence’ for the inverse association between ADH1B*2 and HNC risk and ‘weak evidence’ for the inverse association between ADH1C*1 and HNC risk, according to the ‘Venice Criteria’.

In conclusion, we observed an inverse association between the fast metabolising ADH1B*2 and ADH1C*1 alleles and the risk for HNC. The magnitude of protection was strongest in Pacific Rim Asian populations, where the ADH1B and ADH1C fast alleles are more prevalent compared to Caucasians. Because ADH1B and ADH1C are only the first step in ethanol metabolism, future studies assessing their joint effects with the ALDH2 gene are warranted, particularly in Asian populations. In addition, the influence of oral microflora and other genes such as ADH4 and ADH7 in the ethanol metabolising pathway need to be investigated by levels of alcohol consumption and tobacco smoking to more completely capture the carcinogenic effect of alcohol and acetaldehyde in the development of HNC.

Supplementary data
Supplementary Tables 1 and 2 and Figure 1 are available at Mutagenesis Online.
References


