Alcohol & Alcoholism Vol. 45, No. 1, pp. 6–12, 2010 doi: 10.1093/alcalc/agp078
Advance Access publication 3 November 2009

GENETICS

Polymorphisms in Alcohol Metabolizing Genes and the Risk of Head and Neck Cancer in a Brazilian Population

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(*Received* 16 *May* 2009; *first review notified* 24 *July* 2009; *in revised form* 22 *September* 2009; *accepted* 2 *October* 2009)

Abstract — Aims: The incidence of head and neck cancer (HNC) in Brazil has increased substantially in recent years. This increase is likely to be strongly associated with alcohol and tobacco consumption, but genetic susceptibility also should be investigated in this population. The aim of this study was to evaluate the association of polymorphisms in genes of alcohol metabolism enzymes and the risk of HNC. **Methods:** A hospital-based case–control study was conducted in São Paulo, Brazil. We here investigated *ADH1C* Ile^{350}Val, *ADH1B* Arg^{48}His, *ADH1B* Arg^{370}Cys and *CYP2E1*5A Pst*I polymorphisms by PCR-RFLP Polymerase Chain Reaction - Restriction Fragment Length Polymorphism in 207 histopathologically confirmed HNC cases (184 males and 23 females) and 244 cancer-free controls (225 males and 19 females) admitted as in-patients in the same hospital. **Results:** Chronic alcohol intake increased approximately four times the risk of HNC. The mutant genotype *ADH1B* Arg^{48}His was more frequent in controls (12.7%) than HNC patients (5.8%) conferring protection for the disease (odds ratio (OR) = 0.42; 95% confidence interval (CI), 0.21–0.85). Similar results were observed for individuals with *ADH1B*2* (OR = 0.41; 95% CI , 0.20–0.82) or *ADH1B*2/ADH1C*1* (OR = 0.32; 95% CI , 0.13–0.79) mutated haplotypes. Multiple regression analyses showed that individuals with the mutant genotype *ADH1B* Arg^{48}His who consume alcohol >30 g/L/day have more than four times the risk for HNC (OR = 4.42; 95% CI, 1.21–16.11). **Conclusions:** The fast alcohol metabolizing genotypes may prevent HNC when the amount of alcohol intake is <30.655 g/L/day.

INTRODUCTION

The susceptibility of individuals to the ill effects of heavy alcohol consumption appears to be a result of complex interactions between genes and the environment. Since the beginning of the last century, researchers have known about the association between alcohol consumption and the increased risk for esophageal and upper digestive and respiratory tract cancers, and more recently for liver, colon rectum, and breast cancer in women (Boffeta and Hashibe, 2006).

Worldwide, an estimated 644,000 new cases of head and neck cancers (HNC) are diagnosed each year, with two thirds of the cases occurring in developing countries (Marur and Forastiere, 2008). HNC is the fifth most common cancer among the male population and the seventh most common among the female population in Brazil. The estimates for the incidence of HNC in 2008 were 10,380 new cases a year for males and 3780 for females per 100,000 inhabitants in Brazil (INCA, 2008).

Chronic excessive alcohol intake and tobacco use have a synergistic effect on the risk of HNC of the upper digestive and respiratory tract, although gene polymorphisms may contribute to the final risk (Argiris *et al*., 2008). The two classes of alcohol metabolism enzymes, alcohol dehydrogenase (ADH) which oxidizes ethanol to acetaldehyde and aldehyde dehydrogenase (ALDH) which oxidizes acetaldehyde to acetate, have functional polymorphisms that may alter the rate of synthesis of the toxic metabolite acetaldehyde or decrease its further oxidation (Edenberg, 2007).

In addition to ADH, when alcohol consumption is high, cytochrome P450 2E1 (CYP2E1, a member of the cytochrome P450 superfamily) also can catalyze ethanol into acetaldehyde while producing reactive oxygen species (ROS). Less than 10% of ingested alcohol is metabolized by CYP2E1 enzymes, which are part of the mitochondrial microsomal ethanol oxidizing system (Asakage *et al*., 2007).

DNA single nucleotide polymorphisms (SNPs) have shown to produce enzymes with distinct kinetic properties that may represent transcription, posttranscriptional or posttranslational modifications that alter the final enzyme phenotype (Carlton *et al*., 2006). There are ~240 SNPs in the 365 kb map at the chromosome 4q21–23 region were the seven *ADH* genes are localized. The two polymorphic sites *ADH1B* Arg^{48}His (previously Arg^{47}His) and Arg^{370}Cys (previously Arg^{369}His) are associated with different alcohol metabolism rates. SNPs in the *ADHB* genes may result in haplotypes with different rates of metabolism. The *ADH1B*2* haplotype, that encodes the enzyme subunit $\beta 2$, results in enzymes that have a 70- to 80-fold higher alcohol turnover rate than the *ADH1B*1* haplotype (Edenberg, 2007). The *ADH1C*1* haplotype ($\gamma 1\gamma 1$ enzyme) has a turnover rate that is ~70% higher than of the $\gamma 2\gamma 2$ enzyme (Ho *et al*., 2007).

Genetic variability of enzymes in the metabolism of alcohol may protect some individuals from alcohol addiction but may lead to differences in exposure to acetaldehyde, especially among individuals with chronic high levels of alcohol intake. The accumulation of acetaldehyde in the blood due to intense metabolism of alcohol or failure to eliminate the substrate at an adequate rate seems to be associated to cancer risk (Seitz and Becker, 2007). Studies have shown controversial results regarding *ADH* and *ALDH* polymorphism and the risk of HNC (Druesne–Pecollo *et al*., 2009). There are few studies in Brazilian populations that evaluate the *ADH* and *ALDH* polymorphisms and HNC, exception to polymorphisms in *ADH3*, which were found to be associated with upper aerodigestive tract cancers and polymorphisms in *ADH4*, which were found to be associated with alcohol dependence (Nishimoto *et al*., 2004; Guindalini *et al*., 2005).

Table 1. Main characteristics of the studied population including 244 controls and 207 HNC patients

	Groups							
	HNC		Controls			CI (95%)		
Variable	*n*	%	*n*	%	OR	Lower	Upper	*P**
Sex								
Male	184	88.9	225	92.2	1.00			
Female	23	11.1	19	7.8	1.48	0.78	2.80	0.228
Age								
Average ± SD	54.3 ± 7.8		53.6 ± 9.3					
Skin color								
White	149	72.0	148	59.8	1.00			
Mulatto	38	18.4	68	27.5	0.56	0.35	0.88	0.012
Black	20	9.7	29	11.9	0.68	0.37	1.25	0.211
Tobacco smokers								
Still	165	79.7	121	49.6	11.10	4.89	25.19	<0.001
Never	7	3.4	57	23.4	1.00			
In the past	35	16.9	66	27.0	4.32	1.78	10.47	0.001
Average packages/year	35		24					
Alcohol drinkers								
Still	127	61.4	106	43.4	4.39	2.35	8.22	<0.001
Never	15	7.2	55	22.5	1.00			
In the past	65	31.4	83	34.0	2.87	1.49	5.54	0.002
Average (grams/liter/day)	157		58					
Total	207	100	244	100				

*P values statistically significant (<0.05) by Fisher exact test.

The main objective of this study was to investigate polymorphic differences in the SNPs *ADH1B* Arg^{48}His, *ADH1B* Arg^{370}Cys, *ADH1C* Ile^{350}Val and *CYP2E1*5B* in a hospital-based population of HNC cases and controls. The associated risk of alcohol and tobacco use was also evaluated.

MATERIALS AND METHODS

Subjects

A total of 451 participants in this study were recruited from the Heliópolis Hospital, São Paulo, Brazil (Table 1). All 207 consecutive patients with histologically confirmed squamous cell carcinoma of the head and neck were preoperatively selected in the Department of Head and Neck Surgery and Otolaryngology and invited to participate, after been informed about the research. Eligible patients were 88.9% men (184/207) and 11.1% women (23/207), with ages varying from 24 to 81 years old (median 54.3 ± 9.5 years old). The patients were classified according to the International Classification of Diseases (ICD-10) as follows: 157 (76%) cases with carcinoma of the oral cavity and oropharynx (C00 to C14) and 50 (24%) cases with carcinoma of the larynx (C32).

For the control group, 244 patients, 92.2% men (225/244) and 7.8% women (19/244), varying from 20 to 82 years old (median 53.6 ± 10.6 years old), were selected in the same hospital from different clinics without any cancer diagnoses. These patients mainly had non-cancerous digestive (44%) and cardiovascular diseases (33%).

Subjects were interviewed and asked about their ethnicity and also about their parents' and grandparents' ancestry, according to their self-definition of skin color. Subjects were classified as Black when they defined themselves and their parents and all the grandparents as Black and presented the characteristic phenotype. The same classification was used for White and Asiatic origin individuals. Individuals of mixed ethnicity, mainly White and Black, were defined as Mulatto.

All subjects were also interviewed in-person using a standardized questionnaire regarding smoking habits, alcohol drinking history, dietary habits and occupational activities. The tobacco consumption was expressed in packs/year, which included variables of both intensity and duration of consumption. Information about alcohol consumption habits including frequency and quantity were estimated for various beverages, including beer (5% of ethanol), wine (20% of ethanol), and beverages with high concentration of ethanol, such as 'cachaça', a Brazilian spirit consumed in large scale by our population, that has 41% of ethanol from sugar cane.

The study protocol and the questionnaire were approved by the Institutional Ethics Committees, and all patients gave written informed consent to participate.

Genotyping

DNA extraction was performed by the salting-out method (Miller *et al*., 1988) from 5 mL of peripheral blood lymphocytes. After DNA extraction, gene polymorphisms were detected by PCR-RFLP polymorphisms by PCR-RFLP Polymerase Chain Reaction - Restriction Fragment Length Polymorphism protocols specific to each gene: *ADH1B* Arg^{48}His (previously Arg^{47}His) in exon 3 (rs1229984), *ADH1B* Arg^{370}Cys (previously Arg^{369}His) in exon 9 (rs2066702), *ADH1C* Ile^{350}Val (previously Ile^{349}Val) in exon 8 (rs698) and *CYP2E1*5A* in the 5′ flanking region of the gene (-1295G>C —rs3813867). After DNA amplification and DNA

Table 2. Genotyping conditions for *ADH1B* Arg^{48}His, *ADH1B* Arg^{370}Cys, *ADH1C* Ile^{350}Val and *CYP2E1**5A identification

Genes	Primers	Polymorphism (RefSNP)	PCR/RFLP restriction enzymes	DNA fragments size	Reference
ADH1B Arg^{48}His	Sense: 5′-ATT CTA AAT TGT TTA ATT CAA GAA G-3′, Antisense: 5′-ACT AAC ACA GAA TTA CTG GAC-3′	G to A transition (rs3813867)	M*sl*I	Wild type 685 bp, Variant 443 bp, 242 bp	Xu *et al*., 1998
ADH1B Arg^{370}CyS	Sense; 5′-TGG ACT TCA CAA CAA GCA TGT-3′, Antisense: 5′-TTG ATA ACA TCT CTG AAG AGC TGA-3′	C to T transition (rs3813867)	A*lw*NI	Wild type 201 bp, Variant 130 bp, 71 bp	Xu *et al*., 1998
ADH1C Ile^{350}Val	Sense: 5′-TTG TTT ATC TGT GAT TTT TTT TGT-3′, Antisense: 5′-CGT TAC TGT AGA ATA CAA AGC-3′	A to G transition (rs3813867)	S*sp*I	Wild type 378 bp, Variant 274 bp, 104 bp	Xu *et al*., 1998
*CYP2E1*5A*	Sense: 5′-CCA GTC GAG TCT ACA TTG TCA-3′, Antisense: 5′-TTC ATT CTG TCT TCT AAC TGG-3′	G to C transition (rs3813867)	*Pst*I	Wild type 410 bp, Variant 290 bp, 120 bp	Anwar *et al*., 1996

Table 3. Alcohol and tobacco considered together in the risk of HNC

		Groups							
		HNC		Controls			CI (95%)		
Alcohol	Tobacco	*n*	%	*n*	%	OR	Lower	Upper	*P**
Still	Never	3	2.4	14	13.2	1.00			
	Still	112	88.2	67	63.2	7.80	2.16	28.15	0.002
	In the past	12	9.4	25	23.6	2.24	0.54	9.31	0.267
	Total	127	100	106	100				
Never	Never	1	6.7	33	60.0	1.00			
	Still	9	60.0	15	27.3	19.80	2.30	170.70	0.007
	In the past	5	33.3	7	12.7	23.57	2.37	234.34	0.007
	Total	15	100	55	100				
In the past	Never	3	4.6	10	12.0	1.00			
	Still	44	67.7	39	47.0	3.76	0.96	14.66	0.056
	In the past	18	27.7	34	41.0	1.76	0.43	7.24	0.430
	Total	65	100	83	100				

* *P* values statistically significant (<0.05) by Fisher exact test.

specific enzyme digestion following standard protocols (Table 2), PCR products were separated by high-voltage electrophoresis on a 2% agarose gel and stained with ethidium bromide for visualization.

Statistical analysis

The statistical significance of the association between HNC and SNP polymorphisms was analyzed using the Fisher exact test (two-tailed; Agresti, 1992). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated as approximations of relative risk (Breslow and Day, 1980). The risk of oral cancer was estimated comparing subjects with *ADH1B* Arg^{48}His, *ADH1B* Arg^{370}Cys, *ADH1C* Ile^{350}Val and *CYP2E1*5A* mutated genotype (homozygous and/or heterozygous) against those without mutation, which was considered to be reference genotype. Alcohol consumption was evaluated based on categories of cumulative consumption expressed according to dose estimated as grams by day; the risk point for the consumption of the alcohol was found by use of the receiver operating characteristic (ROC) curve. The OR estimates were obtained by non-conditional logistic regression modeling adjusted for potential confounders. Statistical significance was assessed by the maximum likelihood ratio test. The disease effect of variables that showed $P < 0.02$ in the univariate analysis was compared by multiple logistic regression analysis. The statistical significance of the association was determined by chi-square tests using the statistical computer software SPSS (version 15.0), and the critical level of rejection of the null hypothesis was considered to be 5%.

RESULTS

Table 1 shows the major demographic characteristics of the 207 patients with HNC and the 244 controls. The study groups were very similar regarding sex and age; both consisted mainly of men aged 60 years old or less. Regarding ethnicity, 72% of HNC patients and 59.8% of the controls were classified as White, as showed in Table 1.

Among the HNC cases, only 3.4% reported that they did not smoke, compared to 23.4% in the control group. The difference was considered significant ($P < 0.001$), resulting in more than a 10-time increased risk of cancer among smokers (OR, 11.1; 95% CI, 4.89–25.19), as showed in Table 1. HNC patients reported median alcohol consumption (157 g/L/day)

Table 4. The diary alcohol intake and the risk of HNC considering 207 patients and 244 controls

	Groups								
	HNC		Controls		Total			CI (95%)	
Diary alcohol intake (grams/liter/day)	*n*	%	*n*	%	*n*	%	OR	Lower	Upper	*P*
<30.655[a]	57	27.5	158	64.8	215	47.7	1.00			<0.001
≥30.655	150	72.5	86	35.2	236	52.3	4.83	3.23	7.23	
Total	207	100	244	100	451	100				

[a]Value estimated by ROC curve.

Table 5. *ADH1B*, *ADHIC* and *CYP2E1* genotypes and haplotype frequencies in 244 control individuals and 207 HNC patients and OR (95% CI) of cancer associated with genotypes

	Groups							
	HNC		Controls			95% CI		
Variables	*n*	%	*n*	%	OR	Lower	Upper	*P**
ADH1B Arg[48]His								
Arg/Arg	195	94.2	213	87.3	1.00			
Arg/His	12	5.8	29	11.9	0.45	0.22	0.91	0.026
His/His	–	–	2	0.8	[a]			–
Arg/His+ His/His	12	5.8	31	12.7	0.42	0.21	0.85	0.015
ADH1B Arg[370]Cys								
Arg/Arg	197	95.2	225	92.2	1.00			
Arg/Cys	10	4.8	17	7.0	0.67	0.30	1.50	0.332
Cys/Cys	–	–	2	0.8	[a]			–
Arg/Cys+ Cys/Cys	10	4.8	19	7.8	0.60	0.27	1.32	0.206
ADH1C Ile[350]Val								
Ile/Ilê	99	47.8	136	55.7	1.00			
Ile/Val	89	43.0	91	37.3	1.34	0.91	1.99	0.138
Val/Val	19	9.2	17	7.0	1.54	0.76	3.10	0.232
Ile/Val+ Val/Val	108	52.2	108	44.3	1.37	0.95	1.99	0.094
*CYP2E1 Pst*I								
c1/c1	188	90.8	227	93.0	1.00			
c1/c2	19	9.2	17	6.1	1.53	0.76	3.09	0.237
Haplotypes								
*ADH1B*1*	185	89.4	195	79.9	1.00			
*ADH1B*2*	12	5.8	31	12.7	0.41	0.20	0.82	0.012
*ADH1B*3*	10	4.8	18	7.4	0.59	0.26	1.30	0.189
Haplotype interactions								
*ADH1B*1/ADH1C*2*	98	47.3	89	36.5	1.00			
*ADH1B*2/ADH1C*1*	7	3.4	20	8.2	0.32	0.13	0.79	0.013
Other combinations	102	49.3	135	55.3	0.69	0.47	1.01	0.055
Total	207	100	244	100				

[a]It was not possible to calculate.
*P values statistically significant (<0.05) by Fisher exact test.

higher than that declared by the control group (58 g/L/day), resulting in more than a four-time increased risk of cancer among alcoholics (OR, 4.39; 95% CI, 2.35–8.22) and former alcoholics ($P = 0.002$) as showed in Table 1.

Alcohol consumption is socially acceptable in different countries, including Brazil. As identified in other studies, alcoholics also often are smokers, making it difficult to analyze separately the effects of each one of these habits individually. To assess the combined effect of tobacco and alcohol on cancer, all the possible comparisons between alcohol and tobacco use were evaluated, as showed in Table 3. The consumption of cigarettes combined with the use of alcohol increased by more than seven times the risk for HNC (OR, 7.80; 95% CI, 2.16–28.15), and the difference was also significant for smokers and never alcoholics ($P = 0.007$, Table 3).

Using an ROC curve, it was possible to estimate the amount of alcohol that maximizes the development of HNC. It was found that individuals who reported consuming over 30.655 g/L/day of alcohol had approximately five times greater risk of developing HNC (OR = 4.83; 95% CI, 3.23–7.23) compared with controls (Table 4).

The frequencies of *ADH* and *CYP2E1* allele polymorphism observed in the HNC patients and controls are presented in Table 5. The observed allele and genotype frequencies matched very closely the expected Hardy–Weinberg equilibrium frequencies for both cases and controls (data not shown).

Table 6. Multiple logistic regression analysis for the polymorphisms using consumption of alcohol in 30.655 g/L/day with alcohol and tobacco like habits variables

Variable	OR	CI (95%)		*P*
		Lower	Upper	
Age	1.02	1.00	1.04	0.106
Tobacco				
Never	1.00			
Still	8.31	3.49	19.75	< 0.001
In the past	3.82	1.52	9.61	0.004
Skin color				
Mulatto	1.00			
White	2.22	1.32	3.74	0.003
Black	1.14	0.53	2.44	0.736
Alcohol consumption/genotype				
<30.655/*ADH1B*Arg^{48}Arg	1.00			
≥30.655/*ADH1B*Arg^{48}Arg	3.01	1.90	4.78	< 0.001
<30.655/*ADH1B* Arg^{48}His or *ADH1B*His^{48}His	0.12	0.03	0.52	0.005
≥30.655/*ADH1B* Arg^{48}His or *ADH1B*His^{48}His	4.42	1.21	16.11	0.024

The autosomal dominant model was assumed for all gene polymorphism evaluated, considering together the homozygous and heterozygous genotypes for the variant alleles. The *ADH1B* histidine allele was associated with a decreased risk of HNC (OR = 0.42; 95% CI = 0.21–0.85). There was no significant association between carriers of *ADH1B* Arg^{370}Cys, *ADH1C* Ile^{350}Val and *CYP2E1*5A* polymorphism and the risk of HNC, as showed in Table 5. Potentially protective effect (OR = 0.41; 95% CI, 0.20–0.82) was detected for the variant haplotype *ADH1B*2* (that encodes histidine at position 48 and an arginine at position 370) and for the association of the two fast alcohol metabolizing *ADH1B*2* and *ADH1C*1* haplotypes (OR = 0.32; 95% CI, 0.13–0.79 —Table 5).

When the analysis was further stratified by variables that showed high significance ($P < 0.02$) in the univariate test, adjusted by age and sex, strong main effects were observed for HNC using multiple logistic regression testing (Table 6). The association was higher for individuals with the *ADH1B* Arg^{48}His variant genotype who consumed <30.655 g/L/day of alcohol (OR = 0.12; 95% CI, 0.03–0.52 —$P < 0.005$). In contrast, daily consumption of alcohol at amounts above 30.655 g/L/day increased the risk of HNC regardless of the presence (OR = 4.42; 95% CI, 1.21–16.11 —$P = 0.024$) or absence (OR = 3.01; 95% CI, 1.90–4.78 —$P < 0.001$) of the *ADH1B* protective genotype (Table 5).

DISCUSSION AND CONCLUSIONS

In the present investigation, we have identified that the *ADH1B* Arg^{48}His rapid alcohol metabolism variant genotype seems to be protective against upper aerodigestive tract cancer. Similar results were observed for the haplotype *ADH1B*2* that also has the histidine at the position 48. The other studied polymorphisms including *ADH1B* Arg^{370}Cys, *ADH1C* Ile^{350}Val and *CYP2E1*5A Pst*I showed no association with HNC risk.

Polymorphisms in *ADH* and *ALDH* genes or in the control of their expression may contribute to variation in human alcohol metabolism. The fast metabolizing *ADH1B* Arg^{48}His mutant allele encodes an enzyme whose homodimers have approximately 40 times higher maximum velocity than the heterodimers *ADH1B*1/*2* when compared to *ADH1B*1/*1* (Bosron and Li, 1986). Individuals who are homozygous for the variant alleles *ADH1B*2* and *ADH1C*1* have an alcohol oxidizing capacity almost eight times higher when compared to the reference haplotypes *ADH1B*1* and *ADH1C*1* (Edenberg, 2007). Our data showed a statistically significant difference when the frequencies of *ADH1B*2* and *ADH1C*1* haplotypes were considered together conferring protection for the disease ($P = 0.013$). One possible explanation for this is that the primary association is with *ADH1B*, and the association with *ADH1C* is partially due to linkage disequilibrium. As linkage disequilibrium patterns can be different between populations, this fact can also explain why different studies have produced inconsistent results. In fact, in a pooled analysis including 3876 patients with upper aerodigestive cancer and 5200 controls, *ADH1C* Ile^{350}Val polymorphism was considered significantly associated with cancer risk (OR = 1.14; 95% CI = 1.06–1.23), although this result was not observed in different meta-analysis studies (Brennan *et al*., 2004; Hashibe *et al*., 2008).

The Arg^{48}His variant allele is more frequent in individuals of Asian origin (60–80%), when compared to Caucasians (0–10%) as described by several authors (Goedde *et al*., 1992; Druesne–Pecollo *et al*., 2009). In the present investigation, the frequency of *ADH1B*2* variant allele in individuals who self-reported White (7.9%) and Mulatto (6.71%) ethnicity was similar to that observed in other Brazilian studies (Hashibe *et al*., 2008). No individuals of Asian descent were evaluated in the present prospective study.

The use of >30 g of alcohol per day per individual increased almost five times the risk of HNC in our studied population. This amount of alcohol was considered less than the expected amount of alcohol per day (50 g/L/day), thought to increase the risk of developing HNC by two to three fold (Seitz and Stickel, 2007). Individuals that consumed <30.655 g/L/day of alcohol and had the *ADH1B*2* genotype seemed to be protected from the development of HNC ($P = 0.005$). On the other hand, individuals that used >30.655 g/L/day of alcoholic beverages showed an almost 4-fold increase in cancer risk, independently of the *ADH1B*1* genotype.

All the studies in Asian populations that have assessed risk of HNC found a significantly higher risk of HNC in heavy drinkers with *ADH1B*1* genotype than in heavy drinkers carrying the *ADH1B*2* allele (Asakage *et al*., 2007; Hiraki *et al*., 2007). In European countries, significantly reduced odds ratios were recorded in never or moderate and in heavy drinkers who carried the *ADH1B*2* genotype (Hashibe *et al*., 2006). The observed results in our population can be related to the amount and quality of alcohol and individual habits that may contribute to the increased cancer risk. In fact, the dietary intake of vitamin A associated with the fast metabolizing *ADH1B* genotype may modify the risk of upper aerodigestive cancer due to ADH multiple substrate metabolism (Duester *et al*., 2003). The homemade spirits largely consumed in Brazil like 'cachaça' likely have a high concentration of alcohol and may have contaminants such as nitrosamines that also may contribute to the observed cancer risk. In this case, polymorphisms in genes that metabolize carcinogenic contaminants may also be related to HNC risk (Brennan *et al*., 2004; Gattás *et al*., 2006).

In contrast to *ADH* and *ALDH* genes, the induction of *CYP2E1* in the heaviest drinkers increases the conversion of other xenobiotics, including procarcinogens like nitrosamines, aflatoxins, vinychloride, PAHs polycyclic aromatic hydrocarbons, and hydrazynes to their ultimate carcinogens, probably modifying some cancer risk (Pöschl and Seitz, 2004). The *CYP2E1*5A Pst*I polymorphism is rare in our population (Gattás and Soares–Vieira, 2000) and did not show any correlation to HNC risk in the present investigation. Although the available data on *CYP2E1* polymorphism are limited, previous studies indicated that the *CYP2E1*5A* mutant genotype was associated with oral cavity and pharyngeal cancer (Bouchardy *et al*., 2000; Gattás *et al*., 2006), but the same was not observed by other case–control studies (González *et al*., 1998; Liu *et al*., 2001; Li *et al*., 2005; Ho *et al*., 2007). Recent data indicate a statistically significant difference associated with *CYP2E1*5B Rsa*I polymorphism in Brazilian patients with HNC (Olivieri *et al*., 2009).

Environmental and genetic factors play an important role in the development of the HNC. Differences in exposures and genetic polymorphisms among various ethnic groups suggest that studying a large population would yield important information for preventing HNC. Controlled studies on polymorphisms in genes for metabolism of alcohol that asses not only the genotypic variants, but also the drinking behavior of the population, are needed to better understand the gene–environment interaction in the risk for HNC.

Acknowledgements — This work was partially supported by FAPESP: Fundação de Apoio a Pesquisa do Estedado de São Paulo and Laboratório de Investigação Médica -40/Hospital de Clinicas Faculdade de Medicina da Universidade de São Paulo. I, Silvia Marçal Nunes Garcia declare that I had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The authors are grateful to Dr Karen Huyck who reviewed the manuscript carefully.

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