Hum Genet (1997) 99 : 319–325

ORIGINAL INVESTIGATION

C. Coutelle · P. J. Ward · B. Fleury · P. Quattrocchi
H. Chambrin · A. Iron · P. Couzigou · A. Cassaigne

Laryngeal and oropharyngeal cancer, and alcohol dehydrogenase 3 and glutathione S-transferase M1 polymorphisms

Received: 5 July 1996 / Revised: 15 October 1996

Abstract In this study the GSTμ phenotype and ADH genotype at the ADH3 locus were investigated in a group of 39 alcoholic men with upper respiratory/digestive tract cancer: 21 with oropharyngeal cancer and 18 with laryngeal cancer. The results are compared with those of a control group of 37 alcoholic men without alcohol-related medical complications. Of the control subjects, 48% were found to be GSTμ deficient [GSTμ(–)] and 19% carried the ADH_3^1/ADH_3^1 genotype. In the laryngeal cancer patients, a significantly elevated frequency of both the GSTμ(–) (78%) and ADH_3^1/ADH_3^1 genotype (56%) was observed, relative to the control group. On the basis of this result, the risk of laryngeal cancer associated with the GSTμ(–) and ADH_3^1/ADH_3^1 genotypic combination within the population of alcoholics was estimated to be 12.9 with a 95% confidence interval of 1.8–92 ($P < 0.01$) relative to alcoholic individuals who have GSTμ [GSTμ(+)] and are not ADH_3^1/ADH_3^1. Thus, alcoholics who are GSTμ(–) and ADH_3^1/ADH_3^1 have at least an 80% greater risk of developing laryngeal cancer than alcoholics who are GSTμ(+) and who are not ADH_3^1/ADH_3^1. In addition, the oropharyngeal cancer patients had excess frequencies of both GSTμ(–) (62%) and ADH_3^1/ADH_3^1 (43%) relative to the control group, but these excess frequencies were not statistically significant. The GSTμ(–) and ADH_3^1/ADH_3^1 genotypic combination may be a constitutional risk factor for laryngeal cancer among alcoholics.

C. Coutelle (✉) · B. Fleury · P. Quattrocchi · H. Chambrin
A. Iron · P. Couzigou · A. Cassaigne
Université de Bordeaux II, B.P. 10, 146 rue Léo Saignat,
F-33076 Bordeaux Cedex, France
Tel.: +33-557-57-13-74; Fax: +33-556-99-03-80
e-mail: christiane.coutelle@dbm2.u-bordeaux2.fr

P. J. Ward
Unité INSERM de Recherches en Epidémiologie des Cancers,
U351, Institut Gustave Roussy, 39 Rue Camille Desmoulins,
F-94805 Villejuif Cedex, France

Introduction

Long-term alcohol abuse is associated with a high incidence of cancer of the upper respiratory/digestive tract. It has been estimated that individuals with a high alcohol intake but low tobacco consumption have an eight times greater lifetime risk of upper respiratory/digestive tract cancer than comparable individuals of low alcohol intake (Bassendine 1986; Adami et al. 1992; Kato et al. 1992; Doll et al.1993; Kato and Nomura 1994). Recalculation of this relative risk according to the site of the cancer reveals a wide variation, from as high as 50 for cancer of the supraglottis, to a relative risk of the order of 2.4 for both the oropharynx and the larynx (Kato and Nomura 1994).

Nevertheless only a minority (10–20%) of heavy drinkers develop such a cancer (Wynder and Bross 1957). Part of the reason may lie in constitutional factors, predisposing some alcoholics to develop upper respiratory/digestive tract cancer. Evidence for this is scant, probably due to the complexity of the gene/environment interaction underlying the progression of this disease: carcinogenesis of the upper respiratory/digestive tract epithelium is an extremely complex multistep process (Volkes et al. 1993). Although family studies provide weak evidence for familial aggregation for oral and pharyngeal cancer (Goldstein et al. 1994), they do not support the hypothesis of any single gene component. Any hereditary component is likely to implicate variation at more than one gene locus.

One obvious candidate gene is the GSTM1 gene locus, which codes for the gluthatione S-transferase μ enzyme (GSTμ). This enzyme is well known for its role in the detoxification of carcinogens in tobacco smoke, and as a factor preventing smoking-related cancers (Wolf 1990; Tsuchida and Sato 1992; Beckett and Hayes 1993). It also plays a more general role than this, however, detoxifying many carcinogenic or xenobiotic species, including ethanol or its metabolites (Wolf 1990; Hayes JD et al. 1991). The GSTM1 locus is polymorphic, carrying at least three alleles, one of which is a null allele (Seidegard et al. 1988).

Individuals homozygous for the null allele ($GSTM_1^0$) lack the GSTμ enzyme and are classified as GSTμ(–).

It has been estimated that approximately 50% of French people are GSTμ(–) (Laisney et al. 1984; Groppi et al. 1991). Possession of the GSTμ(–) phenotype is a risk factor for cancer of the larynx (Lafuente et al. 1993) as well as for many other cancers (Seidegard et al. 1986; Harada et al. 1987; Strange et al. 1991; Zhong et al. 1991; Harada et al. 1992; Daly et al. 1993).

A second candidate gene that has not hitherto been considered in the context of upper respiratory/digestive tract cancer is the ADH3 gene locus. This gene is one of three coding for the Class-I alcohol dehydrogenase (ADH) enzymes, which are primarily responsible for the oxidation of ethanol to acetaldehyde. Acetaldehyde is cytotoxic and has been implicated in ethanol-induced cell damage, and production of free radicals and DNA hydroxylated bases (Wickramasinghe et al. 1986; Tan et al. 1988). In alcoholic patients, the concentration of this metabolite within cells might be an important factor inducing cell proliferation, and increasing the risk of neoplastic transformation, particularly within epithelial cells in direct contact with the ingested ethanol. The ADH3 locus carries two alleles coding respectively for the γ_1 and γ_2 subunits of the dimeric ADH enzyme. The frequencies of the two alleles have been estimated to be 0.55 and 0.45, respectively, within the French population (Coutelle 1992). The various isozymes formed by combinations of these two different allelic products show different in vitro kinetic properties with respect to the rate of ethanol oxidation (Edenberg and Brown 1992). Populations of alcoholic patients have been described as differing significantly from nonalcoholic controls with respect to the distribution of the ADH3 genotype (Poupon et al. 1993; Chao et al. 1994), raising the possibility that the ADH3 genotype might govern the individual's in vivo rate of ethanol elimination. The ADH3 gene is expressed in many epithelial tissues, including the mucosal layer of the upper digestive tract (Edenberg and Brown 1992; Yin et al. 1993; Moreno et al. 1994). No investigation has yet considered the ADH3 polymorphism as a potential cancer risk factor among excessive drinkers.

The present study investigates the possibility of an hereditary predisposition to alcohol-induced cancer of the larynx and/or the oropharynx. Particular attention was focused on individuals with excessive alcohol intake: a group of French alcoholics with laryngeal and/or oropharyngeal cancer. A control group of French alcoholics with neither cancer, nor any other alcohol-related disease was used for comparison. These groups were compared with respect to the genotype distribution simultaneously at two loci: ADH3, and GSTM1 as reflected by the GSTμ phenotype.

Materials and methods

Subjects

All subjects were French Caucasian men residing within the Bordeaux region; all of them were heavy smokers. Genotyping was carried out in two groups.

The control group consisted of 37 subjects, all classified as "alcoholics", i.e., each subject had consumed in excess of 100 g of ethanol per day for more than 10 years. They were recruited at a local alcoholism clinic where they had undergone consultation concerning withdrawal. No clinically diagnosed cancer was detected or other alcohol-related medical complication. The mean age of this group was 42 years.

The cancer group consisted of 39 patients, each having consumed more than 100 g of ethanol per day for more than 10 years. Each subject had developed a Malpighian cancer of either the oropharynx or the larynx that was verified histologically. The mean age of this group was 54 years.

This study was approved by the Ethics Committees of the relevant institutions. All subjects agreed to participate in this study.

Blood samples and genotyping

Blood drops were taken from each subject by finger prick, deposited onto filter paper, and used for determination of the GSTM1 and ADH3 genotypes. Another 5 ml of blood was collected into a citrated tube from each patient by venous puncture for determination of the GSTμ protein phenotype.

Polymerase chain reaction (PCR)

The GSTM1 and ADH3 genotyping were both carried out via PCR amplification. A 5-mm disk of filter paper impregnated with dry blood was placed directly into 100 μl of PCR buffer (0.01 M TRIS-HCl, 0.05 M KCl, 0.0015 M $MgCl_2$, 0.1% Triton × 100, pH = 8.8) together with the deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP, as well as two oligonucleotide primers each at 1 μM. The primers were those described by Groppi et al. (1991) for GSTM1, and by Groppi et al. (1990) for ADH3.

Genomic DNA was denatured at 92° C for 10 min. The thermostable DNA polymerase extracted from Thermus brockianus was then added (2.5 U per 100 μl). Thirty-five cycles were carried out, each comprising denaturation for 1 min at 92° C, annealing for 1 min at 55° C, and extension for 2 min at 72° C.

For ADH3 genotyping, the PCR mixture was directly digested using the restriction enzyme SspI. Another site for SspI was created by directed mutagenesis, as an internal digestion control (Groppi et al. 1990). The fragments were separated using high-voltage, vertical, polyacrylamide gel electrophoresis. The ADH_3^1 allele was characterized by the presence of fragments of 67 and 63-bp, whereas the ADH_3^2 allele was characterized by a single 130-bp fragment. The bands were visualized in a bath of ethidium bromide and photographed under UV light.

For GSTM1 genotyping, an aliquot of the amplified solution was submitted to high-voltage gel electrophoresis, and the gel was then placed in a bath of ethidium bromide and photographed under UV light. An amplified 165-bp fragment was observed in GSTμ(+) samples, and was absent in GSTμ(–) samples. The GSTμ phenotype was verified by scoring for the presence of the leukocytic GSTμ enzyme on most blood samples via an immunoassay method: the presence of GSTμ in mononuclear blood cells was detected using a commercially available, sandwich enzyme-linked assay (Biotrin International, Dublin).

Statistical analysis

Statistical associations between the risk of cancer and the ADH3 genotype and GSTμ phenotype were tested for significance via likelihood ratio $\chi 2$ tests. These tests reflect changes in the likelihood ratio statistic (reduction of deviance) associated with the removal of explanatory factors from a linear regression model (Breslow and Day 1980). Analyses were adjusted on patient age by including in the model an indicator variable taking the value 1 for a patient older than 45 years, and the value 0 otherwise. Cancer status was regressed logistically on genotype and age using the BMDP statistical package.

Table 1 Observed number of individuals, categorized by pathology, GSTμ phenotype and ADH3 genotype

Group	*n*	Genotype category					
		GSTμ(+)			GSTμ(–)		
		ADH_3^1/ADH_3^1	ADH_3^1/ADH_3^2	ADH_3^2/ADH_3^2	ADH_3^1/ADH_3^1	ADH_3^1/ADH_3^2	ADH_3^2/ADH_3^2
Noncancer	37	4	14	1	3	13	2
Oropharyngeal cancer	21	3	3	2	6	5	2
Laryngeal cancer	18	0	2	2	10	4	0

Genotype/cancer associations were also tested for significance using the T_{AB} statistic (Ward 1993), which is a recent method developed for the analysis of genotype-disease associations within case-control studies.

Results

Table 1 presents the observed joint distribution of GSTμ phenotype and ADH3 genotype in both the control group and the two cancer patient groups. Since there were small numbers of patients within some categories of this cross-classification, it was necessary to pool the two cancer groups for some of the statistical analyses. In order first to verify whether the two cancer groups could be pooled, they were compared with respect to the distribution of age, GSTμ phenotype and ADH3 genotype. The results are illustrated in Table 2, and show that the two groups of cancer patients did not differ significantly with respect to mean age, the distribution of GSTμ phenotype, or the distribution of ADH3 genotype.

The control patients were younger on average than the cancer patients: the age range was 30–73 years (mean = 42) for controls, 36–72 years (mean = 56) for pharyngeal cancer, and 35–72 (mean = 52) for laryngeal cancer. Therefore, before proceeding with the analysis, we investigated whether any effects due to age could be separated statistically from those due to GSTμ phenotype and/or ADH3 genotype (i.e., statistical tests were carried out to find out whether the age distribution was confounded with either the GSTμ phenotype or the ADH3 genotype distribution). A comparison of mean age between categories of GSTμ phenotype and of ADH3 genotype is presented in Table 3, separately for cancer patients and for control patients.

These results illustrate that age was not significantly different across these genotypic categories, either for the cancer patients or the control patients. The distributions of GSTμ phenotype and ADH3 genotype can therefore be considered independently of the age distribution in the present sample.

Table 4 summarizes a comparison of control patients with cancer patients, with respect to the distribution of GSTμ phenotype. Approximately 51% of the noncancer control patients were GSTμ(+), and a relative excess of GSTμ(–) individuals was observed among cancer patients: 62% of oropharyngeal cancer patients and 78% of laryngeal cancer patients were GSTμ(–). The number of GSTμ(–) individuals is significantly elevated among cancer cases relative to control patients (OR = 2.9, $P < 0.06$), and this elevation is largely accounted for by an excess of GSTμ(–) individuals among the laryngeal cancer cases relative to controls (OR = 4.7, $P < 0.036$). The excess of GSTμ(–) individuals among oropharyngeal cancer patients relative to controls is not statistically significant (OR = 1.8, $P < 0.37$).

Table 4 also presents estimates of the relative risk of cancer associated with the ADH_3^1/ADH_3^1 genotype (the ADH_3^1/ADH_3^2 and ADH_3^2/ADH_3^2 categories being com-

Table 2 Comparison of oropharyngeal cancer patients with laryngeal cancer patients with respect to mean age and the distributions of GSTμ phenotype and ADH3 genotype

Variable	Cancer type		*P*-value
	Oropharynx (*n* = 21)	Larynx (*n* = 18)	
Mean age	55.8	51.9	0.26[a]
(standard error)	(11.7)	(8.9)	
Percentage GSTμ(–)	62	78	0.47[b]
Percentage ADH_3^1/ADH_3^1	43	56	
Percentage ADH_3^1/ADH_3^2	38	33	0.42[c]
Percentage ADH_3^2/ADH_3^2	19	11	

[a] Variance ratio *(F)* test
[b] Contingency table χ^2 test
[c] Due to small numbers of patients, the contingency table χ^2 test compared the number of ADH_3^1/ADH_3^1 patients with the number of patients who were either ADH_3^1/ADH_3^2 or ADH_3^2/ADH_3^2

Table 3 Mean age within categories of GSTμ phenotype and of ADH3 genotype, separately for cancer patients and for control patients

Subgroup	Cases		Controls	
	n	Mean age (SE)	*n*	Mean age (SE)
GSTμ(+)	12	54.9 (11.6)	19	43.8 (10.6)
GSTμ(–)	27	53.6 (10.2)	18	40.6 (5.9)
ADH_3^1/ADH_3^1	20	52.6 (11.0)	7	39.6 (6.1)
ADH_3^1/ADH_3^2 or ADH_3^2/ADH_3^2	19	55.5 (10.0)	30	42.9 (9.1)

Table 4 Odds ratio *(OR)* of cancer according to GSTμ phenotype and according to ADH3 genotype

Patient category	No. of individuals		OR[a] (95% CI)[b]	*P* value[c]
	Cases	Controls		
All cancer				
GSTμ(+)	12	19	1	
GSTμ(–)	27	18	2.9 (1.0, 8.6)	0.060
ADH_3^1/ADH_3^2 or ADH_3^2/ADH_3^2	20	30	1	
ADH_3^1/ADH_3^1	19	7	3.6 (0.7, 10.0)	0.180
Oropharyngeal cancer				
GSTμ(+)	8	19	1	
GSTμ(–)	13	18	1.8 (0.5, 6.2)	0.370
ADH_3^1/ADH_3^2 or ADH_3^2/ADH_3^2	12	30	1	
ADH_3^1/ADH_3^1	9	7	2.6 (0.7, 10.0)	0.180
Laryngeal cancer				
GSTμ(+)	4	19	1	
GSTμ(–)	14	18	4.7 (1.0, 21.8)	0.036
ADH_3^1/ADH_3^2 or ADH_3^2/ADH_3^2	8	30	1	
ADH_3^1/ADH_3^1	10	7	6.1 (1.3, 28.6)	0.024

[a] Risk of cancer, relative to reference category, which is set to OR = 1
[b] 95% confidence interval
[c] Unconditional logistic regression adjusted on age of patients

Table 5 Odds ratio *(OR)* of cancer according to a grouping of patients based on their GSTμ phenotype and ADH3 genotype

Patient category[a]	No. of individuals		OR[b] (95% CI)[c]	*P* value[d]
	Cancer	Controls		
All cancer				
Group 1	9	15	1	
Group 2	14	19	1.7 (0.5, 6.1)	0.41
Group 3	16	3	8.0 (1.5, 42.5)	0.02
Oropharyngeal cancer				
Group 1	5	15	1	
Group 2	10	19	2.0 (0.5, 8.7)	0.36
Group 3	6	3	4.3 (0.6, 28.8)	0.16
Laryngeal cancer				
Group 1	4	15	1	
Group 2	4	19	1.5 (0.3, 9.0)	0.64
Group 3	10	3	12.9 (1.8, 92.0)	0.01

[a] Group 1 patients are GSTμ(+) and are not ADH_3^1/ADH_3^1, Group 2 patients are either GSTμ(+) and ADH_3^1/ADH_3^1 or are GSTμ(–) and not ADH_3^1/ADH_3^1, Group 3 patients are GSTμ(–) and ADH_3^1/ADH_3^1
[b] Risk of cancer, relative to reference category, which is set to OR = 1
[c] 95% confidence interval
[d] Unconditional logistic regression adjusted on age of patients. The *P* value refers to a test comparing the relevant population with the reference population for which OR = 1

bined in these analyses in order to avoid small numbers of patients in some categories of the cross-classification). It was found that alcoholic individuals with the ADH_3^1/ADH_3^1 genotype have a 3.6 times greater risk of such cancer compared with individuals who did not carry this genotype ($P < 0.03$). This result largely reflects an excess frequency of ADH_3^1/ADH_3^1 individuals within the laryngeal cancer group (Tables 1, 4). The risk of laryngeal cancer among alcoholics who carry the ADH_3^1/ADH_3^1 genotype was estimated to be 6.1 times that of alcoholics who did not carry this genotype ($P < 0.024$). In contrast, the excess number of ADH_3^1/ADH_3^1 individuals among oropharyngeal cancer cases, relative to controls, was not statistically significant (OR = 2.6, $P < 0.18$).

In order to estimate the relative risk of cancer associated with the combination of GSTμ phenotype and ADH_3^1/ADH_3^1 genotype, some patient categories were further combined to overcome the problem of unacceptably small sample sizes in some categories of the cross-classification. Thus, individuals who were both GSTμ and ADH_3^1/ADH_3^1 were placed in one category (Group 3), individuals who were GSTμ(+) and not ADH_3^1/ADH_3^1 were placed in another category (Group 1), and the remaining individuals were placed in a third category (Group 2).

Table 5 presents age-adjusted estimates for the risk of cancer for individuals in Groups 2 and 3, relative to that of individuals in Group 1. Alcoholics who are GSTμ(-) and who carry the ADH_3^1/ADH_3^1 genotype have an eight times greater risk of cancer relative to individuals who are GSTμ(+) and who do not have genotype ADH_3^1/ADH_3^1 ($P < 0.02$), and this result was largely explained in terms of a 13 times greater risk of laryngeal cancer ($P < 0.01$, bottom line of Table 5). The corresponding effect was not significant within oropharyngeal cancer patients, but it was nevertheless noteworthy that GSTμ(–) and ADH_3^1/ADH_3^1 genotypes increased the risk of oropharyngeal cancer (OR = 4.3, $P < 0.16$) among alcoholics.

These latter results were verified using the T_{AB} test statistic (Ward 1993). Table 6 presents T_{AB} test statistics for association between ADH3 genotype and cancer, presented

Table 6 T_{AB} test statistics for association between genotype at the ADH3 locus and the incidence of cancer among alcoholic patients. The T_{AB} test statistic has an approximately standard normal distribution under the null hypothesis that the genotype distribution is the same in the case population as in the control population. All tests are one sided. See Ward (1993) for further details

Cancer group	GSTμ(+) subjects	GSTμ(–) subjects	All subjects
Laryngeal cancer	1.150	6.869**	2.468*
Oropharyngeal cancer	–0.856	0.486	0.0648

* $P < 0.02$
** $P < 0.001$

across different GSTμ categories, and according to whether cases had oropharyngeal or laryngeal cancer. Comparison of the ADH3 genotype distribution of GSTμ(–) individuals with laryngeal cancer to that of GSTμ(–) individuals without laryngeal cancer leads to a highly significant result of $T_{AB} = 6.9$ ($P < 0.001$). This reflects an excess frequency of the ADH_3^1/ADH_3^1 genotype among GSTμ(–) individuals with laryngeal cancer, relative to GSTμ(–) individuals without cancer (see Table 1). A corresponding analysis of the oropharyngeal cancer patients, using T_{AB} test statistics, revealed no such significant interaction between GSTμ phenotype and ADH3 genotype as a factor determining cancer risk (Table 6).

Discussion

Within a sample of 39 alcoholic patients with cancer of the upper respiratory/digestive tract, approximately equal numbers were found to have oropharyngeal or laryngeal cancer (21 and 18, respectively), consistent with findings of previous studies (Tuyns et al. 1988; Franceschi et al. 1990; Choi and Kahyo 1991; Blot 1992).

When compared with a sample of 37 alcoholics with no evidence of cancer or other alcohol-related medical problems, these cancer patients were found to contain a significant excess frequency of individuals carrying the GSTμ(–) phenotype: 62% of the oropharyngeal cancer patients and 78% of the laryngeal cancer patients were GSTμ(–), compared with 49% of the alcoholic patients without cancer. An excess of GSTμ(–) individuals among laryngeal cancer patients has previously been reported (Davidson et al. 1993; Lafuente et al. 1993) although those studies concerned heavy smokers and not alcoholics. As far as is known, no previous study has reported an excess frequency of GSTμ(–) individuals among oropharyngeal cancer patients, although such an excess has been reported for many other types of cancer (Di Ilio et al. 1989; Howie et al. 1989; Seidegard et al. 1990; Shea et al. 1990; Hayes PC et al. 1991; Strange et al. 1991; Zhong et al. 1991; Bell et al. 1992; Harada et al. 1992; Hayachi et al. 1992; Daly et al. 1993; Davidson et al. 1993; Hirvonen et al. 1993; Kihara et al. 1993; Nakachi et al. 1993; Nazar-Stewart et al. 1993; Anttila et al. 1994; Heagerty et al. 1994).

The present sample of laryngeal cancer patients who are GSTμ(–) also had a significant excess of individuals carrying the ADH_3^1/ADH_3^1 genotype. On the basis of this data, the age-adjusted risk of laryngeal cancer among alcoholics who are GSTμ(–) and who carry the ADH_3^1/ADH_3^1 genotype, relative to GSTμ(+) alcoholics not carrying the ADH_3^1/ADH_3^1 genotype, is estimated to be 12.9 with a lower bound of 1.8, i.e., an alcoholic carrying the GSTμ(–) and ADH_3^1/ADH_3^1 genotypic combination is at least at 80% greater risk of laryngeal cancer than a comparable alcoholic not carrying either of these genetic traits.

A similar excess frequency of the ADH_3^1/ADH_3^1 genotype was also observed among GSTμ(–) oropharyngeal cancer patients, but the excess was not statistically significant.

Previous studies have shown alcohol consumption to increase the risk of oropharyngeal cancer more than the risk of laryngeal cancer (Franceschi et al. 1990; Choi and Kahyo 1991). The converse has also been reported (Tuyns et al. 1988; Blot 1992). Such findings are not to be confused with the relative risks reported here, which concern the risk of cancer associated with a particular genotypic combination among alcoholics, and not the risk associated with an increasing level of alcohol consumption.

In the present sample, the control alcoholics (those without cancer) were younger on average than the cancer cases, and therefore some caution must be expressed concerning the validity of the findings reported here. However, in both the control and cancer groups, neither the distribution of the GSTμ phenotype nor the frequency of ADH_3^1/ADH_3^1 varied significantly with age. In addition, all statistical tests concerning the relative risk of cancer associated with GSTμ phenotype, and/or ADH3 genotype, were adjusted according to the age of subjects. The conclusions of this study did not change when age was not taken into account in the statistical analysis, nor when the analysis was restricted to a subgroup having the same mean age in cases as in controls.

No previous study has investigated ADH3 genotype distribution as a cancer risk factor. The results of the present study place the ADH3 locus forward as a candidate susceptibility gene for laryngeal (and possibly oropharyngeal) cancer within the group of alcoholic patients who are GSTμ(–). These results need to be confirmed on a larger sample, taking into account other risk factors for oral cancer, such as the smoking habits of the subjects. The GSTμ(–) and ADH_3^1/ADH_3^1 combination may constitute a risk factor for oral cancer among alcoholics, and further investigation into this possibility is needed.

Acknowledgements This work was supported by IREB contract number 93/01.

References

Adami HO, McLaughlin JK, Hsing AW, et al (1992) Alcoholism and cancer risk: a population-based cohort study. Cancer Causes Control 3:419–425

Anttila S, Hirvonen A, Husgafvel-Pursiainen K, Karjalainen A, Nurminen T, Vainio H (1994) Combined effect of CYP1A1 inducibility and GSTM1 polymorphism on histological type of lung cancer. Carcinogenesis 15:1133–1135

Bassendine MF (1986) Alcohol – a major risk factor for hepatocellular carcinoma? J Hepatol 3:513–519

Beckett GJ, Hayes JD (1993) Glutathione S-transferases: biomedical applications. Adv Clin Chem 30:281–380

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Miller CR, Lucier GW (1992) Glutathione transferase μ gene deletion is associated with increased risk for bladder cancer. Proc Am Assoc Cancer Res 33:291

Blot WJ (1992) Alcohol and cancer. Cancer Res 52:2119s-2123s

Breslow NE. Day NE (1980) The analysis of case-control studies. In: Davis W (ed) Statistical methods in cancer research. IARC, Lyon, pp 192–200

Chao YC, Liou SR, Chung YY, Tang HS, Hsu CT, Li TK, Yin SJ (1994) Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. Hepatology 19:360–366

Choi SY, Kahyo H (1991) Effect of cigarette smoking and alcohol comsumption in the aetiology of cancer of the oral cavity, pharynx and larynx. Int J Epidemiol 20:878–885

Coutelle C (1992) Bioclinical studies on alcohol metabolizing enzymes (alcohol dehydrogenase, aldehyde dehydrogenase, glutathione S-transferase1): activities, phenotype, genotype. These Etat es Sciences Pharmaceutiques, Université de Bordeaux II, 223 p

Daly AK, Thomas DJ, Cooper J, et al (1993) Homozygous deletion of gene for glutathione S-transferase M1 in bladder cancer. BMJ 307:481–482

Davidson BJ, Hsu TC, Schantz SP (1993) The genetics of tobacco induced malignancy. Arch Otolaryngol Head Neck Surg 119: 1198–1205

Di Ilio C, Aceto A, Zezza A, Ricci G, Federici G (1989) Electrophoretic and immunological analysis of glutathione transferase isoenzyme of human kidney carcinoma. Biochem Pharmacol 38:1045–1051

Doll R, Forman D, La Vecchia C, Woutersen R (1993) Alcoholic beverages and cancers of the digestive tract and larynx. In:Verschuren PM (ed) Health issues related to alcohol consumption. ILSI Press, Washington, pp 126–166

Edenberg HJ, Brown CJ (1992) Regulation of human alcohol dehydrogenase genes. Pharmacogenetics 2:185–196

Franceschi S, Talamini R, Barra S, BarÛn AE, Negri E, Bidoli E, Serraino D, LaVecchia C (1990) Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx and esophagus in northern Italy. Cancer Res 50:6502–6507

Goldstein AM, Blot WJ, Greenburg RS, Schoenberg JB, Austin DF, Preston-Martin S, Winn DM, Bertsein L, McLaughlin JK, Fraumeni JF Jr (1994) Familial risk in oral and pharyngeal cancer. Oral Oncol Eur J Cancer 30B:319–322

Groppi A, Begueret J, Iron A (1990) Improved methods for genotype determination of human alcohol dehydrogenase (ADH) at ADH_2 and ADH_3 loci by using polymerase chain reaction-directed mutagenesis. Clin Chem 36:1765–1768

Groppi A, Coutelle C, Fleury B, Iron A, Begueret J, Couzigou P (1991) Glutathione S-transferase class μ in French alcoholic cirrhotic patients. Hum Genet 87:628–630

Harada S, Abei M, Tanaka N, et al (1987) Liver glutathione S-transferase polymorphism in Japanese and its pharmacogenetic importance. Hum Genet 75:322–325

Harada S, Misawa S, Nakamura T, et al (1992) Detection of GST1 gene deletion by polymerase chain reaction and its possible correlation with stomach cancer in Japanese. Hum Genet 90: 62–64

Hayachi SI, Watanabe J, Kawajiri K (1992) High susceptibility to lung cancer analyzed in terms of combined genotypes of P450IA1 and Mu-class glutathione S-transferase genes. Jpn J Cancer Res 83:866–870

Hayes JD, Bouchier IAD, Beckett CJ (1991) Glutathione S-transferases in human health and disease. Gut 32:813–818

Hayes PC, May L, Hayes JD, Harisson DJ (1991) Glutathione S-transferase in human liver cancer. Gut 32:1546–1549

Heagerty AHM, Fitzgerald D, Smith A, Bowers B, Jones P, Fryer AA, Zhao L, Alldersea J, Strange RC (1994) Glutathione S-transferase GSTM1 phenotypes and protection against cutaneous tumours. Lancet 343:266–268

Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Vainio H (1993) The GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of the lung. Carcinogenesis 14:1479–1481

Howie AF, Miller WR, Hawkins RA, Hutchinson AR, Beckett GJ (1989) Expression of glutathione S-transferase B1, B2, Mu and Pi in breast cancers and their relationship to oestrogen receptor status. Br J Cancer 60:834–837

Kato I, Nomura AMY (1994) Alcohol in the aetiology of upper respiratory/digestive tract cancer. Oral Oncol Eur J Cancer 30B:75–811

Kato I, Nomura AMY, Stemmermann GN, et al (1992) Prospective study of the association of alchol with cancer of the upper respiratory/digestive tract and other sites. Cancer Causes Control 3:145–151

Kihara M, Kihara M, Noda K, Okamoto N (1993) Increased risk of lung cancer in Japanese smokers with class μ glutathione S-transferase gene deficiency. Cancer Lett 71:151–155

Lafuente A, Pujol F, Carretero P, Villa JP, Cuchi (1993) A human glutathione S-transferase μ (GST μ) deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. Cancer Lett 68:49–54

Laisney V, Nguyen VC, Gross MS, Frezal J (1984) Human genes for glutathione S-transferases. Hum Genet 68:221–227

Moreno A, Parés A, Ortiz J, Enríquez J, Parés X (1994) Alcohol dehydrogenase from human stomach: variability in normal mucosa and effect of age, gender, ADH3 phenotype and gastric region. Alcohol Alcohol 29:663–671

Nakachi K, Imai K, Hayashi S, Kawajiri K (1993) Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in Japanese population. Cancer Res 53:2994–2999

Nazar-Stewart V, Motulsky AG, Eaton DL, White E, Hornung SK, Leng ZT, Stapleton P, Weiss NS (1993) The glutathione S-transferase μ polymorphism as marker for susceptibility to lung carcinoma. Cancer Res 53:2313–2318

Poupon RE, Ward PJ, Balkau B (1993) Alcohol dehydrogenase polymorphisms and predisposition to alcoholic cirrhosis. Hepatology 18:231–232

Seidegard J, Ronald WP, Miller DG (1986) A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 7:751–753

Seidegard J, Vorachek WR, Pero RW, Pearson WR (1988) Hereditary differences in the expression of human glutathione S-transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA 85:7293–7297

Seidegard J, Pero RW, Markowitz (1990) MM isoenzyme of glutathione S-transferase class μ as a marker for the susceptibility to lung cancer: a follow up study. Carcinogenesis 11:33–36

Shea TC, Claflin G, Comstock KE, Sanderson BJS, Burstein NA, Keenan EJ, Mannervick B, Henner WD (1990) Glutathione transferase activity and isoenzyme composition in primary human breast cancers. Cancer Res 50:6848–6853

Strange RC, Matharoo B, Faulder CGC, et al (1991) The human glutathione S-transferases: a case-control study of the incidence of the GST 0 phenotype in patients with adenocarcinoma. Carcinogenesis 12:25–28

Tan KH, Meyer DJ, Gillies N, Ketterer B (1988) Detoxification of DNA hydroperoxide by glutathione transferases and the purification and characterization of glutathione transferases of the rat liver nucleus. Biochem J 254:841–845

Tsuchida S, Sato K (1992) Glutathione transferases and cancer. Crit Rev Biochem Mol Biol 27:337–384

Tuyns AJ, Estève J, Raymond L, Berrino F, Benhamou E, Blanchet F, Boffetta P, Crosignani P, del Moral A, Lehmann W, Merletti F, Péquignot G, Riboli E, Sancho-Garnier H, Terracini B, Zubri A, Zubri L (1988) Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragosa and Navarra (Spain), Geneva (Switzerland) and Calvados (France). Int J Cancer 41:483–491

Volkes EE, Weichsbaum RR, Lippmann SM, Hong WK (1993) Head and neck cancer. New Engl J Med 328:184–194

Ward PJ (1993) Some developments on the affected-pedigree-member method of linkage analysis. Am J Hum Genet 52:1200–1215

Wickramasinghe SN, Gardner B, Barden G (1986) Cytotoxic protein molecules generated as a consequence of ethanol metabolism. Lancet i:823–826

Wolf CR (1990) Metabolic factors in cancer susceptibility. Cancer Surv 9:437–474

Wynder EL, Bross IJ (1957) Aetiological factors in mouth cancer. BMJ 1:1137–1143

Yin SJ, Chou FJ, Chao SF, Tsai SF, Liao CS, Wang SL, Wu CW, Lee SC (1993) Alcohol and aldehyde dehydrogenases in human esophagus: comparison with the stomach enzyme activities. Alcohol Clin Exp Res 17:376–381

Zhong S, Howie AF, Ketterer B, et al (1991) Glutathione S-transferase μ locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis 12:1533–1537