Alcohol-dehydrogenase (*ADH1B*) Arg48His Polymorphism in Basque Country Patients with Oral and Laryngeal Cancer: Preliminary Study

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Abstract. *Oral and laryngeal cancer has a high incidence in the Basque Country (Spain), the main risk factors in this pathology being regular consumption of tobacco and alcohol. However, since not all the individuals exposed to these risk factors develop cancer, the individual genetic susceptibility should be investigated in this population. The aim of this study was to determine the distribution of alcohol dehydrogenase-1B polymorphism (Arg48His; rs1229984) in our region and analyze its association with the risk of oral and laryngeal squamous cell carcinoma. Samples from 87 patients with oral or laryngeal cancer and 242 healthy controls were analyzed. Multivariate logistic regression analysis showed that the combined Arg/His and His/His genotypes were associated with a reduced risk of head and neck squamous cell carcinoma (odds ratio: 0.203; 95% confidence interval: 0.052-0.796). In conclusion, the histidine allele was associated with a reduced risk of oral and laryngeal cancer in the Basque Country.*

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common type of cancer worldwide and accounts for 5% of all the new cancer cases diagnosed in the northeast of Europe and the United States (1). In the Basque Country, the incidence of oral and laryngeal cancers are very high when compared to other Spanish regions or other European Union countries (2-4).

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Although the etiology of oral and laryngeal cancer is multifactorial, alcohol and tobacco intake are known to be major risk factors which can act separately or synergistically. Attributable risk of oral cancer due to both tobacco and alcohol is estimated to be more than 80% (4, 5). However, not all individuals exposed to these well-known risk factors develop HNSCC, which may suggest that there is a genetic susceptibility for developing these diseases. Such susceptibility could be related to different genetic polymorphisms among which are those found in enzymes involved in alcohol metabolism (6-8).

While the role of tobacco as a risk factor is well-known, the mechanisms for alcohol intake as a risk factor is unclear. Several hypotheses on the carcinogenic action of alcohol have suggested that alcohol could act by solubilizing tobacco carcinogens or that acetaldehyde (the metabolite of ethanol) could act as the primary carcinogen (9). If the latter hypothesis is correct, functional polymorphisms in the genes that encode enzymes involved in alcohol metabolism could play an important role in the susceptibility for developing an HNSCC in general population. In humans, ethanol is oxidised to acetaldehyde mainly by the enzyme alcohol dehydrogenase (ADH) (10). It has been shown that common genetic variants of the genes that encode for ADH account for differences in the efficiency in converting ethanol to acetaldehyde (11). In this regard, it has been found that there is a major functional polymorphism for the *ADH1B* gene (located in exon 3: Arg48His) which accounts for differences in the oxidation rate of ethanol to acetaldehyde. *In vitro* studies have shown that the 'atypical' allele (histidine allele) increases ethanol oxidation by 40-fold compared to the wild-type allele (arginine allele) (12, 13). The relationship between this *ADH1B* gene polymorphism and the risk of developing HNSCC has been analyzed in different

populations (14-16). In studies conducted in European populations, the histidine allele has been associated with a lower risk of developing HNSCC (14, 17-19).

The high incidence of oral and laryngeal cancer together with the high alcohol intake observed among the general population in the Basque Country (20) suggest the need to carry out studies to analyze genetic polymorphisms that may be involved in the susceptibility for developing a HNSCC. Therefore, the objective of this preliminary study was to determine the distribution of the *ADH1B* Arg48His polymorphism in our region and to analyze its association with the risk of oral and/or laryngeal squamous cell carcinoma.

Patients and Methods

Patients. Samples from 87 patients diagnosed with an HNSCC were taken and classified into two groups: a) Oral group: consisting of 47 patients who had had an oral squamous cell carcinoma; b) Laryngeal group: consisting of 40 patients who had had a laryngeal squamous cell carcinoma. Cases were recruited after obtaining written informed consent at the Oral Medicine Unit of the University of the Basque Country (UPV/EHU) and at the Oncological Institute of San Sebastian.

Additionally, 242 individuals without a history of HNSCC were selected as controls. The DNA samples from the controls were obtained from the Banco Nacional de ADN (University of Salamanca, Salamanca, Spain).

The study was approved by the Ethics Committee in Research of the Faculty of Medicine and Dentistry at the UPV/EHU.

Sample collection and DNA extraction. The cytological samples from the patients diagnosed with a HNSCC were collected by vigorously swabbing a cytology brush (Cytobrush®) over the oral mucosa. The brush was then deposited in a sterile tube with 10 ml of milliQ water and centrifuged at 1500 rpm for 15 minutes. The cell pellet obtained was incubated with proteinase K (0.2 mg/ml) for 3 h at 56˚C. Afterwards, DNA was extracted with phenol-chloroform and ethanol precipitation.

Genotyping. The *ADH1B* gene polymorphism (rs1229984) was analyzed using a 5' exonuclease assay (Taqman assay). The allelic discrimination Taqman assay (ADH1B C_2688467_20 DMG) was purchased from Applied Biosystems (Foster City, CA, USA) and carried out in an iQ5 Real-Time PCR Detection System (BioRad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. In order to ensure the reliability of the genotyping assay, samples that had been previously genotyped by sequencing were run in each plate.

Statistical analysis. Frequency distributions of demographic variables (sex and age), known risk factors (alcohol and tobacco usage) and alleles and genotypes among cases and controls were assessed. In addition, unconditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) adjusted by potential confounders (sex, age, tobacco and alcohol intake). All tests were two-sided and a p-value of <0.05 was considered to be statistically significant. The statistical analyses were conducted using SPSS software v17.0 (SPSS Inc. Chicago, IL, USA).

Results

Characteristics of the cases and controls studied are presented in Table I. The mean age distribution among oral and larynx cancer patients was fairly similar, but HNSCC patients were slightly older when compared with the control group. There was a lower proportion of women in the cases group when compared with the controls. As expected, HNSCC cases were more frequent among men than among women and tobacco and alcohol habits were more common among the cases.

Due to the small number of individuals who were homozygous for histidine, subjects who were heterozygous and homozygous for histidine were combined.

In order to determine if the genotype of the subjects affected their behaviour towards tobacco and alcohol usage, the distribution of these habits among the control group was analysed (Table II). We observed that the proportion of His allele carriers was higher among non-drinkers (19.5%) than among drinkers (10.8%); however, this association was not statistically significant (p=0.073). With regard to tobacco smoking, no significant difference was observed.

The genotype distributions of *ADH1B* Arg48His among cases and controls are shown in Table III. All genotypes were in Hardy-Weinberg equilibrium in the control group (p=0.827). The prevalence of His carriers (Arg/His + His/His) was lower in the cancer cases than in the controls. However, these differences were only borderline statistically significant between controls and cancer cases overall (p=0.063) and between controls and laryngeal cancer cases (p=0.065). When analyzing the allelic frequencies, we observed that the His allelic frequency was lower in the cases (oral: 0.053; laryngeal: 0.025 and HNSCC: 0.04) than in the controls (0.085), and these differences were marginally significant between controls and cancer cases overall (p=0.053) and between controls and laryngeal cancer cases (p=0.062).

ORs and 95% CI for the *ADH1B* Arg48His genotypes and the risk of head and neck cancer overall and oral and laryngeal cancer adjusted for potential confounders are presented in Table III. The *ADH1B* His allele was associated with a lower risk of head and neck cancer overall (OR: 0.203; 95% CI: 0.052-0.796, p=0.017), but no significant association was observed with oral or laryngeal cancer.

Discussion

To our knowledge, this is the first preliminary study carried out to investigate the association between the *ADH1B* Arg48His (rs1229984; G→A) polymorphism and the risk of HNSCC in a Spanish population from the Basque Country.

The frequencies of *ADH* gene polymorphisms differ significantly among different populations. In this respect, it has been shown that the *ADH1B* His allele is common in Asian populations (approximately 90%) but rare in European

Table I. *Clinical characteristics of cases and controls.*

	Oral (n=47)	Laryngeal (n=40)	HNSCC (n=87)	Controls (n=212)
Age (years)				
Mean	62.5	62.8	62.6	43.4
Range	(45.5-87.5)	(48.5-86.5)	(45.5-87.5)	(28.5-70.5)
Gender				
Male	29 (61.7%)	40 (100%)	69 (79.3%)	150 (62%)
Female	18 (38.3%)	0 (0%)	18 (20.7%)	92 (38%)
Alcohol				
No	16 (34%)	7 (17.5%)	23 (26.4%)	149 (61.6%)
Yes*	31 (66%)	33 (82.5%)	64 (73.6%)	93 (38.4%)
Tobacco				
No	8 (17%)	10 (25%)	18 (20.7%)	150 (62%)
Yes**	39 (83%)	30 (75%)	69 (79.3%)	92 (38%)

*≥10 Units alcohol per week; **≥20 cigarettes per week.

Table II. *Genotype distribution among controls according to alcohol drinking and tobacco smoking.*

Controls		Arg/Arg	Arg/His + His/His
Alcohol	No	120 (80.5%)	29 (19.5%)
	Yes	83 (89.2%)	10 (10.8%)
	χ^2 *p*-value		0.073
Tobacco	No	125 (83.3%)	25 (16.7%)
	Yes	78 (84.8%)	14 (15.2%)
	χ^2 *p*-value		0.766

Table III. *Distribution of cases and controls and odds ratios according to ADH1B genotype.*

	Arg/Arg	Arg/His + His/His	*p*-Value
Oral			
Cases	42	5	0.339
Controls	203	39	
OR (95% CI)	1.00	0.320 (0.073-1.403)	0.116
Larynx			
Cases	38	2	0.065
Controls	203	39	
OR (95% CI)	1.00	0.198 (0.028-1.400)	0.082
HNSCC			
Cases	80	7	0.063
Controls	203	39	
OR (95% CI)	1.00	0.203 (0.052-0.796)	0.017

OR: Odds ratio; CI: confidence interval. ORs were adjusted for age, sex, tobacco smoking and alcohol drinking.

populations (14, 16, 17, 21). In our study, the His allele is rare (0.085) in the resident population analyzed. These results are in accordance with previous studies carried out in other European populations (14, 17).

In this preliminary study, the *ADH1B* His allele was associated with a lower risk of HNSCC overall (OR: 0.203; 95% CI: 0.052-0.796), this result being consistent with previous reports (14-16). A large case–control European study showed that His allele carriers had a decreased risk of upper aerodigestive tract cancer overall, the results of the subsite analysis being significant for laryngeal cancer but not for oral cancer (14). In our study, when the results were analyzed by tumor site, the His allele was associated with a decreased risk of laryngeal and oral cancer; however, these associations were not statistically significant.

The *ADH1B* His allele encodes an enzyme characterized by an unusually rapid conversion rate of ethanol to acetaldehyde. Therefore, those individuals who carry the highly active *ADH1B* His allele are able to rapidly oxidize ethanol to acetaldehyde. This rapid oxidation leads to an accumulation of acetaldehyde after alcohol consumption and results in toxic side-effects such as facial flushing with sweating, tachycardia and drowsiness (22, 23). It has been hypothesized that these adverse effects suffered by His allele carriers exert a protective effect against alcohol consumption, and therefore protect against alcohol-related cancer. Several studies (24, 25) carried out in caucasoid population have reported association of the *ADH1B* His allele with lower alcohol intake. In our study, we observed different patterns of alcohol consumption among controls in the different genotype groups (Table II). Although these differences were not statistically significant (p=0.073), there were more His allele carriers among non-drinkers than among drinkers, which may indicate that this polymorphism may change drinking behaviour. This association between genotype and alcohol intake could imply that the real protective effect of the His allele is higher than that observed, since it may prevent His allele carriers from consuming alcohol.

In summary, we observed that the His allele of the *ADH1B* Arg48His polymorphism is associated with a reduced risk of HNSCC in patients from the Basque Country. Due to the preliminary nature of our study, we consider that these results should be interpreted with caution. Further case–control studies with larger sample sizes are necessary to confirm the protective effect of the *ADH1B* His allele for upper aerodigestive tract cancer in our region. Moreover, and in order to explain the high rates of oral and laryngeal cancer, subsequent studies may also focus on other relevant variants of genes implicated in ethanol metabolism.

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