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CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and *NAT2* polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer

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Abstract

Purpose As risk-modifiers of alcohol and tobacco effects, metabolic genes polymorphisms were investigated as susceptibility candidates for squamous cell carcinoma of the head and neck (SCCHN).

Methods A total of 210 cases and 245 hospital controls, age and gender matched, were genotyped for *CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1* exons 3 and 4, and *NAT2* polymorphisms. A measurement of the biological interaction among two risk factors was estimated by the attributable proportion (AP) due to interaction and its 95% confidence interval (CI).

Results SCCHN risk was associated with high-levels of alcohol intake [OR = 3.50 (95%CI: 1.93–6.35) and OR = 6.47 (95%CI: 2.92–14.35) for 19–30 g/day and >30 g/day, respectively], cigarette smoking [OR = 3.47 (95%CI: 1.88–6.41) and OR = 7.65 (95%CI: 4.20–13.90) for 1–25 and >25 pack-years of smoking, respectively] and low-fruit and vegetables consumption (OR = 2.45; 95%CI: 1.53–3.92). No differences were observed for the genotypes or haplotypes distributions among cases and controls, and no biological interaction emerged from gene–gene and gene–environment interaction analyses. An attributable proportion (AP) due to biological interaction of 0.65 (95%CI: 0.40–0.90) was detected for heavy drinkers with a low intake of fruit and vegetables, and an AP of 0.40 (95%CI: 0.10–0.72) resulted forever smokers with low fruit and vegetables consumption.

Conclusions Even in presence of high alcohol consumption or cigarette smoking, a high intake of fruit and vegetables might prevent the development of around one quarter of SCCHN cases. The lack of interaction between the studied polymorphisms and the environmental exposures suggests that chronic consumption of tobacco and alcohol overwhelm enzyme defences, irrespective of genotype.

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Abbreviations

SCCHN	Squamous cell carcinoma of the head and neck
CYP	Cytochrome *P*-450
GST	Glutathione *S*-transferase
NAT	*N*-acetyltransferase 2
mEH	microsomal Epoxide hydrolase
ORs	Odds ratios
CI	Confidence intervals
HWE	Hardy–Weinberg equilibrium
AP	Attributable proportion
DFS	Disease free survival
HR	Hazard ratio

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer worldwide (Hunter et al. 2005). Its multifactorial aetiology involves genetic susceptibility as well as the interaction between exposure to environmental risk and protective factors (La Vecchia et al. 1997). Exogenous exposure to substances such as tobacco, alcohol and betel quid have been repeatedly linked to the development and survival of upper aerodigestive cancer (Franceschi et al. 2000; Dikshit et al. 2005). However, in recent years evidence has accumulated to support the hypothesis that diet may also play an important aetiological role in the diseases development (Chainani-Wu 2002), with 10–15% of SCCHN cases in Europe being associated with a low intake of fruit and vegetables (Pelucchi et al. 2003).

Many chemical carcinogens contained in tobacco smoke or derived from alcohol degradation are metabolized into active forms that have deleterious effect on organisms. Variations in carcinogen metabolizing genes may result in alterations to the enzymatic activity and consequently the carcinogen activation or deactivation processes (Hung et al. 2005). Among phase I enzymes, the microsomal electron-transport system including the Cytochrome *P*-450s (CYP), play the most important role in the oxidation of chemical carcinogens and the activation of tobacco procarcinogens. Activated metabolites are then subjected to conjugation and other detoxification steps by phase II enzymes. A recent meta-analysis reported a modest positive association between SCCHN and *CYP1A1* 3′-flanking region and Glutathione *S*-transferase (*GST*s, phase II enzymes) polymorphisms (Hashibe et al. 2003), while some studies have demonstrated a slight association with *N*-acetyltransferase 2 (*NAT2*) slow variant (phase II enzyme) (Gajecka et al. 2005; Gonzalez et al. 1998; Morita et al. 1999). Nevertheless other studies have produced inconsistent results for *CYP2E1 Rsa*I and *Dra*I (Li et al. 2005), and microsomal Epoxide Hydrolase (*mEH*, phase I enzyme) polymorphisms (Wenghoefer et al. 2003; To-Figueras et al. 2002; Jourenkova-Mironova et al. 2000).

Even though metabolic genes are presumed to modulate cancer susceptibility via their interaction with carcinogens, few studies have investigated the interaction between these polymorphisms and environmental exposures (Risch et al. 2003; Matthias et al. 1998; Olshan et al. 2000; Jourenkova-Mironova et al. 1999). Furthermore, the combined inheritance of several unfavourable genotypes is yet to be fully understood in terms of an individual's susceptibility to cancer (Gajecka et al. 2005; Olshan et al. 2000; Boccia et al. 2007; Katoh et al. 1999). In such cases the inability of the studies to detect an interaction is often due to low statistical power resulting from a limited number of cases (<100). Additionally, another area requiring further research involves the potential influence of common genetic variations on the reoccurrence of neck nodes after the initial diagnosis of SCCHN, a field in which further research could lead to a better understanding of the carcinogenic mechanisms of SCCHN.

As far as the combined effects of unfavourable environmental exposures on SCCHN risk are concerned, relatively few authors have investigated how the interaction of tobacco or alcohol with fruit and vegetables intake affect that risk (De Stefani et al. 2000; Tavani et al. 2001; Rajkumar et al. 2003; Kreimer et al. 2006), and of these no one has estimated the amount of biological interaction that exists between them. Knowledge of causal interactions has important public-health implications: by identifying groups in which interaction occurs, preventive actions can be more effective (Rothman 2002a).

To explore these issues, and to study the associations between several metabolic gene polymorphisms and risk of SCCHN we conducted a hospital-based case-control study in Italy, comprising of 210 cases and 245 non-cancer controls.

Materials and methods

Study population

Subject recruitment was restricted to Caucasians born in Italy admitted during the period May 2002–April 2006 at the "A. Gemelli" teaching hospital of the Università Cattolica del Sacro Cuore in Rome. Cases were consecutive primary untreated head and neck cancer patients admitted to the Department of Otorhinolaryngology, with histopathologically confirmed SCCHN. We defined SCCHN as including International Classification of Disease Ninth revision codes 140–148 and 161. All patients were staged according to the UICC-TNM classification; 50.6% were staged I–II and 54.2% were classified T1–T2 (Sobin and Wittekind 1997). Based on tumor site, 66.7% of SCCHN were laryngeal, 16.9% oral, 9.7% oral-pharyngeal, 5.8% hypo-pharyngeal and 1.0% involved the paranasal sinuses. In order to identify controls representative of the exposure distribution in the source population for the cases, and to dilute any bias that might result from including a specific diagnostic group related to the exposures under study (Rothman 2002b), controls were randomly selected from cancer-free patients admitted to the same hospital during the identical time period including a broad spectrum of diagnoses (including mainly benign biliary tract disease, abdominal surgery, haemorrhoids, non-malignant thyroid conditions, pancreatic disease, metabolic disorders, inflammatory respiratory conditions, cardiac decompensation, hypertensive, and around 15% of blood donors). Controls

were frequency matched to cases for age (±5 years) and gender. The participation rate amongst cases and controls was 98 and 93%, respectively, and resulted in the recruitment of 210 cases and 245 controls. After written informed consent was obtained each subject donated a venous blood sample which was collected into EDTA-coated tubes from which DNA was isolated from the peripheral blood lymphocytes. This study has been performed according to the Declaration of Helsinki and has been approved by the ethics committee of the University.

Data collection

Cases and controls were interviewed by trained medical doctors using a standard questionnaire for demographic variables, cigarette smoking and drinking history, fruit and vegetables intake and occupational exposure to solvents and paints (for at least 1 year). Lifestyle questions focused on the time period ending 1 year prior to diagnosis. Pack-years were calculated as years smoked multiplied by the current number (or previous number, for those who had quit) of cigarettes smoked/day divided by 20. Fruit and vegetables intake was considered to be ≥14 portions/week if the individual consumed at least one portion of fruit and vegetable per day, or two portions of fruit or two portions of vegetables per day. We used 70 g as the approximate average "portion size" for vegetables (all vegetables both fresh and cooked) and 100 g for fruit (Ashfield-Watt et al. 2004). Cases were actively followed-up after the histopathological diagnosis of cancer was confirmed, with a mean follow-up time of 20 months. Furthermore, information on local and/or regional neck node recurrences were also collected. The proportion of lost to follow-up was 4.6%.

Genotyping

GSTM1 and *GSTT1* null alleles were identified by a multiplex-Polymerase Chain Reaction (PCR)-based method as described by Arand et al. (1996). Determination of the *mEH* exon 3 (Tyr113His) and exon 4 (His139Arg) polymorphisms was performed using an RFLP-based method (Sarmanova et al. 2000). *CYP1A1* 3′-flanking region *Msp*I polymorphism (m2 less common allele), *CYP2E1 Rsa*I polymorphism (c2 less common allele) and *CYP2E1 Dra*I (C less common allele) were also determined by PCR-RFLP analyses (Sarmanova et al. 2000). Three known slow acetylator alleles, NAT2*5A, *6A and *7A were identified as described by Peluso et al. (1998). Fast acetylator genotypes are homo-heterozygous wild-type alleles (*4A), slow acetylator genotypes are those with two slow acetylator alleles (Hung et al. 2004). Quality control for each genotyping was performed in each experiment, and 10% of the total samples were randomly

selected and reanalyzed with 100% concordance. The analyst was blinded to the case or control status of the samples.

Statistical analysis

The relationship between SCCHN and putative risk factors were measured using the adjusted odds ratios (ORs) and their 95% confidence intervals (CI) derived from logistic regression analysis using STATA software (version 8.2). We considered possible risk factors for SCCHN as potential confounders if addition of that variable to the model changed the OR by 10% or greater. Confounding checks were performed in both univariate and final multivariate models. If a factor was identified as a confounder of any estimated main effect, it was kept in all models. Based on these criteria, we controlled for age, gender, pack-years of cigarette smoking, alcohol consumption and fruit and vegetables intake, when appropriate. With exception of gender, we adjusted for confounders as continuous variables. Tests of Hardy–Weinberg equilibrium (HWE) were carried out for all of the polymorphisms among cases and controls separately. The genotypes of *GSTM1* and *GSTT1* were dichotomized according to presence versus absence of the null allele, and *NAT2* was dichotomized according to the inferred phenotype (fast vs. slow). We analyzed exons 3 and 4 *mEH* genotypes by "imputed phenotype" as suggested by Smith and Harrison (1997). Lastly, we conducted haplotype analysis for *EPHX1* exons 3 and 4, and *CYP2E1 Rsa*I and *CYP2E1 Dra*I polymorphisms using Cocaphase software (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased).

We also conducted gene–environment interaction analysis, using as a reference group those homozygous wild-type individuals who had not been exposed to environmental factors. In this analysis smoking status was categorized as ever/never smokers, with ever smokers including both ex and current cigarette smokers. Alcohol consumption was classified as heavy-drinkers/moderate-drinkers, whereby heavy-drinkers consumed as at least 19 g of alcohol/day. Occupational exposures were not considered, in this analysis, due to the small numbers. Gene–gene interaction analysis was conducted, using the homozygous wild-type individuals for both genes as the reference group. Furthermore, interaction analyses between fruit and vegetable intake and cigarette smoking habits, as well as fruit and vegetable intake and alcohol consumption were performed. Biological interaction between two risk factors was estimated using departure from additivity of the joint effects as criterion for interaction, as suggested by Rothman (2002a). To quantify the amount of interaction, the attributable proportion (AP) due to interaction was calculated together with the 95%CI as described by Andersson et al. (2005). The AP

due to interaction is the proportion of individuals among those exposed to the two interacting factors that is attributable to the interaction per se and it is equal to 0 in the absence of biological interaction (Rothman 2002a).

The risk of recurrence related to the studied polymorphisms and some clinicopathological variables was estimated by Cox's proportional hazards model, using the wild-type genotypes as the reference group. Disease free survival (DFS) was calculated from the day of histological diagnosis to the date of local recurrence of disease and/or regional lymph-node involvement.

Results

General characteristics of the study population are presented in Table 1. A moderate consumption of alcohol was not found to be linked to an increased risk of SCCHN. However, in heavy drinkers the OR increased to 3.50 (95%CI: 1.93–6.35) and 6.47 (95%CI: 2.92–14.35) for those with an alcohol intake of 19–30 and >30 g/day, respectively. Wine drinkers were 75% and 52% among cases and controls (p value <0.01), with 27% and 13% also contemporarily spirits drinkers, respectively (p value <0.01). Our results show an increased OR for cigarette smokers, with an OR of 3.47 (95%CI: 1.88–6.41) increasing to 7.65 (95%CI: 4.20–13.90) for 1–25 and >25 cigarette pack-years smoked, respectively (Table 1). A low consumption of fruit and vegetables (<14 portions/week) was significantly associated with the risk of SCCHN (OR = 2.45; 95%CI: 1.53–3.92). No differences were observed for occupational exposures to solvents and paints among the two groups (data not shown). The genotype frequencies of our control group were in keeping with those of Caucasians (Hung et al. 2005; Garte et al. 2001) and were in HWE for both cases and controls (p > 0.05). As shown in Table 2, the distribution of the polymorphisms was similar amongst the two groups, as was the distribution of the *mEH* "imputed phenotype" (data not shown). Haplotype analyses indicated that there was no significant linkage disequilibrium between *EPHX3* and *EPHX4*, as well as between *CYP2E1 Rsa*I and *CYP2E1 Dra*I, amongst the cases and the controls. Furthermore, the frequency of the estimated haplotypes was the same among the two groups. Even when the analysis was restricted to laryngeal cancer, or to moderate-alcohol users or never-smokers, the distribution of genetic polymorphisms remained identical amongst the groups.

From our analysis there was no evidence of gene–gene, gene–smoking, gene–drinking, and gene–fruit/vegetables interaction in relation to SCCHN risk (data not shown). Table 3 shows the effect on SCCHN for the combination of fruit and vegetables intake with cigarette smoking status or

Table 1 Odds ratios (95%CI) for SCCHN according to the collected variables and their frequency distribution among 210 cases and 245 controls

	Cases % (n)	Controls % (n)	OR (95%CI)
Age (years ± SD)	63.6 ± 11.3	63.3 ± 13.4	–
Male gender	71.4 (150)	72.5 (177)	–
Alcohol consumption			
0–6 g/day	26.7 (56)	55.9 (137)	1[a]
7–18 g/day	8.6 (18)	24.5 (60)	0.80 (0.40–1.60)
19–30 g/day	33.8 (71)	15.1 (37)	3.50 (1.93–6.35)
>30 g/day	30.9 (65)	4.5 (11)	6.47 (2.92–14.35)
Pack-years of cigarette smoking			
0	15.2 (32)	56.5 (138)	1[a]
1–25	22.4 (47)	23.8 (58)	3.47 (1.88–6.41)
>25	62.4 (131)	19.7 (48)	7.65 (4.20–13.90)
Fruit and vegetables intake			
≥14 portions/week	34.8 (73)	65.7 (161)	1[a]
<14 portions/week	65.2 (137)	34.3 (84)	2.45 (1.53–3.92)

OR adjusted for age, gender, alcohol consumption, pack-years of cigarette smoking and fruit and vegetables intake (as continuous variables)

[a] Reference category

Table 2 Odds ratios (95%CI) for gastric cancer according to the studied polymorphisms and their frequency distribution among 210 SCCHN cases and 245 controls

	Cases % (n)	Controls % (n)	OR (95%CI)
GSTM1 null[a]	54.6 (112)	52.9 (128)	1.07 (0.74–1.56)
GSTT1 null[a]	23.5 (48)	23.9 (58)	0.97 (0.63–1.51)
EPHX1 exon 3[a]			
Tyr/His	34.3 (72)	34.4 (83)	1.06 (0.70–1.60)
His/His	15.7 (33)	11.2 (27)	1.52 (0.86–2.69)
EPHX1 exon 4[a]			
His/Arg	28.2 (59)	36.4 (88)	0.69 (0.46–1.03)
Arg/Arg	3.4 (7)	2.5 (6)	1.21 (0.40–3.72)
*CYP1A1 Msp*I m2 carriers[a]	19.5 (41)	23.3 (56)	0.80 (0.51–1.27)
*CYP2E1 Rsa*I c2 carriers[a]	4.8 (10)	6.6 (16)	0.72 (0.33–1.63)
*CYP2E1 Dra*I C carriers[a]	7.2 (15)	8.2 (20)	0.87 (0.43–1.76)
NAT2 Slow[b]	52.0 (109)	52.9 (128)	0.98 (0.67–1.45)

OR adjusted for age and gender

[a] Reference groups are the homozygous wild genotypes for each gene

[b] Reference group is fast acetylators (homo-heterozygous for the wild-type allele)

alcohol consumption. An OR of 13.26 (95%CI: 6.40–27.46) appeared for ever-smokers with a low fruit and vegetables consumption (<14 portions/week) compared with never-smokers

Table 3 Odds ratios for SCCHN and 95%CI according to combined cigarette smoking and alcohol intake with fruit and vegetables consumption

		Fruit and vegetables consumption	
		≥14 portions/week	<14 portions/week
Smoking status			
Never	No. cases/	15/96	17/44
	No. controls	1[a]	2.45 (1.05–5.70)
Ever	No. cases/	58/65	120/40
	No. controls	6.50 (3.68–15.62)	13.26 (6.40–27.46)[c]
Alcohol consumption			
Moderate[b]	No. cases/	37/133	37/64
	No. controls	1[a]	2.03 (1.12–3.67)
High[c]	No. cases/	36/28	100/20
	No. controls	3.42 (1.72–6.78)	12.78 (6.35–25.72)[d]

OR adjusted for age, gender, alcohol consumption and pack-years of cigarette smoking (as continuous variables)

[a] Reference category

[b] Moderate = < 19 g/day; High = ≥ 19 g/day

[c] AP = 0.40 (95%CI: 0.10–0.72)

[d] AP = 0.65 (95%CI: 0.40–0.90)

with an intake of fruit and vegetables ≥14 portions/week. Thus an AP of 0.40 (95%CI: 0.10–0.72) SCCHN cases, among the ever-smokers with a low intake of fruit and vegetables, appears to be related to the interaction between the two risk factors. Similarly, an OR of 12.78 (95%CI: 6.35–25.72) appeared for heavy drinkers with a low intake of fruit and vegetables, compared with non-drinkers with an intake of fruit and vegetables ≥14 portions/week. An AP of 0.65 (95%CI: 0.40–0.90) SCCHN cases, amongst the heavy drinkers with a low intake of fruit and vegetables, is therefore be related to the presence of both risk factors.

During the follow-up period local recurrence was observed in 42 cases (1.07/100 person-months; 95%CI: 0.75–1.40), among them 3 patients also experienced metastatic regional lymph-node involvement, with a DFS rate of 81% after 20 months of follow-up. The survival analysis did not show any significant association between any of the polymorphisms and DFS. Similarly, no gene–gene or gene–environment interactions were detected (data not shown). Multivariable analysis showed an improved DFS for the early stages (I–II) of disease and low tumour extensions (T1–T2), with an adjusted Hazard Ratio (HR) of 0.36 (95%CI: 0.18–0.74) and 0.33 (95%CI: 0.16–0.66), respectively.

Discussion

Our study shows a significantly increased risk of SCCHN in relation to cigarette smoking, alcohol consumption and low intake of fruit and vegetables (<14 portions/week). In particular, we found that a strong biological interaction between high alcohol consumption and a low fruit and vegetable intake, as well as between cigarette smoking and a low fruit and vegetable intake, may account for a large proportion of SCCHN cases. Despite the biological plausibility of genetic polymorphisms for phase I–II enzymes as risk modifiers of alcohol and tobacco-related carcinogenesis, we found no difference in the frequency of the studied genotypes between the cases and the controls. Also there was no evidence of biological interaction from the gene–gene and gene–environment interaction analyses. These results are in keeping with previous findings in Caucasian populations (Risch et al. 2003; Matthias et al. 1998; Olshan et al. 2000), confirming that both chronic and high consumption of tobacco and alcohol overwhelm metabolic enzyme defences, (Taningher et al. 1999), irrespective of the genotype. The power to detect interaction in these studies is low, therefore these results should be confirmed by larger studies or by performing a meta-analysis in which the original data is pooled. Finally, we were not able to detect any effect for the metabolic gene polymorphisms on disease recurrence, which provides additional evidence to support the hypothesis that the studied polymorphisms do not effect SCCHN carcinogenesis. The interpretation of this result, however, is not straightforward, since disease progression is also influenced by the effect of the chemotherapic drugs, whose data has not been collected in the present report, which are usually metabolized by the enzymes under investigation.

Our report confirms the well-known effects of alcohol and tobacco-derived carcinogens on SCCHN risk (La Vecchia et al. 1997), and the more recent research findings pertaining to the potential anticarcinogenic effects of fruit and vegetables on laryngeal, oral and pharyngeal cancers (Chainani-Wu 2002; Pelucchi et al. 2003; De Stefani et al. 2000; Tavani et al. 2001; Rajkumar et al. 2003; Kreimer et al. 2006; Pavia et al. 2006). This protective effect is due to the synergistic interaction of a number of the antioxidants micronutrients, such as β-carotene, vitamin C and E and retinol. Furthermore, fruit and vegetables are the main sources of folate, whose deficiency may increase the risk of cancer by inducing an imbalance in DNA precursors, and altering normal DNA methylation (Pelucchi et al. 2003).

The main results of our study shows that an intake of fruit and vegetables <14 portions/week has a strong effect in increasing SCCHN risk in heavy drinkers and ever-smokers, with more than just an additive effect among the two component causes being demonstrated. We used AP due to interaction as a measure to quantify the biological interaction between the unfavourable combined risk factors and showed a strong interaction between high levels of alcohol consumption and fruit and vegetable intake, as well

as for cigarette smoking status and fruit and vegetables. Assuming that the relationships studied are causal and based on the definition of biological interaction between two component causes (Rothman 2002a; Rothman and Greenland 2005), our results suggest that 65% of SCCHN cases among heavy alcohol-drinkers with a low intake of fruit and vegetables have arisen because of the synergistic interaction amongst the two component causes. Similarly, 40% of SCCHN cases among ever-smokers with a low fruit and vegetables intake are caused through a mechanism in which both risk factors are biological dependent in the same disease process. In other words, since biological interaction among two causes occurs when the effect of one is dependent on the presence of the other, if either of the two components (low fruit and vegetables intake and high alcohol consumption or smoking) is absent than a substantial number of the SCCHN cases would not occur. Given that in our population more than half of SCCHN cases were heavy alcohol drinkers or ever-smokers (mostly both), considering the point estimate of the APs, then around a quarter of the SCCHN cases would have never developed if their consumption of fruit and vegetables was adequate. This result has important implications from a public health point of view, since it shows that by increasing fruit and vegetable intake at the population level, even in the presence of harmful lifestyle behaviours, many cases of SCCHN cancer might be prevented.

Biologically speaking, the combined effect of tobacco smoking/alcohol consumption with fruit and vegetable intake could be explained as follows: ethanol might alter the intracellular metabolism of the target epithelial cells, and this impairment can be aggravated by the coexistence of nutritional antioxidant deficiencies. Furthermore, high alcohol consumption interferes with folate absorption and increases folate excretion by the kidney, therefore the combination of alcohol with low fruit and vegetables intake may result in folate deficiency which has repeatedly been linked to SCCHN risk (Pelucchi et al. 2003; Almadori et al. 2002). Similarly, ever-smokers are chronically exposed to genotoxic carcinogens contained in tobacco, and inappropriate peroxidation reactions and detoxification processes, catalysed and controlled by the antioxidants contained in fruit and vegetables, may dramatically increase SCCHN risk (Weisburger 1999).

Several limitations should be taken into account in the interpretation of our results. First, as in all case-control studies information bias may exist, which could lead to a biased OR related to the environmental exposures. Therefore, it would be interesting to compare our results on the interaction between the environmental exposures on SCCHN risk with those coming from cohort or experimental studies. Second, based on the prevalence of the analyzed genotypic variants in our population (Table 2), our study

was powered to detect a minimum OR of 1.8 for common polymorphisms (with a significance level of 5%), but not for *CYP2E1 Rsa*I, *CYP2E1 Dra*I and the homozygotes variants of *EPHX3* and *EPHX4*. The study's sample size limits its ability to explore the combined effects of the genotypes, or gene–environment interactions, which highlights the need for further research, based on larger sample sizes, in order to confirm our results.

In summary, the main result of our study supports the protective role of fruit and vegetable intake in the prevention of SCCHN, particularly in heavy alcohol drinkers and ever-smokers. Specifically it shows that, even in presence of high alcohol consumption levels or cigarette smoking, an intake of fruit and vegetables ≥ 14 portions/week might prevent the development of around one quarter of SCCHN cases. Furthermore, our results suggests the possible lack of association between key metabolic gene polymorphisms and SCCHN, thus emphasizing the importance of alcohol consumption, tobacco smoke and fruit and vegetable intake as major risk factors for SCCHN.

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