Review Article

Polymorphisms in the Human Cytochrome P450 and Arylamine N-Acetyltransferase: Susceptibility to Head and Neck Cancers

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer worldwide and is associated with low survival and high morbidity when diagnosed in advanced stage [1, 2]. This type of cancer accounts for almost 500,000 newly diagnosed cancer cases per year [3, 4]. Epidemiological studies have shown that HNSCC occurs through a complex multistage process that may involve exposure to a combination of carcinogens from cigarette smoking [5, 6], alcohol consumption [7], or tobacco chewing [8, 9]. As in some regions of the world, these toxic agents are responsible for about 75% of all cancer cases. HNSCC is used to be considered as a tobacco-induced and a preventable cancer [4, 10]. The hypothesis that genetic susceptibility or predisposition is of important role in head and neck cancer (HNC) etiology is highly supported by case-control studies of several phenotypic and genotypic assays [11–13]. Some studies stated that gene-environment interactions in relation to HNSCC are linked to genes involved in metabolism enzymes for alcohol and tobacco smoke constituents [14]. Polymorphisms in the genes encoding these enzymes, by altering their expression and function, may increase or decrease carcinogen activation/detoxification, followed by modulation of cancer risk [15, 16].

Polymorphisms in the carcinogen-metabolizing genes have been analyzed on individual basis [17]. Several studies have addressed the relationship between the genetic polymorphisms of enzymes involved in the metabolic activation of carcinogens and the occurrence of HNSCC [15, 18, 19]. Genetic polymorphisms in cytochrome P450 (i.e., CYP1A1, CYP1B1, CYP2D6, and CYP2E1) and N-acetyltransferase isozymes (NAT1 and NAT2) in order to summarize and analyze findings from the literature related to HNC risk by focusing on (i) the interaction between these genes and the environment, (ii) the impact of genetic defect on protein activity and/or expression, and (iii) the eventual involvement of race in such associations.

The occurrence of head and neck cancer (HNC) is associated with smoking and alcohol drinking. Tobacco smoking exposes smokers to a series of carcinogenic chemicals. Cytochrome P450 enzymes (CYP450s), such as CYP1A1, CYP1B1, and CYP2D6, usually metabolize carcinogens to their inactive derivatives, but they occasionally convert the chemicals to more potent carcinogens. In addition, via CYP450 (CYP2E1) oxidase, alcohol is metabolized to acetaldehyde, a highly toxic compound, which plays an important role in carcinogenesis. Furthermore, two N-acetyltransferase isozymes (NATs), NAT1 and NAT2, are polymorphic and catalyze both N-acetylation and O-acetylation of aromatic and heterocyclic amine carcinogens. Genetic polymorphisms are associated with a number of enzymes involved in the metabolism of carcinogens important in the induction of HNC. It has been suggested that such polymorphisms may be linked to cancer susceptibility. In this paper, we select four cytochrome P450 enzymes (CYP1A1, CYP1B1, CYP2D6, and CYP2E1), and two N-acetyltransferase isozymes (NAT1 and NAT2) in order to summarize and analyze findings from the literature related to HNC risk by focusing on (i) the interaction between these genes and the environment, (ii) the impact of genetic defect on protein activity and/or expression, and (iii) the eventual involvement of race in such associations.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Substrate</th>
<th>Reference</th>
<th>Functional effect</th>
<th>Polymorphism</th>
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<tbody>
<tr>
<td>CYP1A1</td>
<td>TC (e.g., benzo[a]pyrene dimethylbenz[a]anthracene), 6-nitrochrysene,</td>
<td>[33]</td>
<td>Phase I oxidative and reductive</td>
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<td>Arylamines and heterocyclic aromatic amines</td>
<td>[181, 184, 186, 187]</td>
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*aTobacco carcinogens; --: undefined.
hydrocarbons including the prototype of this chemical class, benzo(a)pyrene [24, 25], and tobacco-specific nitrosamines (TSNAS) have been implicated in HNC etiology in smokers [26]. It was previously suggested that acetaldehyde, the first metabolite of alcohol when orally ingested, is involved in alcohol-related cancer induction. Nevertheless, carcinogenic pathway of alcohol is not elucidated [27].

In the present paper, we summarize results of studies (published up to February, 2013) dealing with the association between the genetic variations in genes coding for phases I and II carcinogen metabolism enzymes (CYP1A1, CYP1B1, CYP2D6, CYP2E1, NAT1, and NAT2) and the increased risk of head and neck cancer development.

2. Cytochrome P450 (CYP450)

The determinant factors for HNSCC development remain unclear. Although the importance of tobacco and alcohol consumption as risk factors suggests that genes encoding detoxifying enzymes are susceptibility candidates, several data have not confirmed associations between these enzymes and the occurrence of HNSCC. Previous studies suggest that various CYP genotypes are linked with its outcome rather than its susceptibility [28, 29].

2.1. CYP1A1. The human enzyme CYP1A1 is the most active among the CYPs in metabolizing procarcinogens, particularly, the polycyclic aromatic hydrocarbons (PAHs), into highly reactive intermediates [30]. When these compounds bind to DNA and form adducts, they may contribute to carcinogenesis. Despite the fact that PAHs are ubiquitous in the environment, remarkable sources of exposure such as smoking, certain occupations, and air pollution may lead to the greatest concern [31]. The aromatic hydrocarbon receptor is a key activator of the CYP1A1 gene [32, 33]. PAHs were classified among important toxicants as they induce CYP1A1 gene and act as procarcinogenic substrates [34, 35]. The relationship between CYP1A1 variants and cancer risk has been investigated in several studies [18]. CYP1 enzymes are coupled to phase II detoxification in vivo. It has been proposed that, compared with other CYP enzymes, CYP1A1 is more tightly coupled to phase II metabolism and plays a more important role in vivo in detoxification than toxin activation [36].

A recent study confirmed the importance of tobacco smoking as the main risk factor for the upper aerodigestive tract (UADT), indicating that about 68% of cancers can be attributed to this risk factor. A significant association between metabolizing phase I genes (CYP1A1) and UADT cancers was found [37]. Nagaraj et al. [38] identified molecular factors which contribute to the increased risk of smokers for oral squamous cell carcinoma (OSCC). In fact, they evaluate gene expression profile change according to cigarette smoke condensate in normal epidermal keratinocytes, oral dysplasia cell lines Leuk1 and Leuk2, and a primary oral carcinoma cell line 101A. Their results have shown that treatment by cigarette smoke condensate acts on several cell types and usually leads to overexpression of CYP1A1. These findings support the hypothesis that cigarette smoke condensate is widely involved in the activation of procarcinogens. These results are similar to those of Chi et al. [39] and those of Wen and Walle [40].

A functional role has been previously assigned to two nonsynonymous polymorphisms in the CYP1A1 gene. The first one is an adenine (A) to guanine (G) substitution at codon 462 in exon 7 (Ile462Val, rs1048943). The second one is a thymine (T) to cytosine (C) transition (rs4646903) [41]. This last mutation changes a restriction site for the MspI enzyme, thus resulting in three genotypes: a predominant homozygous allele (genotype A, TT), a heterozygous allele (genotype B, TC), and a homozygous rare allele (genotype C, CC) [42]. Contrary to genotype C, genotype A abolishes the restriction enzyme site of MspI. The exon 7 restriction-site polymorphism resulted in three genotypes: the predominant homozygous (Ile/Ile), the heterozygous (Ile/Val), and the rare homozygous (Val/Val). Another mutation, CYP1A1 T6235C (m1), located in the 3’ end of this gene, is considered as a polymorphism for the restriction endonuclease MspI and results in a mutant CYP1A1 allele designated as CYP1A1∗2A. Three additional polymorphisms have also been reported in exon 7 of the CYP1A1 gene. The first one is a CYP1A1 4889AG (m2) transversion responsible for the replacement of Ile by Val at position 462 in the mutant form of the protein, and it is known as CYP1A1∗2B. The second polymorphism is caused by a CYP1A1 T5996C (m3) transition in the 3’ noncoding region of the gene which is known as CYP1A1∗3. The last one is located at position 4887 and is a transversion, a CYP1A1 4887C/A (m4), that results in a mutation of Thr to Asn at codon 461 (CYP1A1∗4) [41, 43, 44]. Among these four polymorphisms, the MspI at the 3’flanking region has been reported in many epidemiological studies to be associated with cigarette smoking-related cancer risk in some but not all studies [18, 41, 43, 45–52].

Several studies have been since performed examining the potential association between the polymorphic CYP1A1 (MspI and/or exon 7) and the HNC occurrence (Figure 1). In the Brazilian patients, a tendency of increased oral cancer risk among CYP1A1 genotypes (426Val/Val) that compared both with the wild-type homozygous (OR = 2.85) and heterozygous (OR = 2.61) ones was found by Marques et al. [53]. The CYP1A1 (426Val/Val) genotype was found three times more frequent than in controls in 3% of oral cancer patients. In spite of the absence of any statistical significance, these results strongly supported the previous ones showing that the mutant allele CYP1A1 426Val is related to an increased risk of oral cancer in Caucasians, in the United States [47], among Asian populations [54], and in Indians [19]. It was also reported that the CYP1A1 4889 A/G genotype (Ile462Val (rs1048943)) is more frequent in the group of white HNSCC patients (10%, n = 108) than in white controls (7%, n = 165) [15]. However, for genotype heterozygous, the moderate increase in the HNSCC risk was not statistically significant. Nevertheless, it was reported an overrepresentation of the CYP1A1 4889G allele among the nonsmoking Caucasian patients with oral cancer [47] and among the Japanese HNSCC patients [55]. For the Polish patients, the increased frequency of the CYP1A1∗4 allele and
the CYP1A1*4/*4 genotype (CYP1A1 Thr461Asn) supports its association with HNC and might be specific for laryngeal SCC [56]. However, Reszka et al. [57] and Amthra et al. [58] suggested no significant increase in HNC risk in the Polish and Indonesian patients, respectively, with the CYP1A1 462Val alleles (OR = 1.60 and 0.70, resp.). Moreover, in a recent meta-analysis study, no association between Ile462Val polymorphism and HNC risk was found [59].

Polymorphisms located in the CYP gene result in the enzyme activity increase [60]. The homozygous CYP1A1 (MspI) mutations are present in 7% to 10% of the white population and in up to 33% of the Japanese population. These homozygous (m2/m2) polymorphisms were associated with a high susceptibility to SCC of the lung or UADT according to some researches [61, 62]. To investigate the association between CYP1A1 polymorphism (MspI) and risk for OSCC in the Korean [63] and the Indian [64] populations, many studies have been conducted, and they found that the risk for oral cancer was significantly increased in subjects of these populations with the homozygous CYP1A1 (m2/m2) genotype (Indian: OR = 3.2, 95% CI = 1.10–10.28, and P = 0.05; Korean: OR = 3.8, 95% CI = 1.9–7.7, and P = 0.023), regardless of smoking history (smokers: OR = 4.4, and 95% CI = 1.2–16.3; nonsmokers: OR = 4.9, and 95% CI = 1.9–12.5). Recently, in Liu et al. [59] meta-analysis, a significant association between MspI SNP and HNC risk was found (95% CI = 1.15–1.57; P < 0.001). This effect was found to be more pronounced in smokers (OR = 2.98, 95% CI = 1.69–5.26, and P < 0.001), thus demonstrating that gene-smoking interaction that intensifies carcinogenesis might exist [59]. Additionally, Sam et al. [65], found that the individuals polymorphic for CYP1A1 MspI revealed an increased risk for UADT cancers than that ascribed to a single susceptible gene among tobacco users in the Indian population (OR = 6.43; 95% CI = 3.69–11.21). Moreover, in a previous study, Sam et al. [66] found that CYP1A1*1A/*2A and *2A/*2A polymorphic genotypes are associated with an enhanced risk to UADT cancers, in particular, among the habitual tobacco smokers and chewers carrying mutant genotypes in the Indian population (OR = 1.76; 95% CI = 1.19–2.60 and OR = 2.83; 95% CI = 1.43–5.61, resp.). Furthermore, Olivieri et al. [67] Figaro Gattà et al. [68], Tanimoto et al. [69], and Singh et al. [23] reported that the Brazilian, the Japanese, and the North Indian patients carrying CYP1A1 (*1A/*2A) genotype presented an increased HNSCC risk. However, no statistically significant difference in the CYP1A1*2A allele and in the CYP1A1*2A/*2A genotype frequency was found in Gajecka et al. study [56].

Many researches focused on the association of CYP1A1 polymorphism with susceptibility to laryngeal cancer. Unfortunately, their results were inconsistent and inconclusive. CYP1A1 MspI polymorphism was found to be a risk factor for laryngeal cancer in Caucasians (OR = 1.29) but not in Asians (OR = 1.38) [70]. Variant genotypes of CYP1A1 might not be considered as risk factors for oral cancer [70]. Moreover, Táí et al. [71] studied CYP1A1 polymorphisms in the Chinese patients with laryngeal and hypopharyngeal SCC and control subjects. They found an increased risk associated with the CYP1A1 5798CC genotype (OR = 2.39; 95% CI = 1.11–5.16), compared with the TT genotype [71]. In other investigations, no such association was found [72–74]. In the Gronau et al. report [75], a German case-control study, the authors found that the homozygous mutation and the MspI restriction site in exon 7 are present only once in the control group and that no patient revealed this genotype. Furthermore, the genotype frequencies at the CYP1A1 gene loci investigated in other German case-control studies showed no differences between these groups, suggesting a lack of influence of these genes in the susceptibility to laryngeal cancer [28, 76]. The association of nasopharyngeal cancer (NPC) in Taiwan with CYP1A1 MspI genetic polymorphism was studied [77], and no significant associations of the examined genotypes with NPC risk were noted. Moreover, a recent study [78] of two SNPs in CYP1A1 m1 [MspI (rs4646903)] and CYP1A1 m2 [Ile462Val (rs1048943)] in a total of 457 Cantonese nuclear families, consisting of 2134 members, has concluded that there is no absence of any statistical significance between m1 polymorphism and susceptibility to NPC. However, m2 polymorphism might be associated with NPC in the Cantonese nuclear families (P = 0.045) [78].

It is noteworthy that all studies on the relationship between CYP1A1 genotype and cancer have focused on each polymorphism separately. Having global information regarding the individual haplotype could give better clarification of such associations. Recently, Sabitha et al. [79] examined for the first time the association of three SNPs in the CYP1A1 MspI locus (m1/m1, w1/w1, and m2/m2) with HNC risk. They found that individuals carrying at least one CYP1A1 m1 or m2 variant allele were at a 2-fold elevated risk for HNC and concluded that CYP1A1 is an important determinant in susceptibility to tobacco-induced HNC among Indians [79]. Cigarette smoke has been shown to upregulate CYP1A1 under in vitro conditions as well as in smokers [38, 39, 80]. In five earlier different studies investigating CYP1A1 genotype-smoking interactions [48, 81–84], two have reported evidence of an interaction [81, 84]. Further few studies [28, 72, 85, 86] did not find a relationship between pack-years of smoking and risk of HNSCC among cases with the CYP1A1 MspI polymorphism. But recently, Sabitha et al. [79] found association...
between pack-years of smoking and risk of HNSCC among cases with the CYP1A1 MspI polymorphism. Heavy smokers showed an increased risk for HNC in association with both m1 and m2 mutations. The OR of HNC for the variant CYP1A1 m1 genotype, the tobacco smoking, and both factors combined were OR = 4.93, 95% CI = 1.83–13.68; 1.07, 95% CI = 0.16–7.34; 0.60, 95% CI = 0.30–1.18, respectively. Sabitha et al. [79] findings support that CYP1A1 m1 and CYP1A1 m2 polymorphisms were associated with smoking-related HNC in India.

Association of more than one SNP in one individual may additively or synergistically contribute to the increased cancer risk. Furthermore, the impact of xenobiotic-metabolizing enzymes and transporters could determine the functional results in the risk of HNC over the independent effects of each single susceptibility gene. It is becoming clearly evident that single gene or single environmental factor cannot explain susceptibility to diseases with complex etiology such as HNC. Expression of these enzymes might be one of the reasons for interindividual differences in HNC risks. In a recent study, Masood et al. [87] studied the expression of CYP1A1 MspI in HNC tumor and normal healthy tissues, and the relationship with stages of HNC in the Pakistani population. They found that the CYP1A1 mRNA is less expressed in head and neck carcinoma compared with adjacent normal tissue (OR = 4.5, 95% CI = 1.5–13.4). CYP1A1 expression was downregulated according to tissue stage as follows: 62.5% in tissues of stage 1, 72.7% in tissues of stage 2, 60% in tissues of stage 3, and 100% in tissues of stage 4. Therefore, it is very obvious to conclude that CYP expression is involved in the carcinogenesis by a pathway that is still not elucidated.

Recently, Sharma et al. [88] explored the North Indian population by a multifactor dimensionality reduction method in order to determine potential gene-environment and gene-gene interactions that predispose to HNC. They observed significant gene-gene interactions among GSTM1 copy number variants and CYP1A1 T380IC (rs464903) variant among smokers. This method showed that the combining three factors, smoking status, CYP1A1 T380IC, and GSTM1 copy number variants, conferred more than 4-fold increased risk of HNC (OR = 4.89; 95% CI = 3.15–7.32; P < 0.01). Therefore, genetic variants in tobacco-metabolizing genes may contribute to HNC risk through gene-gene and gene-environment interactions. In a previous study of Sharma et al. [89] research group, epigenetic modifications of genes involved in carcinogen metabolism pathway, CYP1AI, CYP2AI3, and GSTM1, were assessed by evaluating the role of aberrant hypermethylation as well as its relation to tobacco and alcohol consumption. In addition, CYP1AI and CYP2AI3 polymorphisms were also investigated in the Indian population. Results of this study showed that hypermethylation of CYP1AI and GSTM1 showed significant association with HNC (P = 0.027, and P = 0.010, resp.). They also showed a significant interaction between smoking and methylation status of CYP1AI and CYP2AI3 in HNC (P = 0.029, and P = -0.034, resp.). So hypermethylation of carcinogen metabolism pathway genes is associated with an increased risk of HNC regardless of the smoking status [89].

In a recent case-control Indian population study [90], the CYP1AI (∗2A and *2C), CYP2EI (∗1B, *5B, and *6), and GST (M1, T1, and P1) adenosine triphosphate-binding cassette B1 3435C>T (ABCB1) polymorphisms were studied. Results showed a high risk of gene-gene interactions with the concurrent deletions of GSTT1 and GSTM1 genotypes associated with variant genotypes of CYP1AI∗2A (OR = 8.21; 95% CI = 1.91–49.48), GSTT1, and GSTM1-deficient genotypes with CYP2EI∗1B variant genotypes (OR = 6.73; 95% CI = 1.32–22.81), and a very high risk with the combined variant genotypes of CYP1AI∗2A, GSTT1, and ABCB1 (OR = 11.14; 95% CI = 2.70–46.02). Thus, showing that interaction with many drug-metabolizing enzymes and transporter proteins is of a high risk for UADT cancers compared with that of a single susceptible gene [90]. The interaction between phase I deficient enzymes and a phase I hyperactive enzyme (CYP1AI) is of interest as it can lead to a larger amount of toxic compounds that may play a crucial role in the initiation or progression of UADT cancers. The risk of cancers is frequently higher in individuals with combined mutant genotypes of CYP1AI∗2A and GSTM1 null genotype than in those with CYP1AI or GSTM1 gene alone. The interaction between CYP1AI and GSTM1 is so important. In fact, it can be related to CYP1AI induction [91]. The significant risk for oral cancer among carriers of both CYP1AI∗2A homozygous variant and GSTM1 null genotype previously suggested by Anantharaman et al. [64] was also supported by Indian, Japanese, Korean, and Brazilian studies [62, 63, 68, 69].

2.2. CYP1B1. Human CYP1B1 is located on chromosome 2 at the 2p21-22 region [92, 93]. The length of its genomic DNA is 12 kilobases (kbs), and the length of its mRNA is ~5.2 kb. The CYP1B1 enzyme (cytochrome P450, family 1, subfamily B, and polypeptide l) is a hemihelat monoxygenase involved in metabolizing xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs) [92]. At transcriptional level, CYP1B1 gene is activated by PAHs that constitute the major constituents of cigarette smoke and tobacco, hence making it responsive to smoked and smokeless (chewing) tobacco [40, 92, 94]. As CYP1B1 is crucially involved in the bioactivation of chemically diverse tobacco-related procarcinogens to reactive metabolites, its expression is considered as a significant parameter of carcinogenesis [95]. Other expression studies showed that CYP1B1 is overexpressed in several human tumors in comparison with normal tissues [94, 96, 97]. It was also demonstrated the implication of many allelic variations in CYP1B1 in modulating the incidence of several types of cancers [98, 99]. Therefore, CYP1B1 played an important role in carcinogenesis.

In humans, CYP1B1 locus has been demonstrated to be genetically polymorphic where many mutations have been identified in CYP1B1 gene so far [100]. Four nonsynonymous single-nucleotide polymorphisms (SNPs) have been described: (i) Arg to Ser at codon 48 (CYP1B1∗2) (rs100012), (ii) Ala to Ser at codon 119 (CYP1B1∗2), (iii) Leu to Val at codon 432 (CYP1B1∗3) (rs1056836), and (iv) Asn to Ser at codon 453 (CYP1B1∗4) (rs1800440) [101]. The association
of SNPs in CYP1B1 with the increased risk of ovarian, endometrial, renal, and prostate cancers as well as smoking-related lung cancer has been reported in the Caucasian and the Japanese populations [102]. Contradictorily, Aklillu et al. [101] have shown that CYP1B1 variant enzymes differ in their catalytic activity according to the metabolism of 17β-estradiol. It has been reported that proteins presenting one of the four common SNPs (Arg48Ser, Ala195Ser, Leu432Val, and Asn453Ser) had slight effects on benzo[a]pyrene-7,8-diol metabolism [103, 104]. This genotype is then considered as a susceptibility factor to develop PAH-induced cancers. Few epidemiological studies aimed at evaluating a possible association between genetic polymorphisms of CYP1B1 and susceptibility to HNSCC have been conducted [105, 106].

Two authors have studied the CYP1B1 3 polymorphism and identified the susceptibility factor for HNSCC [21, 26]. In fact, genotype and haplotype frequencies of the four SNPs in CYP1B1 have been evaluated in HNSCC patients of the Indian population [21]. Singh et al. [21] study indicates a several-fold increase in cancer risk among cases that use tobacco chewing with the variant genotypes of CYP1B1 2 (OR = 8.80; 95% CI = 2.60–29.87; P < 0.05) and CYP1B1 3 (OR = 2.74; 95% CI = 1.12–6.70; P < 0.05) suggesting that interaction between genes and environment plays an important role in susceptibility to HNSCC. Another significant interaction between the variant genotypes of CYP1B1 2 and cigarette smoking was also found in smoking patients (OR = 2.37; 95% CI = 1.62–4.85; P < 0.05). However, for CYP1B1 3 and CYP1B1 4 genotypes (heterozygous and homozygous mutants), no significant interaction regarding smoking with relation to HNSCC has been observed [21]. In contrast to Singh et al. [21], findings, Ko et al. [26] reported the presence of variant genotypes of CYP1B1 3 at a significantly higher frequency in smoking patients compared with healthy smokers, thus suggesting that genotypes of CYP1B1 3 significantly interact with smoking and likely represent a susceptibility factor in smoking related to HNSCC (OR = 4.53; 95% CI = 2.62–7.98; P < 0.001). Li et al. [106] failed to find any significant interaction between tobacco smoking and CYP1B1 3 in HNSCC and explained their different results by ethnic backgrounds (Europeans versus American Caucasians). Indeed, there are significant differences in the allele frequency of CYP1B1 2 and CYP1B1 3 variants between Caucasians and Asians [107] and Indians [21]. The variant allele of CYP1B1 2 was more frequent in the Indian controls compared with the Caucasians. This could explain the higher risk for HNSCC in Singh et al. [21] study. However, there were no significant associations between the risk of hypopharyngeal and laryngeal SCC development and CYP1B1 Leu432Val genotypes [71]. The difference in the genetic background related to the ethnic origin of each population or the involvement of other confounding genetic factors responsible for HNSCC might explain absence of associations.

It is well known that the use of tobacco is often accompanied by alcohol consumption [108]. Many studies have reported a high risk of HNC in alcohol drinkers (adjusted for smoking). Depending on the consumed alcohol amount, this risk varies from less than 2 to 12 folds [9, 109]. Despite the fact that interaction between alcohol and CYP1B1 genotypes in promoting HNSCC risk is still unknown, it is suggested that tobacco carcinogens are dissolved in alcohol, thus facilitating their access to the mucosa of upper aero-digestive organs [110]. A strong interaction between alcohol consumption and the CYP1B1 2 genotypes for the increased risk to HNSCC was also established [21]. This interaction was associated in patients with a heterozygous genotype of CYP1B1 2 (OR = 6.07; P < 0.05) and in patients with the homozygous mutant allele of CYP1B1 2 (OR = 5.24; P < 0.05) [21].

Although many polymorphisms of the CYP1B1 gene have been associated with different cancers, less is known about changes in mRNA expression levels in tumor tissue. The CYP1B1 gene encodes for a monoxygenase involved in phase I of xenobiotic metabolism. Levels of CYP1B1 mRNA vary widely from decreased levels in mesothelioma and melanoma to increased levels in prostate and nonsmall cell lung cancer. Hence, CYP1B1 enzyme may be an anticarcinogen or an onco-protein. This depends on what pro-carcinogens are the frequent cancer-causing agents in these tissue types and whether CYP1B1 serves to activate or inactive them [111–114]. Assessment of CYP1B1 expression levels in healthy and cancerous tissue types has been well studied. Results showed that CYP1B1 is upregulated in numerous cancers such as esophagus, lung, skin, breast, brain, testis, and colon cancers [115]. However, CYP1B1 has been detected at low levels in liver, kidney, brain, and eye in healthy adult tissues [92, 95, 116]. In a recent study, Chi et al. [39] evaluated CYP1B1 mRNA expression in OSCC lines exposed to dibenz[a]pyrene and in healthy oral tissues from smokers and non-smokers. They noticed that the interindividual variation in inducible CYP1B1 expression may account in part for the variation in tobacco-related OSCC risk. Furthermore, Schwartz et al. [117] found that RNA from brush cytology of hamster oral SCC showed differential CYP1B1 expression in dibenz[a]pyrene-induced OSCC. Moreover, Kolokythas et al. [118] demonstrated a downregulation of CYP1B1 at the mRNA level only in OSCC from oral brush cytology samples. Similar to Kolokythas et al. [118] findings, Pradhan et al. [119] observed downregulation of CYP1B1 in cancerous tissues in comparison with their corresponding healthy tissues as well as in the epithelial dysplasia lesion compared with its matched healthy tissue at the transcriptional level, and in cancerous tissues at the protein level [119]. This difference might be due to different kinds of oral lesions examined by Pradhan et al. [119] and Shatalova et al. [120]. However, an upregulation of CYP1B1 which included only 19.5% of oral lesions was observed in a recent HNSCC study [120]. These contrasting observations might be due to differences between examined oral lesions by Pradhan et al. [119] and Shatalova et al. [120]. Levels of CYP1B1 in oral tissue were approximately 2–4 folds higher in smokers than in non-smokers according to a recent report by Boyle et al. [121]. In addition, Sacks et al. [122] stated that the approximate level of 3–5 μg/mL of tobacco smoke particles would enhance epithelial oral cells. Thereby, regarding to the ability of tobacco smoke particles to induce CYP1B1 in cultured human cells and hence in smoker oral tissue, there is a good correspondence between the established lower
concentration range in the Sacks et al. [122], research and levels in oral tissue in smokers.

2.3. CYP2D6. Cytochrome P450s consist of the major enzymes required for phase I metabolism of xenobiotics. Cytochrome P450 2D6 (CYP2D6) is one of the enzymes that catalyze about 20% of commonly prescribed drugs. Cytochrome P450 2D6 has also a variety of activities among human populations. In fact, the interindividual metabolism rates differ more than 10000 folds [123–125]. Furthermore, the CYP2D6 gene is activated by some xenobiotic carcinogens such as nicotine which is the major constituent of tobacco [126]. Several predictive computer models have been published in which the distance between a basic nitrogen atom and the site of oxidation in the substrates determines whether a compound is metabolized by CYP2D6 or not [127]. The CYP2D6 gene is localized on chromosome 22q13.1 [128]. The variant CYP2D6 alleles can be classified into categories, which cause catalytic activity abolish, decrease, to stay normal, increase, or to be qualitatively altered.

Some of the known allelic variants of CYP2D6 are not functional or have a reduced catalytic activity (http://www.imm.ki.se/cypalleles/). CYP2D6*4 (G1934A) is the most common poor metabolizer (PM) in Caucasians; however, its frequency is very low in Asians [129, 130]. CYP2D6*3 (2549delA), CYP2D6*5, and CYP2D6*6 (1707delT) are also frequent PMs in Caucasians. Yet, they were described in a less frequency in the Asian population [129–131]. CYP2D6*10 allele (C100T at exon 1), related to a reduced catalytic activity, was found in 50% of the Asian populations and in 2% among Caucasians [129, 130, 132].

The role of the CYP2D6 gene as a risk factor for tobacco-related cancers has been extensively studied since early reports suggested an association between the high-metabolizing CYP2D6 phenotype and HNC risk in smokers [28, 133, 134]. However, no association between CYP2D6 genotype and smoking dose has been observed in terms of risk for UADT cancer in another study [29, 76]. Recently, Yadav et al. [135] found a difference in the risk of developing HNSCC depending on the genotype. In fact, patients with CYP2D6*4 allele present an increased risk, while those with CYP2D6*10 allele have no change or even a small decrease in risk in the Indian patients when comparison is done between consumers of tobacco or alcohol and nonconsumers. Thus, CYP2D6 genotypes are not the only genetic factors that interact with environment in determining the susceptibility to HNSCC. Furthermore, it was shown that patients with poor metabolizer genotypes of CYP2D6 did not respond to the treatment. The fact that the majority of patients present either CYP2D6*4 or CYP2D6*10 genotypes indicates that individuals with PM genotypes of CYP2D6 are more prone to develop HNSCC [135]. In addition, it was reported that CYP2D6 ultrarapid metabolizer patients from Spain and Germany have an increased risk to develop HNSCC [75, 136]. Nevertheless, patients with laryngeal SCC and breast cancer have an increased frequency of PM genotypes [56, 137]. These observations are consistent with previously reported results [138, 139]. However, Kato et al. [140] have reported that patients carrying inactivating alleles of the CYP2D6 gene have reduced levels of DNA nitrosamine. Caporaso et al. [141] have demonstrated that CYP2D6 is not involved in nicotine dependency, and hence this gene is not likely to have a major effect on tobacco smoking.

2.4. CYP2E1. The CYP2E1 human gene is located on chromosome 10 (10q24.3-qter), contains 9 exons, and encompasses several polymorphisms. Some of them have an effect on the protein expression [142]. The CYP2E1 enzyme is responsible for the metabolism of alcohol and some tobacco carcinogens such as low-molecular weight nitrosamines [24, 143, 144]. CYP2E1 enzyme activity is needed during the metabolic activation of many carcinogens such as nitrosamines. CYP2E1 is expressed in oral epithelial cell lines cultures, in human oral mucosa, and in tongues of rats [145, 146]. Two linked polymorphisms (CYP2E1 5B) have been described in the CYP2E1 gene at nucleotides -1259 and -1019. They are located in the 5’ regulatory region and are detectable by Rsal or PstI restriction enzyme digestion [Rsal is 21053C>T (rs2031920), and PstI is 21293G>C (rs3813867), resp.] [142, 147]. According to the presence or absence of these two restriction sites, two alleles have been defined: the common “wild-type” allele (Rsal/PstI−), known as c1, and the variant allele (Rsal/PstI+) known as c2. It was suggested that the Rsal polymorphism, located in a putative HNF-1 transcription factor-binding site, might play a role in the expression of CYP2E1 [142]. In fact, in vitro studies have demonstrated that the regulatory region of the c2 homologous allele shows a significant increase in transcriptional acetylationtransferase reporter gene if compared with that of the c1 allele [142, 148]. It was also reported that the CYP2E1*6 polymorphism (rs6413432) is suspected to alter transcription of the CYP2E1 gene [149].

Over the last two decades, several studies have explored the association of the CYP2E1 polymorphism with the risk of lung cancer [150], gastric cancer [151, 152], and pancreatic cancer [154]. Recently, several studies on the association between the CYP2E1 polymorphism and HNC have also been published, but those studies have yielded contradictory results. Four separate epidemiological studies showed no association between the c2 allelic variant (Rsal−/PstI−) and the risk for UADT cancer in Brazilian [153] or Japanese [55] subjects. Furthermore, Cury et al. [85] and Balaji et al. [154] observed absence of any association with CYP2E1 PstI and HNC in Brazilian patients and oral cancer in South Indians. Moreover, Gajecka et al., [56] Tai et al. [71] did not reveal any association between the CYP2E1 Rsal polymorphism and the overall risk of larynx cancer in Polish and Chinese patients, respectively. In addition, other studies [53, 55, 134, 155, 156] have not found significant differences in allelic variants in patients with HNSCC, including oral cancer. However, Gajecka et al. [56] found that Rsal+/PstI− variant allele was more frequent in controls (2.8%) than in larynx cancer group (1.6%), which may suggest that the mutated allele is rather “protective”. These results are in agreement with the Swedish study which reported that individuals with Rsal+/PstI− allele may be at lower risk for lung cancer [157]. However, these results are not consistent with previous studies conducted in the Caucasian and Chinese populations, in which the CYP2E1
RasI SNP was shown to be associated with increased risk of HNSCC, OSCC, and esophageal cancer [26, 147, 158–160]. Several Brazilian studies have explored the role of CYP2E1 polymorphisms in the induction of HNC. A later study [68] on Brazilian patients with HNC indicated that the presence of the RasI/PstI allele was associated with an increased risk of suffering, specifically, from oral cancer. Furthermore, in another Brazilian study of HNC [67], it was observed that the CYP2E1 *5A/*5B (c1/c2) genotype was more frequent in oral cavity tumors than in tumors from other anatomic sites (\( P = 0.003 \)) and that the CYP2E1 *5A/*5A (c1/c1) genotype was more frequently detected in white patients (\( P = 0.0031 \)). A study including 289 Brazilian volunteers showed that the frequencies of the CYP2E1*6 alleles (DraI, rs6413432) are similar to those observed in Caucasians and African-Americans, but the frequency of the CYP2E1*5B allele is higher in Brazilians [161]. However, for the Brazilian population, taking into account the small number of nonwhite individuals, conclusions were so limited. Moreover, ancestry informative marker-based reports have concluded that, at an individual level in Brazil, race is a poor predictor of genomic ancestry [162, 163].

The association between CYP2E1 (Rsal/PstI) and CYP2E1 (DraI) polymorphisms and HNC susceptibility has been widely investigated. However, results were inconsistent. Recently, Lu et al. [164] and Tang et al. [149] have assumed that CYP2E1 (Rsal/PstI) polymorphism might be a risk factor for HNC in the Asian population as well as several carcinogenic processes that polymorphism might be a risk factor and could induce carcinogenesis. In contrast to these findings, studies conducted by Liu et al. [165] on a Chinese population have concluded that there is no significant association between CYP2E1 (Rsal or DraI) polymorphisms and susceptibility to esophageal SCC (OR = 1.67, \( P = 0.11; \) OR = 1.11, \( P = 0.74 \), resp.). Therefore, it was suggested that c2 allele and DD genotype represent a risk factor for esophageal SCC. The frequencies of these two mutations in the Chinese population [165] were all higher than those of the Caucasian population, which indicated the ethnic difference in the two polymorphisms of CYP2E1 [166, 167]. Hence, there might be a reliable efficiency to evaluate genetic susceptibility of Rsal and DraI polymorphisms for esophageal SCC in a population with high mutant frequencies.

Tobacco and alcohol consumption represent the most important factors for HNC; hence, genes involved in tobacco carcinogen and alcohol metabolism should play a role in the HNC development. An association between the c2 allele and the increased oral cancer risk was previously demonstrated among nonbetel quid chewing males from Taiwan [168]. In addition, in another Taiwanese study [169], the CYP2E1 (c2/c2) genotype was found to be associated with an increased NPC risk, an effect most pronounced in non-smokers. Recently, Jia et al. [170] found robust evidence for associations between genetic variants of CYP2E1 and NPC risk in the Cantonese population. They observed that individuals aged less than 46 years and who had a history of cigarette smoking present OR of specific genotypes ranging from 1.88 to 2.99 corresponding to SNPs rs9418990, rs1536826, rs3827688, and rs8192780 (\( P = 0.0001 - 0.0140 \)). Furthermore, Liu et al. [146] compared the risk of oral cancer between the Caucasian and African-American patients depending on the genotype. They found that patients with "wild-type" (c1c1) genotype have an increased risk if compared with controls smoking less than 24 pack-years. Nevertheless, this association was absent for patients with CYP2E1 genotypes among heavy smokers. These findings support the hypothesis that impact of genetic factors in cancer risk is more reduced if carcinogen doses are higher [171]. A hospital-based study [172] of CYP2E1 5B and CYP2E1 6 polymorphisms and gene-environment interactions in the risk of UADT cancers among Indians was conducted. Results showed absence of differences between groups for the two polymorphisms if analyzed separately. However, results for CYP2E1 6 polymorphisms showed significant interactions among tobacco smokers (>40 pack-years) and regular tobacco chewers. These results illustrate the interaction between genes and environment and provide an additional genetic risk factor, CYP2E1 6 polymorphisms, for UADT cancers in the Indian population [172].

CYP2E1 metabolizes ethanol and generates reactive oxygen species, and it has been suggested that it is important for the development of alcoholic liver disease and cancer, including hepatoblastoma and HNC. Acetaldehyde dehydrogenases are a group of NAD-dependent enzymes, which catalyze the oxidation of acetaldehyde, being the second enzyme of the alcohol oxidation pathway (Figure 2) [27, 173]. Levels of CYP2E1 are elevated under a variety of physiologic and pathophysiologic conditions and after acute and chronic alcohol exposure [174, 175]. Interestingly, in a recent Chinese study of Guo et al. [160], the polymorphism of CYP2E1 5B gene (c2/c2 genotype) and alcohol consumption and were found to increase the risk of OSCC (\( P < 0.01; \) OR = 2.46, and 95% CI = 1.78–4.04). In addition, in the study of Olivieri et al. [67], among alcohol users, the CYP2E1 5B variant allele was more frequently detected in HNC Brazilian patients than in control subjects (\( P < 0.0001; \) OR = 190.6, and 95% CI = 24.50–1483). Overall, the data suggested that CYP2E1 5B is an independent biomarker of risk in alcohol-related HNC. Recently, Cury et al. [85] confirmed that smoking and alcohol consumption were risk factors for HNC, but the CYP2E1*6 and CYP2E1*5B polymorphisms investigated had no association with the development of HNC in Brazilian patients. Alcohol or tobacco consumptions were also found to interact with variant genotypes of CYP2E1 in significantly enhancing HNC risk [147]. In addition, it was suggested that CYP2E1*5B polymorphism can be quite important in oral carcinogenesis in Brazilians [53] or can be compensated by other genes involved in the ethanol and other carcinogens metabolism in oral mucosa [53]. Absence of association between CYP2E1*5B polymorphism and lung cancer among patients from Rio de Janeiro has been previously observed [155]; this could be explained by the fact that alcohol is not a lung carcinogen. The difference in the genetic background between different ethnicities associated to other genetic factors involved in the etiology of HNC might be behind the variability and the inconsistency of these results. It is well established the involvement of some genetic polymorphisms if combined
3. Arylamine N-Acetyltransferases (NATs)

NAT1 and NAT2 human isoforms are encoded by two genes with intronic less coding regions. The NAT genes are located on chromosome 8p21.3–23.1 and express two highly polymorphic isoenzymes (NAT1 and NAT2) with distinct functional roles. In humans, the products of these two genes appear to have distinct functional roles depending on their substrate, their expression in tissues, and the expression of the different genes during development. Although the two genes are organized in a single open-reading frame, their structure and control vary markedly (Figure 3) [176, 177]. Recent studies on human NAT1 and NAT2 genes have identified interactions within the active site cleft that are crucial for substrate recognition [178]. The specific recognition of the substrate is provided by the C-terminal region of the NAT proteins [179], mainly by residues around positions 124–129 [180].
was conducted to test the oral cancer risk associated with polymorphisms in the NAT1 gene. This study is still until now.

In 1987, Drozdz et al. [190] established a association for O-acetylation of aromatic amine carcinogens from one hand and to produce reactive metabolites from another hand [189]. Because these two genes are involved in metabolic activation via O-acetylation [184–187], their genetic polymorphisms could modify the cancer susceptibility related to carcinogen exposure. Many N-hydroxy heterocyclic amine carcinogens are catalyzed by human NAT2 than NAT1 [185, 187]. Their tissue-specific expression is also a determinant factor for a better efficiency.

So far, 36 NAT2 genetic variants have been identified in human. Among them, NAT2*4 is the most common allele reported to be associated with rapid acetylation [188]. The other alleles are classified into two groups: the rapid alleles that include NAT2*11A, NAT2*12A−C, NAT2*13A, and NAT2*18 and the slow alleles such as NAT2*5, NAT2*6, and NAT2*7. For NAT1, the most common alleles are NAT1*3, NAT1*4, NAT1*10, and NAT1*11. NAT1*4 is the most common allele, while NAT1*10 is the putative rapid allele. Subjects having more than one rapid allele were designated by NAT1 rapid acetylation. For the others, they were classified under NAT1 slow acetylation [188].

As NAT1 and NAT2 genes are characterized by allelic heterogeneity, several haplotypes have been established. They were associated with either the rapid or the slow acetylator phenotype [133]. All SNPs of both genes (slow and rapid alleles) have been associated with an increased risk of cancer. This association could be explained by their ability to detoxify aromatic amine carcinogens from one hand and to produce higher levels of reactive metabolites from another hand [189].

In 1987, Drozdz et al. [190] had established an association between the slow acetylator phenotype and the increased risk for laryngeal cancer. So far, little is known about the role of the NAT gene SNPs and their association with HNC (Figure 4).

Some studies have reported that alteration of NAT enzyme activity might be of risk for UADT cancer. It was previously shown that patients with NAT2 slow acetylator genotypes (homozygous for NAT*5, NAT*6, and NAT*7 alleles) are significantly (P < 0.002) more prone to develop UADT cancer (0.37) as compared with controls (0.22) [133]. In a recent study on the Turkish population, the slow acetylator NAT2*7 allele was correlated to a reduced UADT cancer risk [191] as well as larynx cancer [192], thus suggesting a protective role of NAT2*7 genotype in HNC. NAT2*5 and NAT2*6 alleles seem to be associated with cancer risk [191]. Studies focusing on NAT2 haplotypes have shown an association between NAT2*4 and HNC [191]. These results support the hypothesis of the possible involvement of NAT2*4 combinations (NAT2*4/*6A) in larynx cancer predisposition (OR = 3.24; P = 0.045) [56]. In a Tunisian study, Bendjemena et al. [193] observed that genotypic frequencies of NAT2*6/NAT2*6 were significantly higher in the group of nasopharyngeal carcinoma patients (OR = 6.14; 95% IC = 2.4–14.0). Furthermore, in another Tunisian study [194], a significant difference was found between HNC patients and controls for T341C mutation (NAT2*5, rs1801280) in NAT2 gene (OR = 1.82; P = 0.04). This finding is in accordance with the reported association between squamous cell carcinoma and T341C mutation [133]. This is probably due to the great reduction in acetyltransferase 2 catalytic activity in relation with the T341C mutation (NAT2*5) in NAT2 gene [189]. However, no significant difference was found between HNC Tunisian patients and controls for G590A (NAT2*6) mutation in NAT2 gene [194]. In addition, no association between the NAT2 genotype and NPC was found in the Taiwanese population [77].

An association was found between the homozygous NAT2*4 allele and the increased oral cancer risk in a Brazilian population (OR = 1.95; P = 0.032) [53]. Likewise, many studies have reported an association between rapid acetylator phenotype and the increased risk of oral and laryngeal cancer in Caucasians [195, 196]. At the biological level, this could be explained by the fact that O-acetylation of nitrosamines by NAT2 could be more important as a negative metabolic pathway leading to oral carcinogenesis; therefore, slow acetylators would be protected. Chatzimichalis et al. [197] established the distribution of genotypes and showed that it consisted of 55.68% of rapid acetylators and 44.32% of slow acetylators in laryngeal SCC patients, while it was of 36.27% of rapid acetylators and 63.72% of slow acetylators in controls. This study [197] concluded that rapid acetylator genotypes are significantly associated to laryngeal SCC in the Greek population (OR = 2.207; P = 0.0087). Furthermore, Buch et al. [198] found that fast acetylators (NAT2*4) are more frequent in oral cancer patients (53.7%) than in controls (43.9%; OR = 1.55; 95% CI = 1.08–2.20; P = 0.03).

Several studies have explored the role of NAT1 polymorphisms in the incidence of HNC. The first study [199] was conducted to test the oral cancer risk associated with polymorphism in the NAT1 gene. This study is still until now...
Table 2: Summary of studies on CYPs450 and NATs genes status in HNC.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Population</th>
<th>N (case/control)</th>
<th>SNP (allele or genotype)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>References</th>
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<td>1.19–2.60</td>
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<td>[66]</td>
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<td>[67]</td>
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<td></td>
<td>Indian</td>
<td>150/150</td>
<td>*2 (wt/mt or mt/mt)a</td>
<td>4.47</td>
<td>2.07–9.60</td>
<td>&lt;0.05</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td>150/150</td>
<td>*2 (wt/mt or mt/mt)b</td>
<td>8.81</td>
<td>2.60–29.87</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>Indian</td>
<td>150/150</td>
<td>*2 (wt/mt or mt/mt)c</td>
<td>2.74</td>
<td>1.12–6.70</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>German</td>
<td>195/177</td>
<td>*3 (wt/mt or mt/mt)d</td>
<td>4.53</td>
<td>2.62–7.98</td>
<td>&lt;0.001</td>
<td>[26]</td>
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<td></td>
<td>CYP2D6</td>
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<td></td>
<td>Brazilian</td>
<td>153/145</td>
<td>*5B</td>
<td>190.6</td>
<td>24.50–1483</td>
<td>&lt;0.0001</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>320/320</td>
<td>*5B</td>
<td>2.46</td>
<td>1.78–4.04</td>
<td>&lt;0.01</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td>—</td>
<td>*5B</td>
<td>3.44</td>
<td>1.45–8.14</td>
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<td>[147]</td>
</tr>
<tr>
<td></td>
<td>German</td>
<td>312/299</td>
<td>*6</td>
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<td>1.45–2.41</td>
<td></td>
<td>[147]</td>
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<tr>
<td></td>
<td>Chinese</td>
<td>755/755</td>
<td>~71 G&gt;T</td>
<td>0.49</td>
<td>0.25–0.98</td>
<td>0.04</td>
<td>[159]</td>
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<tr>
<td></td>
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<td>rs9418990d</td>
<td>2.95</td>
<td>1.68–5.17</td>
<td>0.0002</td>
<td>[159]</td>
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<td>755/755</td>
<td>rs8192780d</td>
<td>2.99</td>
<td>1.72–5.21</td>
<td>0.0001</td>
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<td>rs1536826d</td>
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<td>1.69–5.13</td>
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<td>rs3827688d</td>
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<td>1.13–3.13</td>
<td>0.0140</td>
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<td>CYP2E1</td>
<td>Caucasian</td>
<td>c1/c1f</td>
<td>—</td>
<td>—</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td></td>
<td>African-American</td>
<td>113/226</td>
<td>*5B</td>
<td>190.6</td>
<td>24.50–1483</td>
<td>&lt;0.0001</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>24 studies</td>
<td>12,562</td>
<td>c2 allele</td>
<td>1.11</td>
<td>1.00–1.22</td>
<td>0.04</td>
<td>[164]</td>
</tr>
<tr>
<td></td>
<td>(meta-analysis)</td>
<td>—</td>
<td>c2/c2</td>
<td>1.57</td>
<td>1.14–2.15</td>
<td>0.006</td>
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<tr>
<td></td>
<td>24 studies</td>
<td>12,562 (all)</td>
<td>c2/c2</td>
<td>1.57</td>
<td>1.14–2.15</td>
<td>0.006</td>
<td>[206]</td>
</tr>
</tbody>
</table>
the only one that suggested a significant increased risk (OR = 3.72; \(P < 0.01\)) associated with the NAT1*10 allele in the Japanese population [199]. However, the other studies have suggested negative findings [191, 195, 200, 200].

Gene-gene interaction testing has shown several cancer-NAT2 associations. The strongest one was observed among persons without a CYP1A1 variant (\(^*2C\) or \(^*4\)) allele (OR = 1.55, 95% CI = 1.08-2.20, and \(P = 0.03\)) [198]. These results implicate fast NAT2 acetylation as a risk factor for oral cancer in the American population (USA) [198]. Moreover, Demokan et al. [191] and McKay et al. [201] found that the association with NAT1 and NAT2 gene combinations may influence the risk of developing HNC. A significant association was observed between the fast acetylator NAT2*4/NAT1*10 diplotype and risk of HNC [191]. Moreover, the association with NAT1\(^*10\)/NAT2\(^*6A\) haplotypes was correlated to the risk of UADT cancer (OR = 1.54; \(P = 0.03\)) [201].

\(NAT\) gene presents a crucial role in the detoxification and activation reactions of numerous xenobiotics originating not only from tobacco-derived aromatic and heterocyclic amine carcinogens but also from drug metabolism. Its function is undergone through \(N-\) and \(O\)-acytylation pathways [200, 202] via a ping-pong bi-bi mechanism. The initial step consists on acetylation of Cys68 by an acetyl-coenzyme A along with the release of the cofactor product coenzyme A. Secondly, the substrate is linked to the acetylated enzyme. Finally, the acetylated product is released [203]. Since chemical compounds present in tobacco are inactivated by phase II enzymes, it has been proposed that HNC risk could be modified by \(NAT\) genotypes. HNCs are strongly associated with smoking, and a few studies have explored the role of NAT1 polymorphisms in the risk of developing HNC in smokers [199, 204]. However, overall findings are inconsistent, and associations if present are weak and indicate either a decreased risk in carriers of the variant NAT1 [201], an increased risk [205], or a lack of association [191, 195, 200, 202].

The role of NAT1 and NAT2 acetylator polymorphisms in cancer risk from aromatic and heterocyclic amine carcinogens will become clearer with more precise determinations of both exposures and genotypes. Further studies of the haplotype combinations in different populations and with larger cohorts are warranted to determine the range of risks associated with the effect of genetic variation of the NAT genes with regard to HNC.

### 4. Conclusion

The present paper reviews studies that assessed association between genetic polymorphisms of genes encoding carcinogen-metabolizing enzymes and showed their possible involvement by significantly increasing the predisposition for HNC. This risk relies on many factors such as the level of carcinogen exposure (e.g., tobacco smoke), the ethnicity and/or racial groups, and so forth. Various polymorphisms in these genes are summarized in Table 2. Many of the discussed studies described HNC risk for a mixed racial and/or ethnic cohort. As shown previously, cancer susceptibility is different according to the genotype in a given racial group. Thus, even if cases and controls are race-matched, erroneous association might be taken into consideration if different racial and/or ethnic groups are mixed. In addition, differences in genetic backgrounds for metabolic genotypes between races and even between ethnic groups whether located in the same region

---

### Table 2: Continued.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Population</th>
<th>(N) (case/control)</th>
<th>SNP (allele or genotype)</th>
<th>OR</th>
<th>95% CI</th>
<th>(P) value</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>21 studies (meta-analysis)</td>
<td>4,951/6,071</td>
<td>(*5B)</td>
<td>1.96</td>
<td>1.33–2.90</td>
<td>&lt;0.05</td>
<td>[93]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td>1.56</td>
<td>1.06–2.27</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian (meta-analysis)</td>
<td>4,951/6,071</td>
<td>(*5B)</td>
<td>2.04</td>
<td>1.32–3.15</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td>2.04</td>
<td>1.27–3.29</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NAT1)</td>
<td>Japanese</td>
<td>62/122</td>
<td>(10)</td>
<td>3.72</td>
<td>1.56–8.90</td>
<td>&lt;0.01</td>
<td>[199]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10)</td>
<td>3.14</td>
<td>1.09–9.07</td>
<td>0.017</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10)</td>
<td>5.88</td>
<td>1.13–30.6</td>
<td>0.022</td>
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</tr>
<tr>
<td>Brazilian</td>
<td>231/212</td>
<td>(4/4)</td>
<td>1.95</td>
<td>1.05–3.60</td>
<td>0.035</td>
<td>[53]</td>
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<td>(4)</td>
<td>2.18</td>
<td>1.13–4.22</td>
<td>0.018</td>
<td>[195]</td>
<td></td>
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<tr>
<td>Greek</td>
<td>88/102</td>
<td>(4)</td>
<td>2.20</td>
<td>1.23–3.95</td>
<td>0.0087</td>
<td>[197]</td>
<td></td>
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<tr>
<td>American (USA)</td>
<td>203/416</td>
<td>(4)</td>
<td>1.55</td>
<td>1.08–2.20</td>
<td>0.03</td>
<td>[198]</td>
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<tr>
<td>Tunisian</td>
<td>64/160</td>
<td>(5B)</td>
<td>1.82</td>
<td>2.68–12.26</td>
<td>0.04</td>
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<td></td>
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<tr>
<td>Tunisian</td>
<td>45/100</td>
<td>(6A)</td>
<td>6.14</td>
<td>2.4–14</td>
<td>&lt;0.05</td>
<td>[193]</td>
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<tr>
<td>Polish</td>
<td>289/316</td>
<td>(4/6A)</td>
<td>3.24</td>
<td>1.1–9.75</td>
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<tr>
<td></td>
<td></td>
<td>(5B/5B)</td>
<td>3.41</td>
<td>1.6–9.9</td>
<td>0.043</td>
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<td></td>
<td>(6A)</td>
<td>0.30</td>
<td>0.10–0.89</td>
<td>&lt;0.042</td>
<td>[133]</td>
<td></td>
</tr>
<tr>
<td>Spanish</td>
<td>75/200</td>
<td>(5B)</td>
<td>0.48</td>
<td>0.25–0.93</td>
<td>&lt;0.039</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>145/164</td>
<td>2</td>
<td>—</td>
<td>0.039</td>
<td>[55]</td>
<td></td>
</tr>
</tbody>
</table>

\(^4\)Smokers; \(^5\)nonsmokers; \(^6\)chewers; \(^7\)smokers <46 years; \(^8\)subjects smoked <24 pack-years; \(^9\)calculation for \(wt/mt\) genotype versus \(wt/mt\) and \(mt/mt\) genotypes. —: undefined; +: the genotype/allele undefined.
or not should also be taken into consideration before an association study is performed. Furthermore, metabolizing enzyme expression could widely vary at diverse sites within the head and neck.

It is well known that there is a real logistical difficulty that consists in combating at least one of the potential biases listed above. However, careful attention should be given to all elements before conducting an association study in order to ensure accurate and significant results. If well designated, these studies would clarify the impact of xenobiotic-metabolizing enzymes in HNC development and help determine the value of potentially “high-risk” genotypes in HNC prevention strategies.

Abbreviations

CYP450: Cytochrome P450
HNSCC: Head and neck squamous cell carcinoma
NPC: Nasopharyngeal cancer
OR: Odds ratios
OSCC: Oral squamous cell carcinoma
NAT: Arylamine N-acetyltransferase
PM: Poor metabolizer
SCC: Squamous cell carcinoma
SNP: Single-nucleotide polymorphism
TSNAs: Tobacco-specific nitrosamines
UADT: Upper aerodigestive tract.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References


[56] M. Gajeczka, M. Rydzanicz, R. Jaskula-Sztul, M. Kujawski, W. Szyfter, and K. Szyfter, “CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma,” Mutation Research, vol. 574, no. 1-2, pp. 112–123, 2005.


