Review

Genetic Polymorphisms of Smoking-related Carcinogen Detoxifying Enzymes and Head and Neck Cancer Susceptibility

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Abstract. *Smoking and the consumption of alcohol are the main risk factors for head and neck cancer. However, inter-individual variation in the activity of enzymes involved in the detoxification of tobacco smoke (pro)carcinogens, such as microsomal epoxide hydrolase (mEH), glutathione-S-transferases (GSTs) and uridine 5'-diphosphate (UDP)-glucuronosyltransferase (UGTs), may influence the process of carcinogenesis. Genetic polymorphisms of these enzymes may alter their activity and may thus modulate the risk for squamous cell carcinomas of the head and neck (SCCHN). A literature review on the role of mEH, GSTs and UGTs polymorphisms in relation to SCCHN was performed and the results summarized. For mEH polymorphisms, some of the studies revealed a relationship between genetic polymorphisms of these enzymes and an altered risk for SCCHN, whereas others did not. The presence of null polymorphisms in GSTM1 or GSTT1 were associated with an increased risk for SCCHN. For the UGTs, only variants in UGT1A7 and UGT1A10 have been studied, both of which were associated with an altered risk for SCCHN.*

Squamous cell carcinoma of the head and neck (SCCHN) including the cancer of oral cavity, pharynx and larynx, worldwide accounts for about 650,000 new cases annually. Recent estimates have indicated that SCCHN is the fifth most common cancer, resulting in approximately 300,000 deaths annually (1). Exposure to tobacco smoke and the consumption of alcohol are considered to be the most important etiological factors for the development of SCCHN (2). The incidence of SCCHN in tobacco and alcohol consumers is significantly higher compared to non-consumers. There are more than 60 known carcinogens in tobacco smoke and at least 16 in unburned tobacco. Polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BaP) together with tobacco-specific nitrosamines and aromatic amines are important tobacco-related carcinogens (3).

Although the chance of developing SCCHN increases with the level of tobacco smoking and alcohol consumption, it is obvious that not every (heavy) smoker and/or alcohol consumer develops head and neck cancer. As well as the level of smoking, the extent of exposure of the upper aerodigestive tract to carcinogens may also depend on whether the (pro)carcinogens in tobacco smoke are activated or detoxified by phase I and phase II biotransformation enzymes, respectively. The risk for an individual to develop SCCHN after exposure to tobacco carcinogens, may therefore also depend on sequence variations in the genes (genetic polymorphisms) coding for these enzymes. This probably implies that not only the exposure to the potential carcinogens, but also other factors such as genetically determined inter-individual differences in the metabolism and excretion of tobacco smoke carcinogens may play an important role in the development of SCCHN. The presence of genetic susceptibility in the pathogenesis of SCCHN is strongly suggested by the higher incidence of this cancer in first-degree relatives of patients with SCCHN (4).

Carcinogens and activated procarcinogens in tobacco smoke may react with the DNA of exposed human tissues, such as the epithelial cells of the upper aerodigestive tract. This can lead to the formation of DNA adducts and subsequently to mutations in crucial genes such as oncogenes and tumor suppressor genes, ultimately resulting in the development of cancer (5). However, these processes may be under the influence of biotransformation (or

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Key Words: Head and neck cancer, microsomal epoxide hydrolase, glutathione-*S*-transferase, UDP-glucuronosyltransferase, polymorphisms, cancer susceptibility, smoking, review.

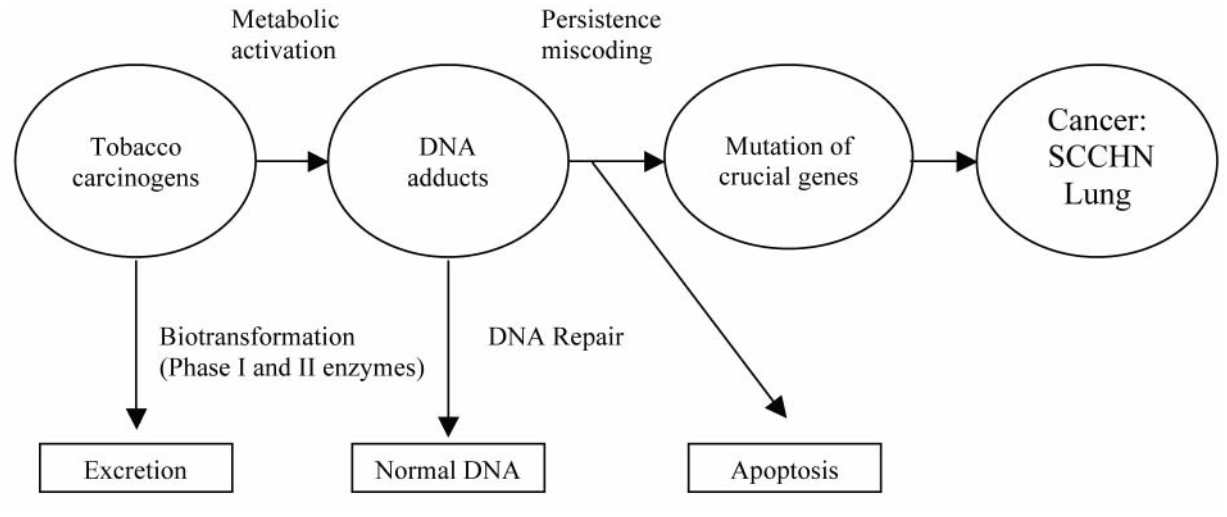

Figure 1. *Simplified scheme of tobacco smoke-related carcinogenesis according to Hecht (3).*

detoxification) enzymes, which are essential for the metabolism and subsequent excretion of carcinogens. Detoxification of tobacco smoke carcinogens, together with DNA repair and apoptotic pathways for cells with deformed DNA, probably are the most important rescue pathways in preventing the development of tobacco-induced SCCHN (3) (see Figure 1).

The biotransformation of many tobacco (pro)carcinogens such as BaP takes place in two phases: transformation of the mostly lipophilic compounds into more polar molecules (phase I) and subsequently a conjugation reaction (phase II). The latter conversion to water-soluble compounds in general makes them less biologically active and facilitates the excretion of the toxins and carcinogens from the body, which diminishes the exposure of the human tissues to these compounds. However, the phase I reactions, mediated by enzymes such as cytochromome P-450 (CYP) and microsomal epoxide hydrolase (mEH), often result in the formation of phenols, epoxides and other reactive intermediates that can be transformed into highly carcinogenic electrophilic compounds such as BaP-diol-epoxides (6, 7). These compounds, in the absence of a rapid further intervention by phase II conjugating enzymes such as glutathione-*S*-transferases (GSTs) and uridine 5'-diphosphate (UDP)-glucuronosyltransferase (UGTs), may form DNA adducts and subsequently initiate carcinogenesis. Since the upper aerodigestive tract is in direct contact with potentially toxic or carcinogenic agents ingested through tobacco smoke, the upper aerodigestive mucosa acts as a first-line barrier. Enzymes of the phase I and II biotransformation pathways present here therefore play an important role in the metabolism and the excretion of tobacco smoke carcinogens (8).

The activity of such enzymes may differ between individuals. It is well known now that genetic polymorphisms occur in the enzymes mentioned above, resulting in functional abnormalities, which may be one of the possible explanations for the differences in inter-individual susceptibility for the development of SCCHN (9-11).

In this review, the role of genetic polymorphisms in mEH, GSTs and UGTs, and their association with the risk of SCCHN is elucidated.

For this review, a PubMed search was used with "microsomal epoxide hydrolase", "glutathione-*S*-transferase" or "UDP-glucuronosyltransferase", in combination with "head and neck cancer" as search terms. Only papers dealing with genetic polymorphisms in the aforementioned enzymes in relation to SCCHN risk published in English and available on PubMed before April 2008 were included in this review.

Microsomal Epoxide Hydrolase

The human mEH is one of the phase I enzymes involved in biotransformation and detoxification of potential tobacco smoke carcinogens. mEH is present in microsomes derived from the endoplasmatic reticulum and is highly expressed in most human tissues, among them also the mucosa of the upper aerodigestive tract (12, 13). This enzyme has a role in both activation and detoxification of environmental (pro)carcinogens. mEH catalyzes the hydrolysis of reactive epoxide intermediates, in preparation for conjugation reactions (phase II detoxification) and excretion. However, in collaboration with CYP enzymes, mEH may activate the PAHs present in tobacco smoke, such as BaP, leading to highly reactive carcinogenic diol-epoxides (7, 14).

Table I. *Predicted mEH enzyme activity based on the classification of Benhamou et al. (17).*

Exon 4	Exon 3 Tyr113His polymorphism		
His139Arg polymorphism	Tyr/Tyr	Tyr/His	His/His
His/His	Intermediate	Low	Low
His/Arg	High	Intermediate	Low
Arg/Arg	High	High	Intermediate

mEH, Microsomal epoxide hydrolase; His, histidine; Arg, arginine; Tyr, tyrosine.

The gene coding for human mEH (*EPHX1*) covers nine exons and is located on chromosome 1q42.1. There are two known amino acid-altering polymorphisms in the *EPHX1* gene which may lead to changes in mEH enzyme activity. The exon 3 polymorphism, with corresponding substitution of histidine for tyrosine at position 113 of the enzyme, is associated with a 40-50% decrease in mEH activity (15). The exon 4 polymorphism, with substitution of arginine for histidine at position 139, may decrease mEH activity by approximately 25% (15, 16). According to Benhamou *et al*., the expected mEH activity can be classified as low, intermediate or high, depending on the combinations of the exon 3 and exon 4 polymorphisms on the two alleles. (17) (see Table I). Because a higher activity of mEH can be associated with higher concentrations of carcinogenic diol-epoxides in the mucosa of the upper aerodigestive tract, a subpopulation of tobacco smokers with the predicted high activity mEH polymorphisms might have a higher risk of SCCHN compared to the subpopulations with intermediate or low mEH activity polymorphisms.

Several studies have investigated the role of mEH polymorphisms in head and neck carcinogenesis (11, 18-21) (see Table II). However, only Jourenkova-Mironova *et al*. found a significant increased risk of oral, pharyngeal and laryngeal cancer in their study population of French Caucasian smokers with predicted high and intermediate mEH activity polymorphisms, as compared to the predicted low mEH activity subpopulation (11). Park *et al*. found that the predicted high mEH activity polymorphisms were significantly associated with an increased risk for oral and laryngeal cancer only in heavy smoking (>35 packyears) Caucasians, but not in African-American subjects (18). Unfortunately, they did not report on the predicted mEH activity (low, intermediate, high) and oropharyngeal cancer risk in their population. Amador *et al*. observed a higher incidence of the high activity mEH Tyr/Tyr genotype in patients with oropharyngeal cancer as compared to a control population (20). Wenghoefer *et al*. found no association between the mEH polymorphisms with predicted high enzyme activity and an increased risk for SCCHN (19). To-Figueras *et al*. reported an increased risk of laryngeal cancer among a Spanish-Caucasian study population with predicted high mEH activity genotypes in combination with the 105Ile/105Ile variant of glutathione-*S*-transferase P1 (*GSTP1*) (21). However, none of the mEH polymorphisms alone were associated with an altered risk of laryngeal cancer.

We recently analyzed blood samples from 429 Caucasian patients with oral, pharyngeal or laryngeal carcinoma and 419 healthy blood donors for the exon 3 and exon 4 mEH polymorphisms (22). Logistic regression analysis did not show any significant differences in the distribution of these polymorphisms between patients and controls, when categorized according to predicted enzyme activity: odds ratio (OR)=O.96; 95% confidence interval (CI)=0.70-1.32 for intermediate mEH activity, OR=0.88; 95% ; CI=0.59-1.23 for high mEH activity, with predicted low activity as reference. No significant differences were found when evaluated with respect to the different tumor localisations, gender or low *versus* high tobacco consumption. However, a significantly higher incidence of the 139 Arg/Arg variant with predicted high activity, was found in patients with hypopharyngeal carcinoma, OR=4.39, 95% CI=1.45-13.35.

Baxter *et al*. (23) recently suggested that the PCR-restriction fragment length polymorphism (PCR-RFLP) assay, which was often used in the exon 3 mEH genotyping research, might be unreliable. Due to the possible presence of an additional polymorphism in codon 119, the use of a primer adhering to the region containing codon 119 might falsely lead to an apparent 113 His/His genotype instead of the 113 His/Tyr variant. The methods applied by Jourenkova-Mironova *et al*. (11), Park *et al*. (18) and Amador *et al*. (20) may be inaccurate because of the use of a primer covering codon 119, whereas the methods for estimating the exon 3 polymorphism applied by Wenghoefer *et al*. (19), To-Figueras *et al*. (21) and ourselves (22) were not potentially inaccurate, since no primer adhering to codon 119 was used here.

Given the potential bias in the method used for the analyses of the exon 3 mEH polymorphisms in the studies with a reported effect, while the two largest studies hardly showed any effect, one may conclude that polymorphisms in mEH are not significantly modulating the risk for head and neck cancer.

Glutathione and Glutathione-*S*-transferases

Glutathione (GSH) is an intracellular thiol that neutralizes (pro)carcinogenic and highly reactive electrophilic compounds, a process catalyzed by GSTs. GSTs are a family of cytosolic enzymes, involved in phase II biotransformation (24, 25). GSH is produced mainly in the liver (hepatocytes), by coupling of the amino acids glycine, cysteine and glutamic acid (26). Hepatic glutathione is transported to most other tissues *via* the blood (26). High levels of

Table II. *Case-control studies on EPHX1 polymorphisms with predicted mEH activity and risk for head and neck cancer in Caucasian populations.*

	SCCHN patients		Controls		OR (95% CI)
	N	%	N	%	
Wenghoefer *et al.* (19)+ (oral/pharyngeal/laryngeal cancer) (19)					
Predicted mEH activity					
Low	90	32.1%	104	36.0%	1 (ref)
Intermediate	135	48.2%	124	42.9%	1.28 (0.84-1.96)
High	55	19.7%	61	21.1%	0.98 (0.58-1.64)
Park *et al.* (18)‡ (oral/laryngeal cancer)					
Predicted mEH activity					
Low+Intermediate	103	76.8%	178	83.6%	1.7 (0.9-3.1)
High	39	23.2%	35	16.4%	
(Smokers ≥35 py)					
Low+Intermediate	55	67.9%	48	87.3%	3.4 (1.2-9.6)
High	26	32.1%	7	12.7%	
To-Figueras *et al.* (21)§ (laryngeal cancer)					
Predicted mEH activity					
Low	76	37.3%	76	37.4%	1 (ref)
Intermediate	91	44.6%	94	46.3%	1.24 (0.76-2.02)
High	37	18.1%	33	16.3%	1.32 (0.69-2.52)
Amador *et al.* (20)* (oral/pharyngeal/ laryngeal cancer)	ever-smokers n=122	never-smokers n=15	n=99	Fischer test *p*-value	
Exon 3 genotypes				ever smokers:	____
Tyr/Tyr	41.7%	60.0%	21.4%	*p*=0.001	
Tyr/His	45.0%	33.3%	46.9%	never smokers:	
His/His	13.3%	6.7%	31.6%	*p*=0.006	
Exon 4 genotypes					
His/His	70.8%	86.7%	73.7%	ever smokers:	____
His/Arg	22.5%	0.0%	21.2%	not significant	
Arg/Arg	6.7%	13.3%	5.1%	never smokers: not significant	
Jourenkova-Mironova *et al.* (11)‡ (laryngeal cancer)					
Predicted mEH activity					
Low	43	33.0%	85	49.4%	1 (ref)
Intermediate	59	45.7%	65	37.8%	1.7 (1.0-3.1)
High	27	20.9%	22	12.8%	2.4 (1.1-5.1)
Jourenkova-Mironova *et al.* (11)‡ (oral/pharyngeal cancer)					
Predicted mEH activity					
Low	42	34.7%	85	49.4%	1 (ref)
Intermediate	55	45.5%	65	37.8%	1.8 (1.0-3.3)
High	24	19.8%	22	12.8%	2.1 (1.0-4.5)
Lacko *et al.* (22)‡ (oral/pharyngeal cancer)					
Predicted mEH activity					
Low	158	36.8%	158	37.7%	1 (ref)
Intermediate	192	44.8%	184	43.9%	0.96 (0.70-1.32)
High	79	18.4%	77	18.4%	0.88 (0.59-1.23)

*6.5% of cases and 9.1 % of controls in the study of Amador *et al.* (20) were not Caucasians. +ORs were adjusted for age and gender. ‡ORs were adjusted for age, gender, smoking and alcohol consumption. §ORs were adjusted for age, gender and smoking. *EPHX1*: human microsomal epoxide hydrolase gene, mEH: microsomal epoxide hydrolase, OR: odds ratio, CI: confidence interval, SCCHN: squamous cell carcinoma of the head and neck, n: number, Tyr: tyrosine, His: histidine, Arg: arginine, py: packyears.

Table III. *Pooled analysis of case-control studies on GSTM1, GSTT1 and GSTP1 genotypes and risk for head and neck cancer (25).*

	GSTM1 (null)	*GSTT1* (null)	*GSTP1* (any105Val)
Number of studies	11	8	5
Cases/controls	2224/2517	1929/1830	1164/982
Summary OR (95% CI)	1.32 (1.07-1.62)	1.25 (1.00-1.57)	1.15 (0.86-1.53)
Test for heterogeneity	0.00	0.22	0.04
Publication bias (Egger'test)	0.15	0.14	0.75
Oral cancer OR (95% CI)	1.20 (0.89-1.63)	1.34 (0.99-1.82)	1.37 (0.88-2.14)
Pharyngeal cancer OR (95% CI)	1.25 (0.98-1.61)	1.11 (0.66-1.87)	1.10 (0.58-2.05)
Laryngeal cancer OR (95% CI)	1.53 (1.17-2.00)	1.10 (0.81-1.49)	1.08 (0.81-1.44)
Never smokers OR (95% CI)	1.58 (1.11-2.23)	1.29 (0.83-1.99)	1.38 (0.46-4.12)
Ever smokers OR (95% CI)	1.33 (1.01-1.74)	1.23 (0.77-1.94)	1.01 (0.76-1.33)
Caucasian OR (95% CI)	1.19 (0.93-1.51)	1.17 (0.91-1.50)	1.15 (0.86-1.54)

GSTM1: Gene coding for glutathione-*S*-transferase (GST) M1, *GSTT1*: gene coding for GSTT1, *GSTP1*: gene coding for GSTP1, Ile: isoleucine, Val: valine, OR: odds ratio, CI: confidence interval. ORs were adjusted for age, gender and race.

glutathione have been demonstrated in mucosal cells of oral/oropharyngeal and laryngeal tissues (27). When the GSH production is reduced or GSH is depleted, reactive electrophilic compounds may freely circulate and may cause damage of DNA or other important biomolecules. Since detoxification by GSH is strongly dependent on the GST enzymes, a reduction or deficiency of GST isoforms may also result in more DNA damage (24, 26, 28)

Any factor that may disturb the process of detoxification can result in increased levels of carcinogens and in a higher cancer risk. In this way GSH and GSTs may regulate the ability of each individual to metabolize environmental carcinogens, such as those of tobacco smoke.

In humans, the cytosolic GST family comprises seven classes and at least 16 different enzymes. The genes corresponding to these enzymes are mapped on different chromosomes (24). A limited number of the GSTs have been shown to be expressed in head and neck tissues. GSTA1/A2, GSTM1 or GSTP1 were detected in 91%, 64% and 100% of normal laryngeal tissues, respectively (27). In contrast, in oral and oropharyngeal normal mucosa, GSTP1 was expressed at high levels in all 14 different specimens investigated, whereas GSTM1 and GSTA1/A2 were expressed at very low levels only (27). In corresponding tumor tissues, GSTP1 was overexpressed in almost all tumors, whereas the expression of GSTM1 and GSTA1/A2 diminished even further (27).

Genetic polymorphisms, mostly resulting in a significant reduction of corresponding enzyme activities, have been described in *GSTM1, GSTT1, GSTP1* and *GSTA1*. For *GSTM1* and *GSTT1*, null polymorphisms may be present, resulting in the complete absence of enzyme activity (29, 30). The fact that *GSTM1* and *GSTT1* null genotypes in Caucasians are common (approximately 50 and 20%, respectively) implies that their co-occurrence is also relatively common. Thus approximately 10% of such individuals are missing both enzymes, which could possibly contribute to their susceptibility to SCCHN (25).

GSTP1-1, the only member of the GSTP class, appears to be the most widely distributed isoenzyme of all GSTs (28) and it is probably also the most abundant form in head and neck mucosal tissues (27). As reported by Sundberg *et al*., GSTP1-1 has selective and high activity towards the carcinogenic epoxide of BaP (31). A functional polymorphism has been described for the *GSTP1* gene at codon 105, where an isoleucine to valine substitution may result in considerable loss of the corresponding GSTP1-1 enzyme activity (32, 33)

The polymorphism in *GSTA1* is also widespread (34) and may have significant consequences for the expression of the corresponding enzyme, but it has not been studied yet in patients with head and neck cancer. Since GSTA is highly expressed in laryngeal tissues (27), a study of this polymorphism would be highly desirable in these patients.

The studies on *GSTM1, GSTT1* and *GSTP1* polymorphisms in relation to head and neck cancer have been recently reviewed by Hashibe *et al*., in a meta-analysis of 31 case-control studies, covering 4635 head and neck cancer patients and 5770 controls (25).The results can be summarised as follows: the *GSTM1* null, *GSTT1* null and *GSTP1 Ile105Val* genotype frequencies were highly variable in the SCCHN case populations that have been studied (range 43-80% for *GSTM1* null, 12-58% for *GSTT1* null and 29-66% for the *GSTP1 Val105* allele frequencies) (25). However, similarly variable frequencies were also seen in the corresponding control populations studied (25-58% for the GSTM1 null genotype, 8-53% for *GSTT1* null genotype and 24-65% for the *GSTP1* 105Val allele frequencies). When patients were selected according to SCCHN tumor site, a similar variation in GST polymorphism frequencies was reported.

Table IV. *Case-control studies of UGT1A7 and UGT1A10 polymorphisms and risk for (upper) aerodigestive tract cancer*

		Cases/controls	OR (95% CI)
UGT1A7	Zheng *et al*. (10)		
	(oral/laryngeal cancer)		
	Caucasian and African-American	194/388	
	Predicted UGT1A7 activity		
	High (genotype: *1/*1)		1.02 (ref)
	Intermediate (genotypes: *1/*2; *1/*3; *1/*4; *2/*2:*2/*3)		1.5 (0.78-2.70)
	Low (genotypes: *3/*3; *3/*4; *4/*4)		3.7 (1.70-8.70)
	Vogel *et al*. (44)		
	(oral/laryngeal/oesophageal/ gastric cancer)		
	Caucasian	76/210	
	Differencies in UGT1A7 gene alleles between the patients and controls		
	*UGT1A7*1*		Not significant
	*UGT1A7*2*		0.44 (0.27-0.71)
	*UGT1A7*3*		2.02 (1.33-3.07)
	*UGT1A7*4		Not significant
UGT1A10	Elahi *et al*. (42)		
	(oral/laryngeal cancer)		
	African-American	115/115	
	Codon 139 polymorphism		
	Glu > Glu		1.0 (ref)
	Glu > Lys		0.20 (0.05-0.87)
	Codon 244 polymorphism		
	Leu > Leu		1.0 (ref)
	Leu > Ile		0.94 (0.26-3.40)

UGT1A7: Gene coding for UDP-glucuronosyltransferase 1A7, *UGT1A10*: gene coding for UDP: glucuronosyltransferase 1A10, OR: odds ratio, CI: confidence interval.

SCCHN susceptibility of individuals with the *GSTT1* or *GSTM1* null genotype separately, appears to be slightly higher as compared with non-null genotype individuals, with pooled odds ratios of 1.25 (95% CI 1.00-1.57) and 1.32 (95% CI 1.07-1.62), respectively, while carrying a *GSTP1* 105Val allele does not seem to increase the risk (see Table III). However, an increased risk of head and neck cancer was observed when several modest risk GST genotypes were present, with an odds ratio of 2.06 (95% CI 1.11-3.81) for the combination of *GSTT1* null, *GSTM1* null and *GSTP1 105Val* (35).

Only three additional studies on GST polymorphisms in association with SCCHN have appeared after the publication of the meta-analysis of Hashibe *et al*.: two studies dealing with very low numbers of patients, 42 and 83 patients in the studies by Unal *et al*. (35) and Konig-Greger *et al*. (36), respectively, and one study by our own group, dealing with 185 patients (37). However, the findings in these three studies do not alter the general conclusions described above.

A recent review by Ho *et al*. (38), in addition to polymorphisms in detoxification enzymes other than mEH and UGTs, also summarized the results of studies on GST polymorphisms in association with the risk for SCCHN, and the conclusions with respect to GSTs were very similar to those presented here.

UDP-glucuronosyltransferases

UGTs belong to a superfamily of membrane-bound phase II enzymes localized in the endoplasmatic reticulum. UGTs catalyze the conjugation of UDP-glucuronic acid with mainly lipophilic substrates (glucuronidation) to form more polar conjugates that can be easily excreted *via* the biliary or renal route. Several members of the UGT family are involved in metabolic and detoxification pathways of (pro)carcinogens present in tobacco smoke, such as the glucuronidation of (pro)carcinogenic BaP metabolites and phenols. Hereby the concentration of such metabolites is diminished, thus reducing the risk of forming DNA-adducts and cancer (3).

The genes encoding the various human UGTs have been assigned to three families: *UGT1, UGT2* and *UGT3* (39).

Because, the catalytic and physiological functions of the human UGT3 family enzymes and their distribution in human tissue have not been characterized yet, the research on UGT genotypes and the susceptibility for SCCHN has until now been limited to the UGT1 and UGT2 genes (39, 40).

The human UGT1 enzymes are all derived from a single combined gene, located on chromosome 2q37 (41) which encodes for nine functional genes: *UGT1A1* and *UGT1A3* - *UGT1A10*. The human UGT2 enzymes are encoded by six separate genes located on chromosome 4q13-q28, resulting in the following enzymes: UGT2B4, 2B7, 2B10, 2B11, 2B15 and 2B17.

The expression of the UGT enzymes is tissue specific, but the factors that govern this specificity remain largely unknown. The expression of UGT enzymes in the mucosa of the upper aerodigestive tract has been studied by semiquantitative reverse transcription polymerase chain reaction, which revealed that UGT1A7 and UGT1A10 mRNAs were well expressed in the tongue, tonsil, floor of mouth, larynx and esophagus, whereas UGT1A8 and UGT1A6 were expressed primarily in the larynx. Out of the UGT2 family, only UGT2B4 and UGT2B17 exhibited significant expression levels in tissues of the aerodigestive tract (8). UGT1A7, UGT1A8, and UGT1A10 were shown to exhibit glucuronidating activity towards metabolites of cigarette smoke carcinogens such as hydroxylated BaP. UGT1A10 exhibiting the highest affinity for this substrate (8, 42).

Only three studies on the relationship between *UGT1A* polymorphisms and head and neck cancer risk have been carried out as yet (see Table IV). The *UGT1A7* gene is highly polymorphic and eleven allelic variants in four different codons of this gene have been described so far: *UGT1A7* *1-*11 (43). Zheng *et al*. (10) found that individuals (Caucasians as well as African-Americans) with any of the predicted low-activity *UGT1A7* genotypes had an increased risk of orolaryngeal cancer, results that were confirmed by a study of Vogel *et al*. (44). However, both studies dealt with only a relatively low number of patients (194 patients, Zheng *et al*.; 76 patients, Vogel *et al*.) and only covered the allelic polymorphisms *1, *2, *3 and *4.

Considering the *UGT1A10* polymorphisms, three functional polymorphisms (codon 139, 240 and 244) have been discovered so far. Elahi *et al*. found that the allelic prevalence of the codon 240 polymorphism in healthy African-Americans as well as in Caucasians was less than 1%, whereas the prevalence of codon 139 and 244 polymorphisms was much higher in African-Americans as compared to Caucasians. None of these polymorphisms were observed in East Asian or Indian individuals (42). By studying 115 African-American patient/control pairs, Elahi *et al*. observed a decreased risk for orolaryngeal cancer in individuals with the codon 139 polymorphism of *UGT1A10*, resulting in a glutamic acid to lysine amino acid change (42).

The polymorphisms in the two UGT genes (UGT1A7 and UGT1A10) studied so far have both been claimed to modulate individual susceptibility to SCCHN. Since many more UGTs are expressed in the mucosa of the upper aerodigestive tract, which are all involved in the metabolism of tobacco smoke carcinogens, polymorphisms in these UGTs need also to be investigated.

Discussion

Only six studies on mEH polymorphisms have been published to date. Three of them showed a higher risk for SCCHN associated with predicted high enzyme activity polymorphisms (11, 18, 20). However, the methods used for detection of the exon 3 mEH polymorphism in these three studies may admit potential inaccuracy. The other two studies, as well as a large study from our group, revealed no relationship between mEH polymorphisms and risk for SCCHN (19, 21, 22). One study reported an increased risk of the high activity mEH polymorphism but only in combination with the less catalytically active 105Val/105Val variant of *GSTP1* (21).

In the GST polymorphism research, a recent meta- and pool analysis showed a modestly elevated risk for SCCHN in individuals with the *GSTM1* or *GSTT1* null genotypes, but no significantly altered risk for carriers of the less active *GSTP1* Val105 allele (25). Individuals bearing a combination of the *GSTM1* null, *GSTT1* null and *GSTP1* Val105 variant showed a synergistic effect on the risk for SCCHN (29).

From the six UGT subtypes which are expressed in the mucosa of the upper aerodigestive tract and which are also involved in detoxification of tobacco smoke carcinogens (UGT1A6, UGT1A7, UGT1A8, UGT1A10, UGT2B4 and UGT2B17), only polymorphisms in *UGT1A7* and *UGT1A10* have been investigated (10, 42, 44). Two studies of *UGT1A7* have found an increased risk for upper aerodigestive tract cancer in individuals with predicted low-activity genotypes and one study of *UGT1A10* showed an association with decreased risk for orolaryngeal cancer. These three studies, all showing a modulating effect of *UGT* polymorphisms, were however performed on relatively small patient groups and much larger populations should be analyzed to confirm these results.

As stated by Brennan, due to an inadequate sample size, most of the studies on gene-environment interaction in cancer etiology are often inconsistent, since much larger patient cohorts are needed to detect the modest effects of these interactions (45). It is possible that genetic polymorphisms in detoxification enzymes, resulting in variations in enzyme activity, may have only a subtle effect on increasing or decreasing the cancer risk of an individual, but may have a much larger impact on a population, because the relevant polymorphisms may be highly prevalent.

Biotransformation and detoxification of tobacco smoke carcinogens by phase I and phase II enzymes is a complex process, commonly consisting of several consecutive reactions catalyzed by different enzymes, where the product of one reaction becomes a substrate for the next reaction. The overall carcinogen detoxification capacity, therefore, in general depends on a cascade of activities of enzymes involved in a particular detoxification pathway. This means that in order to estimate the risk of SCCHN, not only a larger sample size, but also a combination of several different detoxification enzymes should be studied. Identifying such risk-modifying polymorphisms and their combinations may be helpful for estimating the risk for an individual, as well as helping to clarify the process of tobacco-related carcinogenesis of SCCHN.

The survival of patients with SCCHN has not improved in the last decades, despite the diagnostic and therapeutic progress made. Genetic screening may probably help to identify the smokers at high risk for SCCHN and targeted education and smoking cessation programs for these individuals could be started. This may lower the SCCHN incidence rates and subsequently also diminish the mortality rates of tobacco-induced SCCHN.

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Received July 11, 2008
Revised November 26, 2008
Accepted December 1, 2008