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Glutathione S-transferase M1 and T1 genotypes and susceptibility to smoking related larynx cancer

RENATA JASKULA-SZTUL[1], MARIA REINIKAINEN[2], KIRSTI HUSGAFVEL-PURSIAINEN[2], ZYGMUNT SZMEJA[3], WITOLD SZYFTER[3], KRZYSTOF SZYFTER[1] and ARI HIRVONEN[2]*

[1] Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

[2] Finnish Institute of Occupational Health, Helsinki, Finland

[3] Clinics of Ontolaryngology, Medical University, Poznan, Poland

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Susceptibility to smoking related larynx cancer has been suggested to be associated with genetically determined differences in the ability to detoxify carcinogens present in tobacco smoke. The genetic polymorphisms of glutathione S-transferases, involved in the metabolic inactivation of, for example, tobacco derived carcinogens, have been recognized as potential risk modifiers in various environmentally induced malignancies, including larynx cancer. We employed PCR-based methods to determine the distribution of the *GSTM1* and *GSTT1* null genotypes in 171 larynx cancer patients and 180 controls to examine further their potential role in individual susceptibility to this neoplasm. The *GSTM1* null genotype was found in 49·1 % of the cases and 57·7 % of the controls and the *GSTT1* null genotype in 17·5 % of the cases and 21·7 % of the controls, respectively. Larynx cancer risk associated with the lack of *GSTM1* (OR = 0·7; 95 % CI: 0·5–1·1) or *GSTT1* (OR = 0·8; 95 % CI: 0·5–1·3) was not significantly affected by age, smoking status, or cancer progression. Although this study thus suggests no role for the *GSTM1* and *GSTT1* gene polymorphisms in individual susceptibility to smoking-related larynx cancer, due to its relatively small sample size more data are required before any definite conclusions can be drawn.

Keywords: *GSTM1*, *GSTT1*, genetic polymorphism, larynx cancer, tobacco smoke, individual susceptibility.

Introduction

Squamous cell carcinoma (SCC) of the larynx is the most frequent malignancy of the upper respiratory and digestive tract. In Poland an increasing trend in incidence of this neoplasm is seen (IARC 1992); larynx cancer accounts for seven or eight deaths per 100 000 persons per year and it is the third most frequent malignancy in the male population (Zatoñski *et al.* 1991). Although progress has been made in the treatment of laryngeal cancer, the overall 5-year survival rates have increased by only 10 % to 15 % since 1960 (Irish 1994; Jahnke 1995). In fact, in Poland an increase of mortality was observed in the years 1963–1989 due to intensive smoking of poor quality, high tar cigarettes (Zatoñski *et al.*1992).

Larynx cancer is associated with two known risk factors, tobacco smoking and alcohol consumption, which have been shown to have a multiplicative effect (IARC

* Corresponding author: Ari Hirvonen, PhD, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, FIN-00250 Helsinki, Finland.

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1986, 1988). DNA lesions associated with tobacco smoking have been demonstrated along the respiratory tract and, based on epidemiological and molecular studies, tobacco smoking is the primary causative factor in larynx cancer aetiology (Randerath and Randerath 1993, Stern *et al.* 1993, Degawa *et al.* 1994, Cattaruzza *et al.* 1996). Tobacco smoke contains carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines and halomethanes which are capable of forming macromolecular adducts (Löfroth 1989, Hecht *et al.*1993); tobacco smoke-derived DNA adducts in the larynx have been described in several recent studies (Stern *et al.* 1993, Degawa *et al.* 1994, Szyfter *et al.* 1994, 1996). Moreover, the compounds in cigarette smoke have been shown to be toxic to the laryngeal epithelium (Trell *et al.* 1976). However, development of larynx cancer is not an inevitable consequence of smoking. This has stimulated the search for genetic factors modifying the risk for larynx cancer among tobacco smokers (Cloose *et al.* 1994, Copper *et al.* 1995).

Although a number of tobacco derived carcinogens are capable of inducing DNA damage, they must usually be metabolically activated to become ultimate carcinogens. The active carcinogens can subsequently be inactivated and removed from the cell in the course of detoxification. Genetically controlled variations in expression of the detoxifying enzymes may therefore play an important role in individual responses to hazardous agents. The glutathione S-transferases (GSTs) are widely studied for their potential role as modifiers of individual susceptibility to xenobiotic challenge (Ketterer *et al.* 1992, Rushmore and Pickett 1993) and their particular genotypes have been related to increased risk of cancer in several studies (for review see d'Errico *et al.* 1996). The *GSTM1* and *GSTT1* genes are polymorphic so that some individuals have both of the alleles deleted (null genotypes) and are thus devoid of the corresponding enzyme activity. Given that GSTM1 isoenzyme is involved in detoxification of, for example, PAHs and other polycyclic aromatic hydrocarbons abundant in tobacco smoke (Harada and Abei 1992) and GSTT1 metabolizes halomethanes and ethylene oxide also present in tobacco smoke (Guengerich *et al.* 1995), the *GSTM1* and *GSTT1* genotypes are especially interesting candidates for modifiers of individual susceptibility to smoking related larynx cancer. In agreement with this the *GSTT1* null genotype was recently suggested to pose about a two-fold risk of larygeal SCC (Jahnke *et al.* 1996). In this study we investigated this issue further in a Caucasian population in Poland.

Material and methods

Study subjects

The larynx cancer group consisted of 171 subjects (159 men and 12 women) admitted to hospital because of tumours in the upper part of the larynx. Most of the tumours were diagnosed histologically to be the primary type of laryngeal SCC; a few subjects with a relapse were classified as recurrent primary laryngeal SCC. Treatment did not include chemo- or radiotherapy prior to surgery except in the relapse patients, who were ^{60}Co-irradiated after the first surgery. The average age of the cases was 58 years (men aged 34–81 years and women aged 42–70 years). All patients were interviewed for their occupational and life-style (alcohol consumption and smoking habits) histories. The majority of the patients were farmers and none of them reported occupational exposure to either PAHs or *N*-nitrosamines. There was only one non-smoker and nine ex-smokers (stopped smoking at least 1 year before the operation) among the larynx cancer patients; the rest 161 were current smokers who were further divided to moderate (less than 25 cigarettes per day) and heavy (25 and more cigarettes per day) smokers. Since the data concerning alcohol consumption appeared highly unreliable, they were left out from the statistical analyses.

The control subjects were from the same region in Poland as the cases and consisted of 180 healthy unrelated males originally subjected to testing for paternity by DNA fingerprinting. The occupational and life-style data were not available for the controls.

Genotype analysis

Leucocyte DNA was extracted using standard methods from peripheral blood (10ml), drawn from all study subjects into EDTA tubes before surgery and stored at –20 °C until use. *GST* polymorphisms were studied using PCR-based methods, described in more detail elsewhere (Pemble *et al.* 1994, Zhong *et al.* 1994). Briefly, on the basis of the sequences of *GSTM1* and related genes of the same multigene family, three primers for the exon 4 and exon 5 region of the genes were used. Primers 1 and 2 could also anneal to the *GSTM4* gene, while primer 3 was specific for the *GSTM1* gene. When the three primers were used together in a PCR assay, a 158 bp fragment was consistently found, whereas the polymorphic 231 bp fragment could only be seen in the *GSTM1* positive genome. The constant 158 bp fragment was amplified as an internal control, excluding the possibility of a false interpretation due to failure in the amplification reaction.

In the *GSTT1* analysis four primers were used, two of which were complementary to the 3′ coding region of the *GSTT1* sequence and gave a PCR product of 480 bp seen only in the *GSTT1* positive genome, whereas the other two were specific for the vitamin D receptor (VDR) gene and gave a product of 800 bp (Morrison *et al.* 1994), verifying proper amplification from the samples.

Results and discussion

As shown in table 1, the *GSTM1* and *GSTT1* genotypes appeared to be somewhat less frequent in larynx cancer patients than in controls. When the data were stratified by the smoking habits, the frequency of the *GSTM1* null genotype appeared to be lowest among heavy smoker patients, exceeded by moderate smokers and ex-smokers, whereas no clear tendency was observed for the *GSTT1* null genotype.

The concurrent lack of both of the *GST* genes did not contribute significantly to the risk of developing laryngeal SCC (table 2). Neither did age have any significant effect on the *GSTM1* and *GSTT1* genotype distribution among the larynx cancer patients (data not shown). Moreover, the tumour stage was not paralleled with the frequency of these genotypes (data not shown). This is in agreement with the earlier report of Brockmöller *et al.* (1994) where *GSTM1* deficiency did not correlate with staging or grading of the tumours.

The lack of any significant role for the *GSTM1* and *GSTT1* gene polymorphisms in individual susceptibility to larynx cancer observed in this study agree with the finding that GSTs other than GSTP1 are expressed at very low levels in the laryngeal and oral tissue (Mulder *et al.* 1995), suggesting a minor role

Table 1. The distribution of *GSTM1* and *GSTT1* genotypes in the study populations.

	GSTM1 genotype			*GSTT1* genotype		
	No. of subjects	% null	OR (95% CI)	No. of subjects	% null	OR (95% CI)
Control subjects	180	57·7	1·0	180	21·7	1·0
Larynx cancer patients	171	49·1	0·7 (0·5–1·1)	171	17·5	0·8 (0·5–1·3)
Heavy smokers	67	46·3	0·7 (0·4–1·2)	67	19·4	0·9 (0·4–1·8)
Moderate smokers	94	51·1	0·8 (0·5–1·3)	94	16·0	0·7 (0·4–1·3)
Ex-smokers	9	55·6	1·0 (0·2–3·7)	9	22·2	1·0 (0·2–5·2)
Non-smokers	1	0	–	1	0	–

Key: Null = absence of the gene. OR = odds ratio. CI = confidence interval. OR shown is for the null genotype compared with the positive genotype. Reference group consists of the healthy control subjects.

Table 2. The distribution of combined *GSTM1* and *GSTT1* genotypes in the study populations.

	GSTM1-null				*GSTM1*-positive				
	GSTT1-null		*GSTT1*-positive		*GSTT1*-null		*GSTT1*-positive		
	n	%	*n*	%	*n*	%	*n*	%	OR (95% CI)
Control subjects (*n* = 180)	17	9·4	85	47·2	22	12·2	56	31·1	1·0
Larynx cancer patients (*n* = 171)	18	10·5	66	38·6	12	7·0	75	43·9	0·8 (0·4–1·8)
Heavy smoker (*n* = 67)	7	10·4	24	35·8	6	9·0	30	44·8	0·8 (0·3–2·1)
Moderate smoker (*n* = 94)	10	10·6	38	40·4	5	5·3	41	43·6	0·8 (0·3–1·9)
Ex-smoker (*n* = 9)	1	11·1	4	44·4	1	11·1	3	33·3	1·1 (0·1–11·4)
Non-smoker (*n* = 1)	0	0	0	0	0	0	1	100	–

Key: Null = absence of the gene; positive = presence of the gene. OR = odds ratio. CI = confidence interval. OR shown is for the combined *GSTM1*-null and *GSTT1*-null genotype compared with the *GSTM1*-positive and *GSTT1*-positive genotype combination. Reference group consists of the healthy control subjects.

for the GSTM1 and GSTT1 isoenzymes in the detoxification of tobacco smoke-derived carcinogens in these sites. Our findings on *GSTM1* polymorphism are contrasted by two recent phenotyping-based studies (Lafuente *et al.* 1993, Coutelle *et al.* 1997), indicating that functional GSTM1 protects against developing laryngeal cancer. This discrepancy may in part arise from the main limitations of this study, i.e. unavailability of the occupational and life-style data for the controls and inability to adjust for alcohol consumption. On the other hand, adjustment for alcohol consumption was also missing in the study of Lafuente *et al.* (1993) where some additional bias may have been caused by the relatively small study size. Similarly, although the alcohol consumption was properly taken into account in the other phenotype based study (Coutelle *et al.* 1997), their sample size is considered to be too small to give sufficient power for accurate interpretations. In agreement with this, in the sole genotyping-based study so far reported on this topic with a comparable sample size to ours, no significant association between the *GSTM1* null genotype and larynx cancer risk was observed (Jahnke *et al.* 1996). Although they did observe a higher frequency of *GSTT1* null genotypes among larynx cancer patients than among controls, in contrast to our findings, the difference failed to reach statistical significance, and needs to be confirmed in future studies.

The study of Lafuente *et al.* (1993), where GSTM1 deficient larynx cancer patients were reported to be mostly heavy smokers, also disagrees with our observations on *GSTM1* genotype distribution in relation to smoking habits. However, our findings are very similar to those in the study of Alexandrie *et al.* (1996) where higher frequency of *GSTM1* null genotypes was found among light smokers with lung cancer. This supports the hypothesis that the genetic variation in the ability to metabolize a particular carcinogen may be irrelevant at high exposure levels (Vineis *et al.* 1994, London *et al.* 1995). Consequently, this could have caused some bias to the study by Coutelle *et al.* (1997) where all of the subjects were heavy smokers.

Interestingly, cancer incidence was recently found to be almost 20 % lower among Finnish farmers than among the general population (Pukkala and Notkola 1997). The difference was most remarkable in the smoking-related lung cancer and larynx cancer; incidence of these cancers was one-third and one-half of that expected for the male and female farmers, respectively. Although the significantly (30 %) lower prevalence of smokers among the farmers than in the Finnish population on average supposingly accounts for the main portion of the difference in the cancer incidence, other environmental factors may also have played some role. Consequently, this could have affected the outcome of the present study, where most of the cases were farmers. However, since it is not known whether the lower cancer incidence can also be seen among Polish farmers compared with the general Polish population, this issue remains to be evaluated in the future studies.

Although clear differences have been observed between males and females in the susceptibility to smoking related oral cancer (Muscat *et al.* 1996), this is unlikely to explain any of the divergent findings discussed here; all of the above mentioned study populations consisted either solely (Lafuente *et al.* 1993, Coutelle *et al.* 1997), or mainly (Jahnke *et al.* 1996) of males, similarly to the present study population. In contrast, polymorphisms in other genes, especially those involved in the metabolism of ethanol, i.e. *CYP2E1* (Guengerich *et al.* 1991) and alcohol dehydrogenase 3 (Coutelle *et al.* 1997), may well have had some effect on the outcomes of the studies. Establishment of a combined impact of all relevant genes for a given exposure may thus be a prerequisite for more reliable identication of susceptible individuals and subgroups in environmentally-exposed populations (Hirvonen 1997).

Taken together, we found no association between the *GSTM1* and *GSTT1* genotypes and larynx cancer risk, failing thus to support the hypothesis that they would play an important role in individual susceptibility to smoking-related larynx cancer. However, due to the relatively small sample size in this study, the complex nature of the gene–environment interactions, and limitations of the studies so far conducted on this topic, more data from well-designed studies are needed before any definite conclusions can be drawn.

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