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• ESOPHAGEAL CANCER •

GSTM1, *GSTT1*, *GSTP1* and *CYP1A1* genetic polymorphisms and susceptibility to esophageal cancer in a French population: Different pattern of squamous cell carcinoma and adenocarcinoma

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Abstract

AIM: To evaluate the association between *CYP1A1* and *GSTs* genetic polymorphisms and susceptibility to esophageal squamous cell carcinoma (SCC) and esophageal adenocarcinoma (ADC) in a high risk area of northwest of France.

METHODS: A case-control study was conducted to investigate the genetic polymorphisms of these enzymes (*CYP1A1*2C* and *GSTP1* exon 7 Val alleles, *GSTM1*2/*2* and *GSTT1*2/*2* null genotypes). A total of 79 esophageal cancer cases and 130 controls were recruited.

RESULTS: *GSTM1*2/*2* and *CYP1A1*1A/*2C* genotype frequencies were higher among squamous cell carcinomas at a level close to statistical significance (OR = 1.83, 95% CI 0.88-3.83, $P = 0.11$; OR = 3.03, 95% CI 0.93-9.90, $P = 0.07$, respectively). For *GSTP1* polymorphism, no difference was found between controls and cases, whatever their histological status. Lower frequency of *GSTT1* deletion was observed in ADC group compared to controls with a statistically significant difference (OR = 13.31, 95% CI 1.66-106.92, $P<0.01$).

CONCLUSION: In SCC, our results are consistent with the strong association of this kind of tumour with tobacco exposure. In ADC, our results suggest 3 distinct hypotheses: (1) activation of exogenous procarcinogens, such as small halogenated compounds by *GSTT1*; (2) contribution of *GSTT1* to the inflammatory response of esophageal mucosa, which is known to be a strong risk factor for ADC, possibly through leukotriene synthesis; (3) higher sensitivity to the inflammatory process associated with intracellular depletion of glutathione.

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INTRODUCTION

One of the highest incidences of esophageal cancer in Europe is observed in the Northwest of France[1-4]. There are two predominant histological forms of this cancer: squamous cell carcinoma (SCC) and adenocarcinoma (ADC)[4,5]. Recent epidemiological observations showed an important decrease in the incidence of SCC whilst ADC was slightly increased[2,4].

In Western countries, smoking tobacco and drinking alcohol are the main risk factors for SCC. For ADC, exogenous risk factors are not well known. A link was found between this pathology, esophageal reflux and Barret's esophagus[5].

Tobacco smoke contains many carcinogens such as polycyclic aromatic hydrocarbons (PAH) and *N*-nitrosamines that can be activated or deactivated by phase I (cytochromes P-450) and phase II enzymes (glutathione S-transferases). Cytochromes P450 (CYP) are a widely expressed enzyme family, some members of which present genetic polymorphisms (e.g. *CYP1A1*, *2E1*, *2D6*). *CYP1A1* is expressed in esophageal mucosa, which means that activation of tobacco carcinogens can happen *in situ*[6]. Benzo[a]pyrene is activated by *CYP1A1* to diol-epoxide, which is a reactive and carcinogenic product. Four main genetic polymorphisms are described for *CYP1A1*. One of the most studied is Ile/Val polymorphism in exon 7 (*CYP1A1*2C* allele). One study reported that Val-type could be associated with a higher aryl hydrocarbon hydroxylase activity[7].

Many studies have reported the association of *CYP1A1* polymorphisms with lung cancer[8], particularly with SCC of the lung[9,10]. The level of DNA adducts was found to be linked to *CYP1A1*2C* polymorphism[11]. All these results suggest that susceptibility to tobacco-related cancers could be modified by *CYP1A1* polymorphisms.

The glutathione S-transferases are a family of phase II enzymes, which catalyse the conjugation of many endogenous and exogenous electrophilic compounds to glutathione. GSTM1 and GSTP1 are able to detoxify benzopyrene diol-epoxide[12], whereas GSTT1 can conjugate oxidised lipids and halogenated compounds[12]. Both *GSTM1*, *P1* and *T1* are expressed in esophageal mucosa[13,14]. GSTP1 is the mainly expressed GST in this tissue[15]. *GSTP1* presents a substitution polymorphism in exon 7 that results in a substitution of Ile by Val at amino acid position 104[16]. Val variants were found to have a lower activity towards 1-chloro-2, 4-dinitrobenzene[17]. *GSTM1* and *GSTT1* present deletion polymorphisms (*GSTM1*2/*2* and *GSTT1*2/*2*), which are currently at about 50% and 20% among Caucasians, respectively[18,19]. *GSTM1*2/*2* polymorphism has been found to increase the frequency of chromosome aberrations after tobacco-specific *N*-nitrosamine exposure *in vitro*[20]. Many studies have shown that this deletion increases the susceptibility conferred by the *CYP1A1*2C* allele for tobacco-associated cancer[21]. While *GSTT1*2/*2* genotypes have not been clearly

associated with susceptibility to tobacco-linked cancers, an interaction with *GSTM1*2/*2* has often been found[22,23].

The aim of our work was to evaluate the susceptibility conferred by *CYP1A1* and *GSTs* genetic polymorphisms to SCC and ADC of esophagus in a high risk European area.

MATERIALS AND METHODS

Controls and cases were from the geographic area of Basse-Normandie, France. Patients were recruited after endoscopic and histologic diagnosis of primary esophageal cancer. All cases were newly diagnosed and previously untreated patients. Controls were required to be free of any chronic diseases, having no cancer history and living in Basse-Normandie. They were matched with cases in sex and age. Alcohol and tobacco consumption were also evaluated during the recruitment of cases and controls by means of a questionnaire.

The research protocol was approved by the Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédical en Basse-Normandie. A 20 mL sample of venous blood was taken and DNA extraction was performed by phenol/chloroform method.

The primer sequences and product sizes of each gene amplification are shown in Table 1. *GSTM1* and *GSTT1* multiplex PCR was performed according to the Lin *et al*. method[24], with some modifications. A final mixture volume of 25 μL was prepared containing 0.100 μg of DNA, 0.25 μmol/L of dNTP, 0.4 μmol/L of primer for *GSTM1*, 0.8 μmol/L of primer *GSTT1*, 0.8 μmol/L of primer albumin, 5 μL of 10× buffer, 2 mmol/L of $MgCl_2$ and 0.5 U per sample of DNA Gold *Taq* polymerase (Applied Biosystem, Coutaboeuf, France). The first step was performed for 15 min at 95℃ followed by 35 cycles: at 94 ℃ for 1 min (denaturation), at 58 ℃ for 1 min (annealing), at 72 ℃ for 1 min (elongation). PCR ended a final extension for 10 min at 72 ℃. PCR products were visualised on 20 g/L agarose gel with ethidium bromide staining.

Table 1 Primer sequences and length of PCR products

Gene	Primer sequence	Size of PCR product (bp)	Reference
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC-3'	219	Lin *et al*, 1998[24]
	5'-GTTGGGCTCAAATATACGGTGG-3'		
GSTT1	5'-TTCCTTACTGGTCCTCACATCTC-3'	459	
	5'-TCACCGGATCATGGCCAGCA-3'		
Albumin	5'-GCCCTCTGCTAACAAGTCCTAC-3'	350	
	5'-GCCCTAAAAAGAAAATCCCCAATC-3'		
GSTP1	5'-ACCCCAGGGCTCTATGGGAA-3'	176	Harries *et al*, 1997[16]
	5'-TGAGGGCACAAGAAGCCCCT-3'		
CYP1A1	5'-GGCTGAGCAATCTGACCCTA-3'	206	Cascorbi *et al*, 1996[8]
	5'-TTCCACCCGTTGCAGCAGGATAGCC-3'		

GSTP1 PCR restriction fragment length polymorphism (rflp) was performed using a method adopted by Harries *et al*.[16] with slight modifications. The final mixture (40 μL) was prepared containing 0.100-0.500 μg of DNA, 0.25 μmol/L of dNTP, 0.25 μmol/L of each of the primers, 1.25 μmol/L of $MgCl_2$, 4 μL of 10× buffer, 4 μL of DMSO, 1.5 U of *Taq* polymerase (Eurobio, Les Ulis, France). Briefly, the samples were denatured at 94 ℃ for 5 min and submitted to 30 cycles of amplification as follows: for 30 s at 94 ℃ (denaturation), for 30 s at 55 ℃ (annealing), for 30 s at 72 ℃ (extension) and a final extension at 72 ℃ for 5 min. PCR product of 12 μL was digested by 5 U Alw26 I restriction enzyme (Eurogentec, Seraing, Belgium) for 12 h at 37 ℃. Migration was performed on low melting 40 g/L agarose gel (Eurobio, Les Ulis, France), stained with ethidium bromide, in order to separate the 85 and 91 bp fragments.

*CYP1A1*2C* polymorphism was determined by PCR-RFLP as previously described[8].

Each PCR analysis was performed twice in double blind.

Statistical analysis

Chi-square test and *P* value estimation were performed using Stata software (STATA Corporation, college Station, TX). Odds ratio was also evaluated using StataÒ software and adjusted for age, sex and histological type.

RESULTS

The populations of controls and cases are described in Table 2. The patient group consisted of 52 SCCs and 27 ADCs. The mean ages for cases and controls were 62 and 56 years respectively. Unfortunately, we obtained tobacco and alcohol exposure data for only 48 cases. This was insufficient to allow us to study interaction between exposure and polymorphisms. As it could be expected, the vast majority of SCCs were smokers (93%, all with more than 20 years of tobacco consumption) and heavy drinkers (86% drinking more than 229 g/wk). Fewer ADCs were smokers (78%, of which 67 % with more than 20 years of tobacco consumption) and only 50% were heavy drinkers (Table 2).

Table 2 Description of control and case populations

	Control *n* (%)	Case *n* (%)	SCC *n* (%)	ADC *n* (%)
Male	87 (0.67)	69 (0.87)	44 (0.85)	25 (0.93)
Female	43 (0.33)	10 (0.13)	8 (0.15)	2 (0.07)
Mean age (yr)	56 [19; 87]	62 [40; 85]	60 [40; 78]	66 [51; 85]
Tobacco duration[1,2]				
(*years of smoking*)				
Non-smokers	66 (0 .66)	6 (0.13)	2 (0.07)	4 (0.22)
1-19	13 (0.13)	2 (0.04)	0 (-)	2 (0.11)
+20	21 (0.21)	40 (0.83)	28 (0.93)	12 (0.67)
Alcohol consumption[3,4]				
(*g of ethanol per week*)				
0-228	11 (0.64)	13 (0.28)	4 (0.14)	9 (0.50)
228.5 -/+ 470	6 (0.36)	35 (0.72)	26 (0.86)	9 (0.50)

[1]Tobacco duration (year); [2]Data were available for 77% of controls, 61% of cases, including 58% of SCC and 67% of ADC; [3]Alcohol consumption (gram of ethanol per week); [4]Data for alcohol consumption were available for only 13% of controls, 61% of cases, including 58% of SCC and 67% of ADC.

Frequencies of the different genetic polymorphisms in the control group were 0.06 for *CYP1A1*A/*2C* (no homozygous **2C/*2C* subject was found), 0.45 and 0.07 for Ile/Val and Val/Val *GSTP1* genotypes, 0.49 for *GSTM1*2/*2* and 0.26 for *GSTT1*2/*2* (Tables 3, 4).

A high frequency of *CYP1A1*1A/*2C* genotype was found in SCC cancer patients (Table 3). However, the difference did not reach statistical significance (with a *P* value of 0.06). The ADC patient group did not show any significant difference compared to the control group.

*GSTM1*2/*2* genotype (*GST M1* null) was increased among the cases compared to the controls, particularly among SCC patients (Table 4), but this difference was not statistically significant (OR = 1.83; 95% CI = 0.88-3.83). The distribution of *GSTM1*2/*2* genotype among ADCs did not differ from the controls.

The frequency of *GSTT1*2/*2* genotype (*GSTT1* null) was not different between cases and controls (Table 5). However, the ADC group showed a greatly decreased frequency of *GSTT1*2/*2* genotype (4%) compared to the control population (26%) and SCCs (29%) (OR = 13.31, 95 % CI = 1.66-106.92).

Distribution of the *GSTP1* genotype did not differ between SCC, ADC and control groups (Table 6).

Table 3 Repartition of *CYP1A1* genotypes among controls and cases

	n	CYP1A1*1A/*1A		CYP1A1*1A/*2C			OR[1]	95% CI
		n	(%)	*n*	(%)			
Controls	107	101	(94)	6	(6)			
Cases	70	61	(87)	9	(13)	All cases *vs* controls[2]	2.63	[0.84-8.28]
SCC	47	40	(85)	7	(15)	SCC *vs* controls[3]	3.03	[0.93-9.90][5]
ADC	23	21	(91)	2	(9)	ADC *vs* controls[4]	2.06	[0.33-13.04]

[1]Adjusted OR for age and sex; [2]Comparison of *CYP1A1*1A/*2C* genotype repartition in controls *vs* all cases; [3]Comparison of *CYP1A1*1A/*2C* genotype repartition in controls *vs* SCCs; [4]Comparison of *CYP1A1*1A/*2C* genotype repartition in controls *vs* ADCs; [5]*P* = 0.067.

Table 4 Repartition of *GSTM1* genotypes among controls and cases

	n	*GSTM1*2/*2*				
		n	(%)		OR[1]	95% CI
Controls	120	59	(49)			
Cases	68	39	(57)	All cases *vs* controls[2]	1.43	[0.76-2.69]
SCC	43	27	(63)	SCC *vs* controls[3]	1.83	[0.88-3.83][5]
ADC	25	12	(48)	ADC *vs* controls[4]	0.95	[0.38-2.41]

[1]Adjusted OR for age and sex; [2]Comparison of *GSTM1*2/*2* genotype repartition in controls *vs* all cases; [3]Comparison of *GSTM1*2/*2* genotype repartition in controls *vs* SCCs; [4]Comparison of *GSTM1*2/*2* genotype repartition in controls *vs* ADCs; [5]*P* = 0.108.

Table 5 Repartition of *GSTT1* genotypes among controls and cases

	n	*GSTT1*2/*2*				
		n	(%)		OR[1]	95% CI
Controls	115	30	(26)			
Cases	70	14	(20)	All cases *vs* controls[2]	1.78	[0.84-3.80]
SCC	44	13	(29)	SCC *vs* controls[3]	1.03	[0.46-2.27]
ADC	26	1	(4)	ADC *vs* controls[4]	13.31	[1.66-106.92][a]

[1]Adjusted OR for age and sex; [2]Comparison of *GSTT1*2/*2* genotype repartition in controls *vs* all cases; [3]Comparison of *GSTT1*2/*2* genotype repartition in controls *vs* SCCs; [4]Comparison of *GSTT1*2/*2* genotype repartition in controls *vs* ADCs; [a]*P*<0.05.

Table 6 Repartition of *GSTP1* genotypes among controls and cases

	n	*GSTP1*								
		Ile/Ile		Ile/Val		Val/Val			OR[1,2]	95% CI
		n	(%)	*n*	(%)	*n*	(%)			
Controls	124	59	(48)	56	(45)	9	(7)			
Cases	70	31	(44)	33	(47)	6	(9)	All cases *vs* controls[3]	1.02	[0.55-1.89]
SCC	45	21	(47)	21	(47)	3	(6)	SCC *vs* controls[4]	0.95	[0.47-1.91]
ADC	25	10	(40)	12	(48)	3	(12)	ADC *vs* controls[5]	1.17	[0.46-2.97]

[1]Adjusted OR for age and sex; [2]Ile/Val and Val/Val genotypes were compared to Ile/Ile genotype; [3]Comparison of *GSTP1* Ile/Val and Val/Val genotype repartition in controls versus all cases; [4]Comparison of *GSTP1* Ile/Val and Val/Val genotype repartition in controls *vs* SCCs; [5]Comparison of *GSTP1* Ile/Val and Val/Val genotype repartition in controls *vs* ADCs.

DISCUSSION

Esophageal cancer presents a very variable incidence in different regions and ethnic groups. In France, different levels of environmental exposure to carcinogens could not fully explain this high variability[25-27], a fact which suggests a genetic susceptibility. Many epidemiological studies have established that exposure to tobacco smoke and alcohol is a major risk factor for SCC in Western countries, whereas ADC is not strongly linked to exogenous factors. As far as we know, only one study concerning the genetic susceptibility to esophageal cancer was performed among Caucasians[28]. Moreover, the cases for this study were recruited in a low risk area in Europe.

The repartition of different polymorphisms in our control group agrees with available data for a Caucasian population[8,16,18,19,29]. Recently, frequencies of these polymorphisms among a healthy population were evaluated and published by International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC)[18].

Among SCC cases, *CYP1A1*2A/*2C* frequency was increased when compared to controls and adjusted OR was 3.03 (95% CI 0.93-9.90), however this result was not statistically significant (*P* = 0.067). The deletion of *GSTM1* gene was also more frequent among SCC cases when compared to controls (63% and 49% respectively, OR = 1.83; 0.88-3.83). But this result was also not statistically significant (*P* = 0.108). *CYP1A1*2A/*2C* and *GSTM1*2/*2* genotypes were found to increase the risk of SCC in a previous study in an Asian population, particularly among cases with higher tobacco consumption. However, some studies did not find *CYP1A1* and *GSTM1* gene polymorphisms to be related to SCC. No association was found between other genetic polymorphisms studied (*GSTT1, GSTP1)* and esophageal SCC, which is in accordance with the data in literature[24,30,31]. It should be emphasized that, concerning *GSTT1*, our study is the first report about a Caucasian population.

No differences were found among ADC cases regarding the

frequencies of *CYP1A1*, *GSTM1* and *GSTP1* polymorphisms when compared to controls. This observation is in accordance with the weak association of tobacco smoke, alcohol consumption and ADC. In the ADC group, an unexpected protective effect of *GSTT1* deletion was found (OR = 13.31; 95% CI 1.66-106.92). Such results have been previously described for other sites such as renal or prostate carcinoma[32,33]. It is well known that the risk of renal carcinoma is increased by exposure to small halogenated compounds such as dicholoromethane or trichloroethylene. Activation of these compounds in electrophilic species implies GSTT1[29], which could explain these results. However, to our knowledge, no studies have demonstrated a role of small halogenated compounds in esophageal ADC carcinogenesis. Exposure to these compounds is possible through occupational factors, chlorinated tap water consumption or tobacco smoke. The latter, which is a weakly associated risk factor for ADC, contains methyl chloride[34]. However, our present data did not allow us to estimate exposure to halogenated compounds in our population.

Another hypothesis is that GST could participate in chronic inflammation through leukotriene synthesis[35]. In particular, leukotriene A4 to C4 (LTC4) conversion requires GST activity. Inflammation is a major etiologic factor for ADC and leukotrienes have been found to be mediators implicated in this process[5]. Furthermore, leukotriene LTD4, which is biosynthesized from LTC4, was found to induce contraction of the oesophagus and lower esophageal sphincter in animal models[35,36]. This phenomenon is likely to be involved in gastro-oesophageal reflux, which constitutes the strongest risk factor for ADC. However, though GSTT1 is also expressed in esophageal mucosa[14], it remains unclear whether this enzyme contributes to LTC4 synthesis in this tissue.

The association between susceptibility to cancer and *GSTT1* genotypes could be also explained by depletion in intracellular glutathione in the presence of GSTT1 enzyme. In this case, cells would be more sensitive to radical species produced during the inflammatory process observed among adenocarcinoma patients.

In conclusion, our study shows a different pattern of susceptibility to SCC and ADC of esophagus in a European high risk population. Whereas a slight susceptibility to SCC could be conferred by *CYP1A1*1A/*2C* and *GSTM1*2/*2* genotypes, a high frequency of *GSTT1*1/*1* genotype was found among ADC. These results are consistent with the association of SCC with tobacco exposure, as other tobacco-related cancers such as lung cancer were found to be moderately linked to *CYPA1A1*2C* allele and *GSTM1*2/*2* genotype. In ADC, our results suggest 3 distinct hypotheses. (1) The activation of exogenous procarcinogens, such as small halogenated compounds (to which ways of exposure remain to be identified), by GSTT1. Unlike tobacco, the evaluation of exposure to small halogenated compounds remains difficult because of the wide distribution of these compounds. (2) The contribution of GSTT1 to the inflammatory response of esophageal mucosa, which is known to be a strong risk factor for ADC, possibly by way of leukotriene synthesis. (3) Higher sensitivity to the inflammatory process associated with intracellular depletion of glutathione. A new study focusing on esophageal ADC with a larger recruitment would allow us to investigate these issues.

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