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Polymorphic Expression of the Glutathione *S*-Transferase *P1* Gene and Its Susceptibility to Barrett's Esophagus and Esophageal Carcinoma[1]

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ABSTRACT

Factors determining individual susceptibility to esophageal cancer or premalignant Barrett's epithelium are still largely unclear. An imbalance between phase I drug metabolism [*e.g.*, cytochrome P450 (CYP)] and phase II detoxification [*e.g.*, glutathione *S*-transferase (GST)] may contribute to the development of these diseases. Polymorphic variants in the *CYPIA1* gene were described leading to increased levels of bioactive compounds, whereas polymorphisms in GST genes often resulted in impaired detoxification. We studied the frequencies of polymorphic variants in *CYPIA1*, *GSTP1*, *GSTT1*, and *GSTM1* genes in 98 patients with Barrett's epithelium and 34 patients with esophageal cancer. The results were compared with those obtained from 247 healthy blood donors. DNA was extracted, and PCR-RFLP methods were used to detect genetic polymorphisms. χ^2 analysis, Spearman rank correlation, and Wilcoxon rank sum tests were used for statistical evaluation. Polymorphisms in *CYPIA1*, *GSTM1*, and *GSTT1* occurred at an equal frequency in patients and controls. Occurrence of the polymorphic *GSTP1b* variant in the *GSTP1* gene resulted in a significantly lower GST enzyme activity ($P < 0.05$), and *GSTP1b* was found significantly more often in patients with Barrett's epithelium (70%; $P < 0.001$) and patients with esophageal adenocarcinoma (76%; $P = 0.005$), as compared to healthy blood donors (41%). In conclusion, presence of the *GSTP1b* allele leads to lower GST enzyme activity levels and, consequently, impaired detoxification. This most important esophageal GST isoform may, therefore, contribute to the development of Barrett's epithelium and adenocarcinoma.

INTRODUCTION

Barrett's esophagus is a pathological condition in which stratified squamous epithelium of the esophagus is replaced by columnar epithelium (1). Compared to the general population, patients with Barrett's esophagus have a 30–125-fold increased risk of developing esophageal adenocarcinoma (2–4), and therefore, Barrett's esophagus is considered to be a premalignant condition. The prevalence of esophageal adenocarcinoma is increasing rapidly, and its annual 10% rate of increase exceeds that of all other cancers (5).

The reason for this rapid increase in adenocarcinoma of the esophagus is unknown; however, a large proportion of human cancers are related to environmental or dietary chemical compounds, either synthetic or naturally occurring (6, 7). Many of those chemical structures are metabolically activated to forms that have deleterious effects on organisms (8), and this metabolic activation is an obligatory step in the initiation of many human cancers. CYP[3] isoenzymes play a major role in the oxidation of chemical compounds, often resulting in the formation of highly reactive compounds that are the ultimate carcinogens (9, 10). CYP1A1 may be involved in the initiation of lung cancer and other smoking related cancers because it is able to generate benzo[*a*]pyrene-derived mutagens in cigarette smoke (11–14). Two relevant genetic polymorphisms have been demonstrated in the *CYPIA1* gene: in the 3′ flanking region, a T to C substitution alters protein folding, whereas an Ile to Val substitution may occur in exon 7 (15). Both substitutions result in the enhancement of ethoxyresorufin-*O*-deethylase enzyme activity (16, 17) and may contribute to an altered susceptibility to chemical carcinogens.

Human cytosolic GSTs are a family of dimeric biotransformation enzymes comprised of the four main classes; α, μ, π, and θ (18). They catalyze the binding of a large variety of electrophiles to the sulfydryl group of glutathione, are involved in the detoxification of (oxygen) radicals, and have a main function in the binding and transport of a wide variety of harmful compounds. GSTs have a considerably important role in the detoxification of carcinogens (18). GSTs are present in many species and tissues and also in relatively large amounts in the epithelial tissues of the human gastrointestinal tract (19–21). In normal esophageal epithelium, GSTP1-1 is, by far, the most important GST isoform present, at least in a quantitative sense (21). A significant negative correlation was demonstrated between GST enzyme activity and tumor incidence in the mucosa along the human gastrointestinal tract, suggesting the importance of GSTs in tumor prevention (21). *GSTM1* and *GSTT1* null genotypes have been reported to enhance the risk of developing gastric, colorectal, or lung cancer (18, 22–26), although other studies did not show such a genetic predisposition (27–29). An Ile to Val substitution in the *GSTP1* gene (*GSTP1b* variant) was found more often in patients with bladder and testicular cancer (30).

Here, we studied the occurrence of genetic polymorphisms in the *CYPIA1*, *GSTP1*, *GSTM1*, and *GSTT1* genes in Caucasian controls and patients with Barrett's epithelium or esophageal adenocarcinoma or squamous cell carcinoma.

MATERIALS AND METHODS

Ninety-eight patients with Barrett's esophagus (all Caucasian; 61 males and 37 females; mean age ± SE, 61 ± 2 years; age range, 25–93 years) were included in this study during a 4-year period. Biopsies were collected from both normal esophageal (three biopsies) and Barrett's epithelium (three biopsies) at routine endoscopic inspection. Barrett's epithelia were of the intestinal, gastric-fundic, and junctional types in 95, 2, and 1 cases, respectively. Surgical specimens were obtained from patients with adenocarcinoma (all Caucasian; $n = 21$, 18 males and 3 females; mean age ± SE, 64 ± 2 years; age range, 43–74 years) and squamous cell carcinoma (all Caucasian; $n = 13$, 9 males and 4 females; mean age ± SE, 54 ± 3 years; age range, 32–70 years). In all cases, diagnosis was confirmed by a pathologist. All tissues were immediately frozen in liquid nitrogen and stored at −80°C until use. Blood samples were collected from 247 healthy volunteers (Caucasians; 98 males and 149 females; mean age ± SE, 52 ± 2 years; age range, 18–78 years) by venipuncture in sterile siliconized EDTA K3 (15%) 4-ml Vacutainer tubes (Becton Dickinson, San Jose, CA). Whole blood was stored at −20°C until use. The investigations were approved by the local ethical committee on human experimentation.

Genomic DNA was extracted from tissue using phenol, chloroform, and isoamylalcohol according to Maniatis *et al.* (31). Genomic DNA was isolated from whole blood using the Wizard genomic DNA purification kit, according to the instructions of the manufacturer (Promega, Madison, WI). Genetic

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[3] The abbreviations used are: CYP, cytochrome P450; GST, glutathione *S*-transferase.

polymorphisms were detected by PCR-RFLP. For detection of the null polymorphisms in *GSTM1* (primer set G2 and G3; see Ref. 29) and *GSTT1* (primer set T1 and T2; see Ref. 32), one primer was situated in the deleted area. For studying the genetic polymorphism in *GSTP1*, a primer set (105F and 105R) was designed so that the presence of the rare G allele (Val) resulted in the appearance of an *Alw*26I restriction enzyme site (30). Genetic polymorphism in exon 7 of *CYPIA1* was detected using the primer set CYPIA1/1 and CYPIA1/2, and the presence of the rare G allele (Val) resulted in loss of an *Nco*I restriction enzyme site (15). For the polymorphism in the 3′ flanking region of *CYPIA1*, primer set C44 and C47 was designed so that the presence of the rare C nucleotide resulted in the appearance of an *Msp*I restriction enzyme site (15). All primers were synthesized by Pharmacia Biotech (Roosendaal, the Netherlands). All chemicals needed for PCR were purchased from Promega.

Cytosolic fractions from normal squamous mucosa were prepared, and protein concentration and GST enzyme activity toward 1-chloro-2,4-dinitrobenzene were measured essentially as described previously (21).

The statistical significance of differences for individual and combined polymorphisms between different groups was tested with χ^2 analysis with Yates correction in 2×2 (*GSTM1* and *GSTT1*) or 2×3 (*GSTP1* and *CYPIA1*) contingency tables. Spearman rank correlation test was used for assessing the association between GST enzyme activity and GSTP1 polymorphism. Wilcoxon rank sum test was used to compare GST enzyme activity in the GSTP1a-1a group with that in the GSTP1a-1b and GSTP1b-1b groups, respectively.

RESULTS

Results of the analysis of genetic polymorphisms in *CYPIA1*, *GSTP1*, *GSTM1*, and *GSTT1* genes are presented in Table 1. Presence of rare alleles in exon 7 or the 3′ flanking region of *CYPIA1* was found equally often in patients with Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma, as compared to the control population. *GSTM1* and *GSTT1* null genotypes were found in 52 and 20% of controls, respectively, and these frequencies were not different from those in the patient populations studied. In controls, the above-mentioned polymorphisms did not differ between males and females.

The Ile to Val substitution in GSTP1 (GSTP1b variant) was found significantly more often in patients with Barrett's esophagus (59% heterozygous and 11% homozygous; $\chi^2 = 25.14$, $P < 0.0001$) and adenocarcinoma (71% heterozygous and 5% homozygous; $\chi^2 = 10.53$, $P = 0.005$) as compared to controls (36% heterozygous and 5% homozygous). No difference was found between controls and patients with squamous cell carcinoma or between patients with adenocarcinoma and Barrett's esophagus. Occurrence of the *GSTP1b* genotype was significantly correlated with GST enzyme activity in normal esophageal mucosa (Table 2), as studied by the Spearman rank correlation test: $R_s = -0.21$ (95% confidence interval, -0.38–0.03; $P = 0.02$). GST enzyme activity in normal esophagus of Ile/Val and Val/Val subjects (*GSTP1a-1b* and *GSTP1b-1b*, respectively) was significantly lower than the activities in normal esophageal tissue of *GSTP1a-1a* (wild-type) subjects (both $P < 0.05$, Wilcoxon rank sum test).

DISCUSSION

Individual susceptibility to gastrointestinal cancers may be partly due to genetic differences in the metabolic balance between activation and detoxification of (pro)carcinogens. The activation of procarcinogens to carcinogens is an obligatory step in initiation of human carcinogenesis (8–10). Nakajima *et al.* (33) demonstrated that carcinogens can be formed or inactivated in human esophageal epithelium by enzymatic activity of CYPIA1, GST α, GST μ, or GST π. Genetic polymorphisms in the 3′ flanking region or in exon 7 of CYPIA1, both leading to a more active enzyme, are found equally often in patients with Barrett's epithelium, adenocarcinoma, or squamous cell carcinoma of the esophagus as compared to controls. This points toward a lack of association of *CYPIA1* genotype and development of Barrett's esophagus or esophageal cancer. Previously, Murray *et al.* (34) found no difference in the expression levels of CYPIA1 protein between noncancer and cancerous tissues of the esophagus. Similar to our results, Morita *et al.* (35) did not find an association between *CYPIA1* genotype and squamous cell carcinoma of the esophagus (53 patients *versus* 132 controls) in the Japanese population. Nimura *et al.* (36) showed that the *CYPIA1*/exon 7 polymorphism was related to esophageal carcinoma in smokers only (89 patients *versus* 137 controls).

GSTs are involved in the detoxification of a wide variety of chemical carcinogens, and four main subclasses have been identified in humans (18). Approximately 50% of Caucasians lack a functional *GSTM1* gene (37, 38). Previous studies have shown that the *GSTM1* gene deletion is more common among patients with colorectal cancer (22, 24, 27, 39), squamous cell carcinoma of the lung (25), and other lung cancers (23), although results are conflicting in other studies on lung cancer (28, 29). The *GSTT1* null genotype has been correlated with increased risk of colorectal cancer (24, 26), although in another study (27), such a relationship was not found. In our study, the *GSTM1* and *GSTT1* null frequencies did not differ between patients and controls, which indicates that a lack of these enzymes is not crucial for development of Barrett's esophagus or esophageal carcinoma. This agrees well with data of Morita *et al.* (35), which showed no association between GSTM1 deletion and occurrence of squamous

Table 1 *Frequencies of polymorphisms in CYPIA1, GSTP1, GSTM1, and GSTT1 genes in controls and patients with Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma of the esophagus*[a]

Gene	Variant	Controls ($n = 247$)	Patients		
			Barrett's esophagus ($n = 98$)	Adenocarcinoma ($n = 21$)	Squamous cell carcinoma ($n = 13$)
CYPIA1 (exon 7)	Ile/Ile	207 (84)	78 (80)	18 (86)	8 (61)
	Ile/Val	37 (15)	16 (16)	3 (14)	5 (39)
	Val/Val	3 (1)	4 (4)	0 (0)	0 (0)
CYPIA1 (3′ flanking region)	T/T	207 (84)	85 (87)	14 (67)	8 (61)
	T/C	37 (15)	12 (12)	7 (33)	5 (39)
	C/C	3 (1)	1 (1)	0 (0)	0 (0)
GSTP1a-1a	Ile/Ile	146 (59)	29 (30)	5 (24)	5 (39)
GSTP1a-1b	Ile/Val	89 (36)	58 (59)[b]	15 (71)[c]	6 (46)
GSTP1b-1b	Val/Val	12 (5)	11 (11)	1 (5)	2 (15)
GSTM1	Deletion	128 (52)	43 (44)	12 (57)	5 (39)
GSTT1	Deletion	49 (20)	24 (25)	4 (19)	2 (15)

[a] Genetic polymorphisms were detected as described in "Materials and Methods." Percentages are given in parentheses. The statistical significance of differences between patient and control group was assessed in 2×2 (*GSTM1* and *GSTT1*) or 2×3 (*GSTP1* and *CYPIA1*) contingency tables with χ^2 analyses. *n*, number of patients or controls.
[b] $P < 0.0001$.
[c] $P = 0.005$.

Table 2 *Association between GSTP1 genotype and GST enzyme activity in normal esophageal mucosa from patients with Barrett's epithelium*[a]

GSTP1 genotype	GST enzyme activity (nmol/min · mg protein)
GSTP1a-1a	914 ± 32 (n = 29)
GSTP1a-1b	817 ± 21 (n = 58)[b]
GSTP1b-1b	780 ± 37 (n = 11)[b]

[a] *GSTP1* polymorphisms and GST enzyme activity were determined as described in "Materials and Methods." GST enzyme activity is expressed as mean ± SE. Wilcoxon rank sum test was used to compare GST enzyme activity of the *GSTP1a-1a* subgroup with that in the *GSTP1a-1b* and *GSTP1b-1b* subgroups, respectively. *n*, number of patients in each group.

[b] $P < 0.05$ as compared to enzyme activity in the *GSTP1a-1a* subgroup.

cell carcinoma of the esophagus. Nimura *et al.* (36), however, found an association between *GSTM1* null genotype and esophageal carcinoma in smokers. Overall, it may be concluded that *GSTM1* or *GSTT1* null individuals may be more susceptible to certain types of cancer, although lifestyle and dietary habits, without doubt, are of great importance in this respect.

A bp substitution at nucleotide 313 of the *GSTP1* gene (so-called *GSTP1b* variant) was found significantly more often in patients with Barrett's esophagus or esophageal adenocarcinoma, which may point toward a genetic predisposition to these diseases. No higher rates of *GSTP1b* alleles were found in patients with squamous cell carcinoma, which is in agreement with the fact that esophageal adenocarcinomas but not squamous cell carcinomas often develop from Barrett's epithelium. Harries *et al.* (30) demonstrated an association between occurrence of the GSTP1b polymorphism and susceptibility to bladder or testicular cancer, whereas no such association was observed in patients with breast or colon cancer. In the control population from the United Kingdom (n = 155), a very similar percentage of wild-type gene (*GSTP1a-1a*) was found, as compared to our Dutch controls (51 *versus* 59%, respectively).

In normal esophageal mucosa from patients with Barrett's esophagus, we were able to demonstrate that presence of a *GSTP1b* allele significantly correlated with a lower GST enzyme activity. A similar association was recently found in normal lung tissue from patients with lung cancer (40). By expressing the GSTP1b-1b variant in *Escherichia coli*, Ali Osman *et al.* (41) demonstrated a lower affinity for 1-chloro-2,4-dinitrobenzene but not for glutathione, resulting in a reduced V_{max} of this modified enzyme. In a quantitative sense, GSTP1-1 is by far the most important GST isoform in normal esophageal mucosa (21). The observed reduction in GST enzyme activity associated with the occurrence of GSTP1b variants in normal esophageal tissue of patients with Barrett's esophagus may imply that these patients have a decreased capacity to detoxify carcinogens, resulting in an increased risk for development of premalignant and malignant diseases.

In summary, we found no evidence for a possible genetic predisposition to Barrett's esophagus or esophageal carcinoma due to genetic polymorphisms in *CYPIA1*, *GSTM1*, or *GSTT1*. However, higher frequencies of the *GSTP1b* gene variant were present in patients with Barrett's esophagus and adenocarcinoma, with concomitant reduction of GST enzyme activity in normal esophageal epithelium. As a consequence of this partially deficient GST system in these patients, an impaired detoxification of carcinogens may exist, resulting in an enhanced cancer risk.

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