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ORIGINAL ARTICLE

Polymorphisms of glutathione S-transferase M1, T1 and P1 in patients with reflux esophagitis and Barrett's esophagus

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Abstract Reflux esophagitis (RE) and Barrett's esophagus (BE) belong to the most common esophageal complications of gastroesophageal reflux disease. Glutathione S-transferase (GST) enzymes play an important role in cellular protection against oxidative stress and toxic foreign chemicals. Therefore, we investigated the hypothesis that polymorphisms in genes for these detoxifying enzymes could influence susceptibility to RE and BE. GSTM1, GSTT1 and GSTP1 loci were analyzed by PCR-based methods in 64 patients with RE (and an additional group of 22 subjects with BE as the fourth grade of esophagitis) and 173 unrelated controls. There were no significant differences in the distributions of GSTM1 and GSTT1 genotypes between the controls and patients with RE or BE. Similarly, frequencies of GSTP1 alleles were non-significantly different between the control and RE groups. However, GSTP1 B allele carriers were more frequent among the patients with BE compared to those in the reflux esophagitis group ($P = 0.04$, OR = 2.10, 95% CI 0.99–4.44) and most significantly when compared to the controls ($P = 0.0067$, $P_{corr} < 0.05$, OR = 2.56, 95%CI 1.30–5.05). Although the GSTM1 and GSTT1 genes did not show any relationship with reflux disease, the GSTP1 gene might be one of the risk factors associated with susceptibility to RE, especially to BE.

Keywords GERD · GSTs · Glutathione S-transferase · Polymorphism · Reflux esophagitis · Gene · Barrett's esophagus

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Introduction

Gastroesophageal reflux disease (GERD) is defined as a failure of the antireflux barrier, allowing abnormal reflux of gastric contents into the esophagus. Profound knowledge of the ethiology of GERD is limited due to a scarcity of valid population-based data of sufficient statistical power. Furthermore, many intrinsic (TLESR, delayed gastric emptying, etc.) and extrinsic factors (environmental factors, such as tobacco smoking, alcohol drinking, coffee and tea consumption or regular use of non-steroidal anti-inflammatory drugs, etc.) exist. A population-based study comparing reflux symptoms in monozygotic and dizygotic twins showed that also genetic factors might contribute to the ethiology by 30% (Cameron et al. 2002).

Approximately 50% of patients with GERD develop esophagitis. The fourth grade of esophagitis includes strictures, ulcerations and Barrett's esophagus (BE). Patients with BE have a 30–125 times greater risk of developing adenocarcinoma of the esophagus (Hameeteman et al. 1989; Spechler et al. 1984; Pera et al. 1993). Recent studies reported that interactions between the environmental factors and genetic variations of enzymes involved in the detoxification of oxygen radicals through the binding and transport of harmful compounds may represent one of the possible mechanisms of the carcinogenic process (Fitzgerald 2005). These enzymes include glutathione S-

transferases (GSTs) that are involved in the conjugation of a wide range of electrophilic substances with glutathione, thus facilitating detoxification and further metabolisation and excretion. They can also metabolize tobacco-related carcinogens (Berhane et al. 1994). In addition, the relation between tobacco smoking and reflux symptoms was previously reported (Nilsson et al. 2004).

GSTs form a supergene family of enzymes involved in the phase II detoxification of toxins and enzymes (Brabender et al. 2002). GSTs comprise four main classes: A, M, P and T. They are present in many species and tissues and also in the epithelial tissues of the human gastrointestinal tract (Lieshout et al. 1999). Among them, GSTP1 enzyme is the most important form in the esophagus (Brabender et al. 2002). Previously, polymorphism in the GSTP1 gene on chromosome 11q13 was identified with six common phenotypes resulting from homo- and heterodimeric combinations of GSTP1*A, GSTP1*B and GSTP1*C (Ali-Osman et al. 1997). The transition changed codon 105 from ATC (Ile) in GSTP1*A to GTC (Val) in GSTP1*B and GSTP1*C and also codon 114 from GCG (Ala) to GTG (Val) in GSTP1*C. Both amino acid changes are in the electrophile-binding active site of the GSTP1 enzyme, and GSTP1-1 isoforms have been shown to possess different enzymatic activities (Hu et al. 1997; Watson et al. 1998; Zimniak et al. 1994). Decreased GSTP1 enzyme activity has been detected in BE, suggesting that these alterations may contribute to an increased cancer risk in this disease (Brabender et al. 2002).

The most important polymorphism encodes a partial gene deletion at the GSTM1 locus on chromosome 1p13.3 (GSTM1 null genotype) resulting in the complete absence of GSTM1 enzyme activity (Garte et al. 2001). At the GSTT1 locus on chromosome 22q11.2, the GSTT1 null genotype represents a partial gene deletion and is associated with the absence of functional activity of the GSTT1 enzyme. The frequency of the GSTM1 and GSTT1 null genotypes are approximately 50 and 20% in the Caucasians (Garte et al. 2001). Both null genotypes have been reported to enhance the risk of developing gastric, colorectal or lung cancer (Lai et al. 2005; Holley et al. 2006), probably due to a low ability to detoxify several xenobiotics and a decreased defense toward oxidative stress and free radical-mediated cellular damage.

In vivo studies have shown that also *Helicobacter pylori* causes oxidative damage and that *H. pylori* eradication attenuates oxidative stress in human gastric mucosa (Pignatelli et al. 2001). ROS (reactive oxygen species) are believed to be involved in inflammation, expression of oncogenes and cell proliferation. The GST activity was lower before the eradication of *H. pylori* compared to afterwards, and the GSH level was significantly higher after the eradication of this bacterium. This demonstrates the loss of a detoxification mechanism of GST by *H. pylori* infection (Wang et al. 2000). So, the GST polymorphisms, single and/or in combination, are associated with compromised antioxidant capacity, especially in the presence of *H. pylori* infection, and therefore may be considered an additional risk factor for cancers, determining interindividual differences in susceptibility.

Although several studies have been undertaken to examine the association between susceptibility to some type of cancer (Morita et al. 1998; Tan et al. 2000; Lee et al. 2000; De Bruin et al. 2000) and genetic polymorphism in GSTs, there are limited data on their association with reflux esophagitis (Liu et al. 2006). The aim of this study was to analyze associations of the GST polymorphisms with reflux esophagitis and BE, and their interactions with smoking status and *H. pylori* infection in influencing the susceptibility to develop these complications of GERD in the Czech population.

Methods

Study subjects

A total of 259 unrelated, Caucasian people of Czech nationality were enrolled in the study. The investigated population included 64 patients with reflux esophagitis and an additional group of 22 individuals with BE as the fourth grade of esophagitis, all of them recruited from the Department of Gastroenterology, and an independent group of 173 healthy controls from the same geographical region selected according to age, gender and smoking status (Table 1).

The diagnosis of GERD was based on the clinical symptoms such as heartburn and/or acid regurgitation. To objectify the diagnosis, we performed 24-h pH-metry, esophagogastroduodenoscopy and esophageal manometry (Labenz and Malfertheiner 2005; Kroupa et al. 2006). Prolonged 24-h pH monitoring was designed to quantify the actual time the esophageal mucosa was being exposed to gastric juices by placing a pH probe 5 cm above the upper border of the distal sphincter for 24 h. The electrode was positioned 5 cm above the upper border of the LES. The pH signal was recorded in a digital data logger. The patients were instructed to fill in diary cards regarding the time of the meals, recumbent time and the exact time of symptoms experienced during the 24-h study. No restrictions were imposed on food intake or smoking behavior. After 24 h stored data were retrieved from the data logger by a personal computer and analyzed automatically. Our monitoring revealed a De Meester score of 41.0 ± 23.4 for the GERD patients and 38.0 ± 17.6 for the patients with BE. Esophageal manometry was used to measure the LES

Table 1 Clinical characteristics of the studied subjects

Characteristics[a]	Controls (n = 173)	Patients with RE (n = 64)	Patients with BE (n = 22)
Age (years ± SD)	44.7 ± 10.7	46.0 ± 13.6	46.4 ± 12.7
Sex (males/females) (%)	62/38	64/36	82/18
Smoking no/yes (%)	72/28	82/18	89/11
The prevalence of *H. pylori* infection	ND	23%	45%

RE reflux esophagitis; *BE* Barrett's esophagus, *ND* not determined

[a] Data are expressed as means ± SD unless stated otherwise

pressure by using a six-channel water-perfused catheter in University Hospital Brno-Bohunice. The LES serves as a valve to prevent reflux of gastric acid into the esophagus. The LES pressure for GERD patients was 13.5 mmHg ± 4.5 and 13.7 mmHg ± 4.2 for BE. Esophagogastroduodenoscopy was used for searching for the presence of macroscopic signs of esophagitis. We used the Los Angeles classification system for the endoscopic assessment of reflux esophagitis (Armstrong et al. 1996). The diagnosis of BE also had to be proven by the histological finding. *H. pylori* infection was determined by histology. Briefly, two gastric biopsies were done routinely, the first from the gastric corpus and the second from the gastric antrum. All were fixed, routinely processed and stained with Warthin–Starry. Simultaneously, the rapid urease test was performed.

The control group consisted of 173 people randomly recruited from general practitioners' registries in Brno and Breclav. The people had no history and/or clinical symptoms of any gastrointestinal disease, cardiac disorders, hypertension or diabetes and were on no medication.

The study was performed with the approval of the Ethical Committees of the Medical Faculty and University Hospital in Brno.

DNA isolation

Genomic DNA for PCR was isolated from peripheral blood leukocytes by the conventional method using proteinase K digestion of cells.

Genetic polymorphism analysis of the GSTM1 and GSTT1 genes

The genetic polymorphism analysis for the GSTM1 and the GSTT1 genes was performed simultaneously in a single assay using the multiplex PCR approach as reported previously (Abdel-Rahman et al. 1996) with a small modification. Briefly, isolated DNA was amplified in a 25-µl reaction mixture containing 30 pmol of each of the following GSTM1 primers: 5′-GAACTCCCTGAAAAGCT AAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTG-3′; and the following GSTT1 primers: 5′-TTCCTTACTGGTC CTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAG-CA-3′. As an internal control exon 7 of the CYP1A1 gene was co-amplified using the primers 5′-GAACTGCC ACTTCAGCTGTCT-3′ and 5′-CAGCTGCATTTGGAA GTGCTC-3′ in the presence of 200 µmol dNTPs, 5 µl of 10× PCR buffer (10× 500 mM KCl, 100 mM Tris–HCl, pH 9.0) and 1.5 MM $MgCl_2$ and 2 U Taq polymerase.

The PCR conditions included the initial melting temperature of 94°C (2 min) followed by 35 cycles of melting (94°C, 2 min), annealing (59°C, 1 min) and extension (72°C, 1 min). The final extension step (72°C) of 10 min terminated the process. The PCR products from co-amplification of GSTT1, GSTM1 and CYP1A1 genes were then analyzed electrophoretically on an ethidium bromide-stained 2% agarose gel. The presence or absence of GSTM1 and GSTT1 genes was detected by the presence or absence of a band at 480 bp (corresponding to GSTT1) and a band at 215 bp (corresponding to GSTM1). A band at 312 bp (corresponding to CYP1A1 gene) was always present and was used as an internal control to document successful PCR amplification.

Genetic polymorphism analysis of the GSTP1 gene

To detect GSTP1 genotype variants, an assay was applied that used both forward and reverse allele-specific primers enabling identification of *cis/trans* orientation (''PCR haplotyping'') as described previously (Marshall et al. 2000). All reaction mixes included control primers in order to verify successful amplification. Primers and reaction mix composition are detailed in Table 2. Briefly, cycling parameters were as follows: 1 min at 96°C followed by five cycles of 96°C for 25 s, 70°C for 45 s and 72°C for 45 s, followed by 21 cycles of 96°C for 25 s, 65°C for 50 s and 72°C for 45 s, followed by four cycles of 96°C for 25 s, 55°C for 60 s and 72°C for 120 s. PCR products were then electrophoresed on 2% agarose gels containing ethidium bromide and visualized with UV light.

All samples were successfully genotyped and to verify and confirm the results; genotyping was repeated on 10% randomly selected DNA samples for each of the methods.

Table 2 Polymerase chain reaction-sequence primers (PCR-SSP)

	Sense primer	Concentration (μmol/l)	Antisense primer	Concentration (μmol/l)	Product size (bp)
GSTP1105 Ile-114 Ala (GSTP1_A)	5′-ggACCTCCgCTgCAAATACA-3′	0.6	5′-CACATAgTCATCCTTgCCCg -3′	0.6	928
GSTP1105 Val-114 Ala (GSTP1_B)	5′-ggACCTCCgCTgCAAATACg-3′	0.6	5′-CACATAgTCATCCTTgCCCg -3′	0.6	928
GSTP1105 Val-114 Val (GSTP1_C)	5′-ggACCTCCgCTgCAAATACg-3′	0.6	5′-CACATAgTCATCCTTgCCCA -3′	0.6	928
GSTP1105 Ile-114 Val (GSTP1_D)	5′-ggACCTCCgCTgCAAATACA-3′	0.6	5′-CACATAgTCATCCTTgCCCA-3′	0.6	928
GSTP1 reactions also include consensus primers for human growth hormone	5′-gCCTTCCCAACCATTCCCTT-3′	0.4	5′-TCACggATTTCTgTTgTgTTTC-3′	0.4	426

Statistical analysis

Comparisons were made between allelic (gene) and genotype frequencies in the disease and control populations. The allele frequencies were calculated from the observed numbers of genotypes. The significance of differences in the allelic frequencies between each of the two groups was determined by Fisher's exact test. χ^2 analysis was used to test for deviation of genotype distribution from Hardy–Weinberg equilibrium and for comparison of differences in genotype combinations among groups. Correction for possible multiple testing errors was performed using Holm's procedure (Holms 1979). Contingency table analysis, odds ratio (OR), 95% confidence intervals and significance values were estimated with the use of the program package Statistica v 6.0 (Statsoft Inc., Tulsa, OK).

Computer package EpiInfo6 (available from the Centers for Disease Control and Prevention, USA (http://www.cdc.gov/epo/epi/epiinfo.htm) was used to estimate the statistical power to detect significant differences between the study groups. Power calculation estimated the sample size of approximately 232 subjects (the ratio of cases to controls was 1:3) as sufficient to achieve 80% power for detecting difference in allele frequencies of all polymorphisms with OR = 2.67 and significance level $\alpha = 0.05$. Power calculation was performed using allele frequencies as previously published by Izakovicova Holla et al. (2006) (for GSTM1 and GSTT1 variants) and Marshall et al. (2000) (for GSTP1 polymorphisms).

Results

All subjects were Caucasians of exclusively Czech nationality. The characteristics of 259 subjects are given in Table 1. The mean age ± SD of patients and controls was similar. There were more males than females in all groups. The data for smoking habits was available in 51 patients with reflux esophagitis (RE) and 16 patients with BE and 162 controls; 16% patients and 28% controls were cigarette smokers ($P < 0.01$). The prevalence of *H. pylori* infection was higher in patients with BE (45%) in comparison with patients with reflux esophagitis (23%). Unfortunately, healthy controls were not examined for *H. pylori* infection.

The distributions of the three GST genotypes in controls and patients with GERD are shown in Table 3. All groups were in Hardy–Weinberg equilibrium with non-significant χ^2 values comparing the observed and expected genotype frequencies of each of the tested polymorphisms. GSTM1 and GSTT1 null genotypes were found in 52.6 and 23.7% of controls, respectively, and these frequencies were not different from those in both patient groups (59.4 and 28.1% in RE and 36.4 and 31.8% in BE, respectively). To

Table 3 GSTs genotype frequencies in patients with reflux esophagitis (RE), Barrett's esophagus (BE) and control subjects

Genotypes	Controls (N = 173)	Patients with RE (N = 64)	Patients with BE (N = 22)	OR (95% CI) vs. RE	OR (95% CI) vs. BE
GSTM1	*N* (%)	*N* (%)	*N* (%)		
Wild	82 (47.4)	26 (40.6)	14 (63.6)	1.0	1.0
Null	91 (52.6)	38 (59.4)	8 (36.4)	1.32 (0.74 – 2.36)	0.51 (0.21 – 1.29)
GSTT1	N (%)	N (%)	N (%)		
Wild	132 (76.3)	46 (71.9)	15 (68.2)	1.0	1.0
Null	41 (23.7)	18 (28.1)	7 (31.8)	1.26 (0.66 – 2.41)	1.50 (0.57 – 3.94)
GSTM1/GSTT1	N (%)	N (%)	N (%)		
Both wild	62 (35.8)	18 (28.1)	10 (45.5)	1.0	1.0
Either null	90 (52.0)	36 (56.3)	9 (40.9)	1.38 (0.72 – 2.64)	0.62 (0.24 – 1.61)
Both null	21 (12.1)	10 (15.6)	3 (13.6)	1.64 (0.64 – 4.11)	0.89 (0.22 – 3.53)
GSTP1	N (%)	N (%)	N (%)		
*A/*A	92 (52.3)	32 (50.0)	5 (22.7)	1.0	1.0
*A/*B	52 (29.5)	19 (29.7)	12 (54.5)	1.05 (0.54 – 2.04)	4.25 (1.42 – 12.72)
*A/*C	17 (9.7)	6 (9.4)	2 (9.1)	1.01 (0.37 – 2.80)	–[a]
*B/*B	4 (2.3)	4 (6.2)	1 (4.5)	2.88 (0.68 – 12.17)	–[a]
*B/*C	7 (4.0)	2 (3.1)	2 (9.1)	–[a]	–[a]
*C/*C	1 (0.6)	1 (1.6)	0 (0.0)	–[a]	–[a]
B allele	67 (19.4)	29 (22.6)	16 (36.4)	1.22 (0.75 – 2.00)	2.56 (1.30 – 5.05)
Non-B allele	279 (80.6)	99 (77.3)	26 (59.1)	1.0	1.0

Wild genotype: patients homozygous or heterozygous for the GSTM1 or GSTT1 functional allele

OR odds ratio [reference group (healthy controls) designated with an OR of 1.0]; *CI* confidence interval

[a] OR (and 95% CI) were not calculated for small numbers of cases in analysis

investigate whether the profile of GSTM1/T1 genotypes might be associated with the risk of GERD, we examined both genotypes in combination. The distributions of the null genotypes of both GSTM1 and GSTT1 in patients with RE (15.6%) or patients with BE (13.6%) were not significantly different from that of the control group (12.1%) (Table 3).

The genotype and allelic frequencies of GSTP1 in the control population were determined in 173 individuals. The observed frequencies of GSTP1*A, GSTP1*B and GSTP1*C alleles were 73.1, 19.4 and 7.5%, respectively. In 64 patients with reflux esophagitis, the corresponding frequencies of GSTP1 alleles were 69.6, 22.6 and 7.8%, respectively. No significant difference in allelic frequencies was observed between both groups (P = 0.25 for comparison of B allele vs. non-B allele). However, the frequency of B allele was significantly higher in the patients with BE when compared with the controls (P = 0.0067, $P_{corr} < 0.05$) and marginally when compared with the patients with RE (P = 0.04, $P_{corr} > 0.05$). The risk of BE (OR = 2.56, 95% CI 1.30-5.05) was 2.5-fold higher for the GSTP1 *B allele carriers compared with the subjects with the GSTP1 *A or *C alleles. This difference was caused mainly by the effect of nucleotide exchange A → G resulting in a protein with an amino acid substitution (valine instead of isoleucine at codone 105) with a lower catalytic activity (Watson et al. 1998; Zimniak et al. 1994). Results of the analysis of GSTP1 genotypes in both case groups and controls are also summarized in Table 3.

Furthermore, because smoking is one of the known risk factors for GERD (Nilsson et al. 2004), we analyzed all polymorphisms separately in smoking and non-smoking subjects. There was no significant difference in the genotype frequencies of the GST variants between smoking patients with reflux esophagitis, BE and control smokers, similarly as between non-smoking patients and non-smoking controls (data not shown). In addition, the frequencies of GST polymorphisms were not significantly different in subgroups of *H. pylori* positive and negative patients (data not shown).

Discussion

Gastroesophageal reflux disease is one of the most important and frequent gastrointestinal disorders of the

Western world, and may lead to esophageal cancer (Pettit 2005). Recent epidemiologic and biochemical studies have led to the conclusion that polymorphic GSTs are important enzymes in the metabolism and induction of numerous known or suspected endogenous and exogenous compounds (Wormhoudt et al. 1999). GSTs, the first enzymes in the mercapturic acid pathway, catalyze the nucleophilic addition of the thiol of GSH to many possibly harmful compounds, and this is important for detoxification of xenobiotics and for protection of several tissues from oxidative damage. Carcinogenesis is a highly complex multifactorial process, and oxidative damage of the esophagus together with its chronic inflammation in patients with RE may be risk factors for the development of BE (Sarr et al. 1985). Barrett's metaplasia is a major risk factor for esophageal adenocarcinoma development. Peters et al. (1993), Compton and colleagues (1999) and very recently Herszenyi et al. (2007) demonstrated that Barrett's metaplasia has significantly less GSTP enzyme activity and content than the normal esophageal tissue. Lower activity of GSTs that may increase the potential for the mucosa to accumulate genetic alterations should be evident, also in first stages of this disease process, thus in patients with RE.

On the basis of these facts, the role of GST polymorphisms in reflux esophagitis has been hypothesized. The results of the present study indicate that the polymorphic GSTM1 and GSTT1 genes do not have a major effect on the occurrence of reflux esophagitis but, according to our data, allelic variants of the polymorphic GSTP1 gene may be involved in genetic susceptibility to reflux esophagitis, especially to BE. The carriers of the GSTP1*B allele conducted probably more than 2.5-fold higher risk of developing BE; GSTP1*A allele had an opposite effect, protective against the disease. Our results are in accordance with previous findings confirming that although a lack of GSTM1 or GSTT1 enzymes is not crucial for development of BE (Lieshout et al. 1999), GSTP1B allele was found significantly more often in patients with BE (Lieshout et al. 1999), which may point toward a genetic predisposition to this disease. The mechanism by which the GSTP B allele may exert an effect on BE susceptibility is unclear, but a number of in vitro studies provide the evidence that these alleles exhibit functionally different enzyme activity. The amino acid difference from isoleucine (in GSTP1*A) to valine (in GSTP1*B and GSTP1*C) causes steric changes in the enzyme binding site, resulting in different substrate specificity and heat stability in vitro (Zimniak et al. 1994) and in vivo (Watson et al. 1998). Allelic variants of the GSTP1 differ significantly in their ability to catalyze the GSH conjugation, and hence detoxification of the ultimate carcinogenic metabolites (diol epoxides) of polycyclic aromatic hydrocarbons (Hu et al. 1997, 1998a, b), which are suspected human carcinogens, widespread in the environment. This is supported by findings that the catalytic efficiencies of the GSTP1 variants with valine in position 105 in the GSH conjugation of chlorambucil were lower by approximately 90% than that of the GSTP1*A isoform (Pandya et al. 2000) and also significantly less efficient in catalyzing the GSH conjugation of thiotepa, another clinically relevant anticancer agent (Srivastava et al. 1999). In addition, variability of the glutathione S-transferase genes can also play a role in reaction of tissues to oxidative stress. Animal and human studies of gastroesophageal reflux suggest that oxidative damage contributes to esophageal carcinogenesis (Wetscher et al. 1995, 1997). One postulated mechanism is that the inflammatory responses in the esophagus, mainly those involving neutrophils, are a major source of ROS (Olyaee et al. 1995). Under normal circumstances, cellular redox balance is maintained by enzymatic systems that ensure the prevalence of the overall reducing conditions. A shift to a prooxidant state, caused by a lower efficacy of the appropriate enzymes (given inter alia genetically), can cause a more serious damage of tissues. The increased frequency of a less effective allele of the GSTP1 gene in the patients with BE in our study, together with the observed reduction in GST enzyme activity associated with the occurrence of the same variant in esophageal tissue of the patients with BE (Lieshout et al. 1999) may imply that these patients have a decreased capacity to detoxify carcinogens and products of ROS, resulting in an increased risk for development of premalignant and malignant diseases.

Our results, however, are in disagreement with those of a previous study that suggested association between GSTP1 variants (alone or in interaction with *H. pylori* infection) and susceptibility to reflux esophagitis in the China population (Liu et al. 2006). In our study, patients with GSTP1*B allele had no significantly increased risk for development of reflux esophagitis alone. Gene frequencies are different among both racial groups (frequencies of the GSTP1*B allele in patients with RE were 40% in China vs. 23% in Czech patients), which may explain the differences in association between gene polymorphisms and disease in different studies. However, although the prevalence of RE seems to be increasing in China, the occurrence of Barrett's epithelium and esophageal adenocarcinoma in Chinese population is much lower than in Western countries (Wang et al. 2003). The prevalence of *H. pylori* infection in the RE group in China was highest in patients with the lowest grade of inflammation (A-grade according to Los Angeles classification). This is in contrast with our study, where this pathogen was present twice more frequently in patients with BE than in the patients with RE, which is in compliance with a very recent study of Johansson et al. (2007) suggesting a synergetic effect of colonization of *H. pylori* and reflux for the development of BE.

The GSTP1 gene appears to be particularly susceptible to carcinogens from cigarette smoking, and tobacco habit is a risk factor for reflux symptoms (Nilsson et al. 2004). If the mode of action is through the activation and detoxification of tobacco carcinogens, one might expect the relationship between polymorphisms and risk to be stronger among smokers. However, in our study no interactions were identified between the GSTs variants and cigarette smoking.

Searching for genetic contributions to complex conditions such as reflux esophagitis is difficult due to genetic heterogeneity, incomplete penetrance, gene–gene and gene–environmental interactions (Belmont and Leal 2005). Although multiple genetic factors, including the polymorphisms of enzymes involved in detoxification of xenobiotics and in protection of several tissues from oxidative damage, should be simultaneously considered to understand the entire picture of GERD susceptibility, the present study suggests that the GSTP1 gene polymorphisms can act as one of the genetic factors that may participate in the complex process of esophagitis in our patients and, probably, in the development of a severe form of this disease, such as BE. However, indications that the GSTP1 gene might serve as a marker of a more severe grade of esophagitis, BE, in predisposed subjects certainly deserve additional attention and exploration.

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