Genetic polymorphisms of glutathione S-transferases and disease activity of rheumatoid arthritis

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Abstract

Objectives

Glutathione S-transferases (GST); GST-mu1 (GSTM1), GST-pi1 (GSTP1) and GST-theta1 (GSTT1) have peroxidase activity towards cytotoxic metabolites produced in inflammatory reactions, the main feature of rheumatoid arthritis (RA). Genetic polymorphisms in GSTM1, GSTP1 and GSTT1 modify the enzyme conjugation capacity and may be associated with the activity of RA.

Methods

A genotyping approach was used to analyze GSTM1-0, GSTT1-0 and GSTP1 Ile105Val and Ala114Val polymorphisms in 213 RA patients. Disease activity was assessed by the disease activity score of 28 joint counts (DAS28) twice for each patient and mean DAS28 values were calculated.

Results

The patients with GSTT1-0 genotype had a higher risk for developing high activity RA than the patients with GSTT1 genes present (p=0.028, OR=2.761, 95% CI=1.114-6.843). An interaction between the GSTT1 polymorphism and smoking was observed. In the group of smokers, the carriers of a homozygous deletion GSTT1 had an 8.5-fold higher risk for developing high disease activity than the patients with the GSTT1-1 genotype (p=0.004, OR=8.640, 95% CI=1.995-37.426). GSTM1 and GSTP1 polymorphisms were not associated with the disease activity.

Conclusions

Our results suggest that the presence of the GSTT1-0 genotype contributed to higher disease activity in RA patients. The risk for developing highly active RA was the highest in smokers with the GSTT1-0 genotype.

Key words

Disease activity, GST polymorphism, rheumatoid arthritis, smoking.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by mostly polyarticular inflammation, increased cytokine production and pannus development, which subsequently lead to the erosion of the cartilage and underlying bone. Reactive oxygen species (ROS) are involved in RA pathology, since they are generated by neutrophils, monocytes and macrophages in synovial fluid of inflamed joints and cause DNA and lipid oxidation leading to cartilage and bone destruction. The defence mechanism against ROS is complex and involves several enzymes including glutathione S-transferases (GST), a large family of isoenzymes that catalyse conjugation reactions of reduced glutathione with several endogenous and exogenous compounds. GST-mu1 (GSTM1), GST-pi1 (GSTP1) and GST-theta1 (GSTT1) isoenzyme members of the GST family have a peroxidase activity towards cytotoxic secondary metabolites, such as base propenals and organic hydroperoxides and thus have an important role in cellular protection against ROS (1).

Several polymorphisms in these genes have been described; some of them alter enzymatic activity and may modify the ability for the elimination of ROS products (2-4). The most common GSTM1 polymorphism is a gene deletion; approximately half of Caucasians are homozygous for the deletion and hence lack the protein function (3). A homozygous gene deletion that leads to enzyme deficiency has also been reported in GSTT1 and occurs approximately in 20% of healthy Caucasians (5). In GSTP1 two frequent single nucleotide polymorphisms (SNPs) resulting in an amino acid change have been reported. The A313G polymorphism results in Ile to Val change at position 105 (Ile105Val) close to the active site and decreases the enzymatic activity. The functional role of the C341T polymorphism (Ala114Val) is not clear but enzyme with both polymorphisms present had reduced conjugation capacity (6-8). GSTM1, GSTP1 and/or GSTT1 polymorphisms have been associated with increased susceptibility to RA (9-11) as well as to other autoimmune and inflammatory diseases, such as type 1 diabetes (12), asthma (13) and different types of cancer (14-18), especially smoking-related ones (19-22). To our knowledge, only one study investigated the association between GSTM1, GSTT1 and GSTP1 genotypes and disease activity but observed no significant association (9).

Cigarette smoking is another risk factor for RA and has been associated with both susceptibility and severity of the disease (23-25) and also with disease activity (26). Tobacco smoke promotes the generation of ROS. In individuals with limited elimination of ROS products due to GSTM1, GSTP1 and GSTT1 polymorphisms, smoking may have an even greater impact on the disease activity. Indeed, a previous study demonstrated worse radiographic and functional outcome in smokers with GSTM1-0 genotype (27). However, to this date the interaction between smoking and GST polymorphisms and their association with RA disease activity have not been studied yet.

In the present study, we investigated the association of genetic polymorphisms of GSTM1, GSTP1 and GSTT1 with the activity of RA and their interaction with smoking. We report new data on the interaction between the GSTT1-0 genotype and smoking and their association with the disease activity.

Materials and methods

Patients

The study population consisted of a group of 213 unrelated RA patients, 26 male (12.1%) and 188 female (87.9%), median age 60.5 (range 51-69) years. All patients were Central European Caucasian and fulfilled the American College of Rheumatology (ACR) 1987 criteria for the diagnosis (28). Patients were treated at the Department of Rheumatology, University Medical Centre, Ljubljana, Slovenia. Current or past treatment with methotrexate (MTX) was the main criterion for the inclusion into the study. At the entry in the study 156 out of 213 patients were treated with MTX (99 on MTX monotherapy), while the remaining 57 patients discontinued the MTX treatment due to inefficacy and/or toxicity and were treated with

other disease modifying antirheumatic drugs [leflunomide (14), cyclosporine A (six), chloroquine (five), injectable gold (five), sulphasalasine (four), azathioprine (two), mycophenolate mofetil (one), tacrolimus (one), cyclophosphamide (one), sulphasalasine plus chloroquine (one), leflunomide plus sulphasalasine (one), corticosteroids only (seven) or biologics: infliximab (one), etanercept (two), adalimumab (six)]. Cigarette smoking status was obtained for each patient by a questionnaire. All patients gave their written informed consent. The study was approved by the Slovenian Ethics Committee for Research in Medicine and was carried out according to the Helsinki Declaration.

Clinical assessments and laboratory measurements

Based on the examination of the literature we followed the ACR recommendations that erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are one of the core set measures of disease activity in RA clinical trials (29). ESR (mm/h) and CRP (mg/L) were measured twice within three months time. The disease activity score of 28 joint count (DAS28) was calculated for each patient using well known calculations, which included the number of tender joints (TJC), the number of swollen joints (SJC), ESR and patients' global assessment of general health expressed on visual analogue scale (VAS 0-100 mm) (30). To evaluate the disease activity over time two DAS28 scores of two consecutive visits within three months time (DAS1- at the inclusion in the study and DAS2- at month 3 of follow-up) were obtained and mean DAS28 values were calculated for each patient. Patients with mean DAS28 in range between 2.6 and less than 5.1 were classified into the low or intermediate activity group, while patients with mean DAS28 values 5.1 and more were classified in the high activity group. The DAS28 cut off point for remission of RA was set at DAS28<2.6 as defined by American Rheumatism Association (ARA) (31). Patients with DAS28<2.6 were also included into the low disease activity group due to some disagreement of various investigators about the validity of DAS28 for assessing remission (32). Functional disability of each patient was measured at the entry into the study using the Health Assessment Questionnaire (HAQ-DI-Stanford version) (33, 34).

All other patients' data, such as rheumatoid factor (RF), determined by Waaler-Rose and Latex agglutination methods (35), at least twice during the course of the disease, erosive changes of hands and feet observed on x-rays, duration of the disease and drug therapy were collected from patients' files.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using a standard salting-out procedure (36). Multiplex PCR using β-globin gene primers as an internal control was performed to determine GSTM1 and GSTT1 genotypes (37). PCR products were visualised on a 2% agarose gel stained with ethidium bromide. Custom TaqMan SNP genotyping assays (ABI, Foster City, California, USA) were used to determine both GSTP1 polymorphisms. Primer and probe sets were designed for GSTP1 Ile105Val (F: 5'CGGCGTGGAGGACCTC3', R: 5'CAGATGCTCACATAGTTGGTGTAGA3'; 105Ile probe FAM-CTGCAAATACATCTCCCT, 105Val probe VIC-CTGCAAATACGTCTCCC) and for GSTP1 Ala114Val polymorphism (F: 5'GGAGGGATGAGAGTAGGATGATACA-3', R: 5'-GGCAGTGCCTTCACATAGTCAT3'; 114Ala probe FAM-CTTGCCCGCCTCCT, 114Val probe VIC-CTTGCCCACCTCCT). Real time PCR was performed under universal conditions on the ABI 7900HT in a 5 μL reaction mix containing 0.125 μL of TaqMan SNP genotyping assay (ABI, Foster City, California, USA), 2.5 μL of TaqMan Universal PCR Master Mix (ABI, Foster City, California, USA) and 120 ng of DNA. Prior to GSTP1 genotyping TaqMan SNP genotyping assays were validated on 100 samples with a known genotype for each polymorphism.

Statistical analysis

Kruskal-Wallis test and Chi-square test were used to compare median values of ESR, CRP, HAQ, number of swollen joints, number of tender joints, VAS, disease and MTX treatment duration and the frequencies of RF seropositivity, patients with bone erosions and smokers between the disease activity groups. Binary logistic regression analysis with the addition of independent variables, such as sex, disease duration, MTX treatment duration, the presence or absence of erosions and RF seropositivity was used to express the risk for developing high disease activity as an odds ratio (OR) with 95% confidence intervals (CI). Multiple linear regression analysis with the same adjustment was used to determine the association of genetic polymorphisms with mean DAS28 values. Regression coefficient was given by B value with standard error (SE). The level of significance was set to 0.05. All statistical analyses were done using SPSS for Windows version 13.0.1 software (Statistical Package for the Social Sciences, Chicago, IL).

Results

Demographic and clinical data of our study population patients are shown in Table I. Patients were divided into two groups of disease activity according to mean DAS28 values; into a high disease activity group (DAS28 ≥5.1) and a low or intermediate disease activity group (DAS28<5.1). Mean DAS28 score of two consecutive visits within three months time was calculated with the mean variation of 0.186±0.684 between the patients. DAS28 values were normally distributed, while ESR, CRP, HAQ, SJC, TJC, VAS, disease and MTX treatment duration values were not, hence, their averages are presented by median values with range of p25-p75. When included in the statistical analyses variables were transformed to obtain normal distribution. Median ESR, CRP, SJC, TJC, VAS and HAQ values were significantly different between the disease activity groups ($p \leq 0.002$), while no significant differences in median values of disease duration and in the presence or absence of erosions and RF seropositivity between the disease activity groups were observed. The duration of MTX treatment was significantly different between

Table I. Baseline characteristics of RA patients divided in the disease activity groups.

Variable	All	Disease activity n (%)	
		DAS28 ≥5.1	DAS28 <5.1
n (%)	213 (100)	33 (15.5)	180 (84.5)
Sex: male	25 (11.7)	1 (3.0)	24 (13.3)
female	188 (88.3)	32 (97.0)	156 (86.7)
Median age [years] (range)	61 (51-69)	57 (51-67)	61.5 (51-70)
Median disease duration [months] (range)	69 (40.5-137.5)	97 (48-144)	67.5 (39-135)
Median MTX treatment duration* [months] (range)	32 (13-57.5)	17.5 (8-56.3)	35.5 (14.3-57.5)
MTX treatment discontinued	57 (26.8)	25 (75.8)	32 (17.8)
Mean DAS28 (SD)**	4.1 (0.9)	5.5 (0.4)	3.8 (0.7)
Median ESR [mm/h]§ (range)	24 (15-34)	41 (25-55)	23 (13.3-32)
Median CRP [mg/L]§ (range)	6 (5-12)	11 (7-22)	6 (5-11.8)
Median no. tender joints§ (range)	2 (1-4)	5 (4-7)	2 (1-4)
Median no. swollen joints§ (range)	4 (2-6)	8 (5-11)	3 (1-5)
Median VAS§ (range)	35 (24-50)	64 (54-67)	33 (22-44)
No. of patients with joint erosions	169 (79.3)	30 (90.9)	139 (77.2)
Median HAQ§ (range)	1.125 (0.800-1.500)	1.375 (1.000-2.000)	1.100 (0.800-1.500)
RF seropositivity	159 (74.6)	28 (84.8)	131 (72.8)
Smoking status, Non-smokers	122 (57.3)	16 (48.5)	106 (58.9)
Smokers: Past	59 (27.7)	13 (39.4)	46 (25.6)
Current	32 (15.0)	4 (12.1)	28 (15.6)

*p=0.036; **Mean DAS28 was calculated of DAS28 scores of two consecutive visits within three months; §$p \leq 0.002$; p-values were obtained using non-parametric Kruskal-Wallis test.

disease activity groups (p= 0.036) and was included as a covariate in the further statistical analyses. The duration of MTX treatment was the shortest in the high activity group, as 25 out of 33 patients from this group discontinued MTX treatment due to inefficacy and/or toxicity after a median of 15 months (8-52.5). Smoking status was not associated with the disease activity (DAS28 or with the risk for high disease activity), not even when only the patients with RF positivity were included in the analyses (data not shown).

The frequencies of GSTM1, GSTP1 and GSTT1 genotypes are presented in Table II. Since the genotyping approach for GSTM1 and GSTT1 did not allow us to detect heterozygous carriers of GSTM1 or GSTT1 gene deletions, the group GSTM1-0 or GSTT1-0 genotype included only patients homozygous for a gene deletion and the group of GSTM1-1 and GSTT1-1 genotype included patients homozygous or heterozygous for the functional gene. The observed frequencies of GSTM1-0 and GSTT1-0 genotype in RA patients (57.7% and 19.7%, respectively) were similar to the frequencies reported for the healthy Slovenian (38) and other Caucasian populations (39). Three GSTP1 alleles (**A*, **B* and **C*) were detected based on the presence of the polymorphisms in codons 105 and 114 giving rise to five different GSTP1 genotypes. For statistical analysis these genotypes were combined according to the presumed conjugation capacity of each particular allele. The group with presumed high conjugation capacity included patients with GSTP1**A*/**A* genotype. Patients with GSTP1**A*/**B* and/or **A*/**C* genotypes were included in the group with presumed intermediate conjugation capacity, while patients with GSTP1**B*/**B* and/or **B*/**C* were classified in the group with presumed low conjugation capacity. No patient with GSTP1**C*/**C* genotype (105 Val/Val and 114 Val/Val) was identified. The frequencies of GSTP1 genotypes in RA patients were not significantly different from the frequencies in a healthy Slovenian population (38).

In our study we investigated the association of polymorphic GST genotypes with the disease activity. We tested if there is an association of GSTM1, GSTT1 and GSTP1 genotypes with mean DAS28 scores and the risk for developing high RA activity. None of the studied GST polymorphisms was significantly associated with the mean DAS28 values. As DAS28 consists of ESR, SJC, TJC and VAS, we also tested for an association between the studied GST genotypes and the individual components of DAS28 score. We observed a significant association of GSTT1-0 genotype with ESR (p=0.034), but not with SJC, TJC and VAS. When we stratified the patients into a high and a low or intermediate group of disease activity we observed that the patients with homozygous deletion of both GSTM1 and GSTT1 genes had a 3.1-fold higher risk for developing high disease activity as compared to the patients with both genes present (p=0.047, OR=3.142, 95% CI=1.018-9.701). Also the patients with GSTT1-0 genotype had a 2.8-fold higher risk for developing high disease activity as compared to the patients with GSTT1-1 genotype (p=0.028, OR=2.761, 95% CI=1.114-6.843). On the other hand, neither GSTM1-0 nor the polymorphic GSTP1 genotypes leading to intermediate or low conjugation capacity modified the risk for developing high disease activity (p>0.05). We obtained similar results when only female patients as well as when only patients treated with MTX as monotherapy were included in the analyses (data not shown). Integrated past RA activity was characterized by the presence of bone erosions and functional impairment evaluated by HAQ score. However, none of the genetic polymorphisms in the GSTM1, GSTP1 and GSTT1 had a statistically significant impact on both parameters (data not shown).

We also assessed the association of the interaction between GST polymorphic genotypes and smoking with the disease activity as shown in Table III. First, we tested for an association of GST polymorphic genotypes with smoking. We divided the patients into smokers (n=91) and non-smokers (n=122). The frequencies of GSTT1-1 and GSTT1-0 genotypes as well as GSTM1-1 and GSTM1-0 genotypes were not statistically significant different between smokers and non-smokers (p=0.742 and p=0.596, respectively). Secondly, we evaluated the association of GST genotypes and smoking with the disease activity. In the group

Table II. The distribution of GST genotypes in the disease activity groups.

GST genotype			All	Disease activity		
				Mean DAS28 (SD)	DAS28 ≥5.1 n (%)	DAS28 <5.1 n (%)
Combined GSTM1 and GSTT1						
GSTM1-1 and/or GSTT1-1			190 (89.2)	4.1 (0.9)	27 (81.8)	163 (90.6)
GSTM1-0 and GSTT1-0			23 (10.8)	4.2 (1.1)	6 (18.2)*	17 (6.4)*
GSTT1-1			171 (80.3)	4.0 (0.9)	23 (69.7)	148 (82.2)
GSTT1-0			42 (19.7)	4.2 (1.0)	10 (30.3)**	32 (17.8)**
GSTM1-1			90 (42.3)	4.0 (1.0)	14 (42.4)	76 (42.2)
GSTM1-0			123 (57.7)	4.1 (0.9)	19 (57.6)	104 (57.8)
GSTP1	codon 105	codon 114				
**A/*A*	Ile	Ala	83 (39.0)	4.2 (0.9)	14 (42.4)	69 (38.3)
**B/*B*	Val	Ala	14 (6.6)	4.1 (0.7)	2 (6.1)	12 (6.7)
**A/*B*	Ile/Val	Ala	80 (37.6)	4.0 (1.0)	12 (36.4)	68 (37.8)
**B/*C*	Val	Ala/Val	15 (7.0)	4.5 (0.9)	4 (12.1)	11 (6.1)
**A/*C*	Ile/Val	Ala/Val	21 (9.9)	3.9 (0.8)	1 (3.0)	20 (11.1)
GSTP1 presumed conjugation capacity§						
High			83 (39.0)	4.2 (0.9)	14 (42.4)	69 (38.3)
Intermediate			101 (47.4)	4.0 (1.0)	13 (39.4)	88 (48.9)
Low			29 (13.6)	4.3 (0.8)	6 (18.2)	23 (12.8)

*p=0.047, OR=3.142, 95% CI=1.018-9.701; **p=0.028, OR=2.761, 95% CI=1.114-6.843; §GSTP1 presumed conjugation capacity: high = patients with GSTP1*A/*A genotype; intermediate = patients with GSTP1*A/*B and/or *A/*C genotypes; low = patients with GSTP1*B/*B and/or *B/*C genotype.

Table III. Disease activity and the distribution of GST genotypes between smokers and non-smokers.

Genotype	Smokers			Non-smokers		
	Mean DAS28 (SD)	DAS28 ≥5.1 n (%)	DAS28 <5.1 n (%)	Mean DAS28 (SD)	DAS28 ≥5.1 n (%)	DAS28 <5.1 n (%)
GSTM1-1 and/or GSTT1-1*	4.1 (0.9)	12 (70.6)	66 (89.2)	4.1 (0.9)	15 (93.8)	97 (91.5)
GSTM1-0 and GSTT1-0	4.6 (1.2)§	5 (29.4)	8 (10.8)	3.8 (0.9)	1 (6.2)	9 (8.5)
GSTT1-1*	4.0 (0.9)	10 (58.8)	64 (86.5)	4.0 (0.8)	13 (81.3)	84 (79.2)
GSTT1-0	4.5 (1.1)¥	7 (41.2)	10 (13.5)	4.0 (1.0)	3 (18.7)	22 (20.8)
GSTM1-1*	4.0 (0.9)	9 (52.9)	32 (43.2)	3.9 (1.0)	5 (31.3)	44 (41.5)
GSTM1-0	4.2 (1.0)	8 (47.1)	42 (56.8)	4.1 (0.8)	11 (68.7)	62 (58.5)

*reference; §p=0.035, OR=4.849, 95% CI=1.117-21.060; B=0.578, SE=0.286, p=0.047; ¥p=0.004, OR=8.640, 95% CI=1.995-37.426; B=0.587, SE=0.257, p=0.025.
P and OR values with 95% CI were calculated by logistic regression analysis and *P* and B values with SE were calculated by multiple linear regression analysis with addition of independent variables sex, disease duration, MTX treatment duration, the presence of erosions and RF seropositivity.

of smokers GSTM1 deletion alone was not significantly associated with the disease activity (p=0.443). The patients with GSTT1-0 genotype had significantly higher mean DAS28 values (B=0.587, SE=0.257, p=0.025) and an 8.6-fold higher risk for developing high disease activity as compared to the carriers of GSTT1-1 genotype (p=0.004, OR=8.640, 95% CI=1.995-37.426). Also smokers with a homozygous deletion of both GSTM1 and GSTT1 genes had significantly higher mean DAS28 values (B=0.578, SE=0.286, p=0.047) and a 4.84-fold higher risk for developing high disease activity as compared to the patients with both genes present (p=0.035, OR=4.849, 95% CI=1.117-21.060), but this effect was manly due to the effect of GSTT1 genotype. Among the components of DAS28, GSTT1 polymorphism was associated with TJC (p=0.027), and not with SJC, ESR and VAS (p=0.085, p=0.498 and p=0.114, respectively). In the group of non-smokers however, neither GSTM1, GSTT1 polymorphisms nor their combination was associated with the disease activity. All the results were corrected for sex, disease and MTX treatment duration, the presence of erosions, RF and/or anti-CCP seropositivity.

Discussion

In the present study we analyzed GSTM1, GSTP1 and GSTT1 genetic polymorphisms in a well documented group of RA patients and our results suggest that GST genetic polymorphisms may be associated with the disease activity of RA as evaluated by mean DAS28. We observed that GSTT1-0 genotype contributed to higher disease activity in RA patients. The risk for developing highly active RA was the highest in smokers with the GSTT1-0 genotype. On the other hand, no association of GSTM1-0 genotype alone and GSTP1 genetic polymorphisms with disease activity was observed.

To our knowledge only one study investigated the association of GSTM1, GSTT1 and GSTP1 genotypes with disease activity assessed using 5-year area

under curve measurements of ESR and CRP, but observed no significant association (9). In our study we evaluated disease activity by the DAS28 scores of two consecutive visits within three months time and observed that the association of GSTT1-0 genotype with disease activity was mainly due to the association with ESR. ESR and CRP are non-specific measurement of inflammation and vary during the disease progression and the treatment, which may be the reason for the discrepancies observed between our and the previously reported results.

There is extensive evidence that genetic factors may play a role not only in disease susceptibility but also in disease severity in RA (reviewed in references (40. 41). Most of the previous studies investigated the association of GSTM1, GSTP1 and/or GSTT1 polymorphisms with susceptibility and/or severity of RA (9, 11). In a Northern European white population (n=277) no significant association of the GSTM1, GSTP1 and GSTT1 polymorphisms with the susceptibility and/or severity of RA obtained by scoring radiographs of hands and feet using the Larsen score was observed (9). Similarly, no association of GSTT1 or GSTP1 genotypes with either risk or severity of RA was observed in a Korean population (n=258) but GSTM1-0 genotype was associated with increased susceptibility for RA and also with a higher risk for severe RA (11). A significant association of GSTT1 polymorphism with a higher Larsen score in carriers of two manganese superoxide dismutase (Mn-SOD) polymorphic alleles was reported (42). In our study, disease severity was assessed by the presence or absence of erosions on the x-rays of hands and feet. Using data collected from patient files however, the x-rays were not evaluated by the Larsen score because they were not taken at the same time frame after the onset of the disease. Therefore, functional disability assessed with HAQ scores at the entry of the patients in the study was used as one of the indicators of the disease severity. In contrast to the former two studies (9, 11) genetic polymorphisms in the GSTM1, GSTP1 and GSTT1 neither showed correlation with the presence or absence of erosions seen on x-rays which were obtained for each patient in the past nor with HAQ score determined for each patient at the inclusion into the study.

As the female percentage in our study did not represent the sex ratio of the whole RA population all the statistical analyses were performed separately for female patients, but the results obtained were similar. The strength of our findings was that they were not biased by sex, disease and MTX treatment duration, the presence or absence of erosions or RF seropositivity as *p*-values were corrected for these factors. In addition, our study was not biased by genetic heterogeneity since all the patients were recruited in a geographic area with an ethnically homogeneous population (43, 44).

It is well documented that tobacco smoke contains a variety of compounds such as polycyclic aromatic hydrocarbons (PAHs), ethylene oxide and others, which are genotoxic and also promote the production of ROS. Conjugation reactions catalyzed by GSTs are an important step in the biotransformation of these xenobiotics. Although GSTs have overlapping substrate specificities GSTT1 tends to be more reactive towards ethylene oxide, while GSTM1 and GSTP1 have higher affinities towards PAHs (reviewed in references 45, 46). Therefore, we assessed the disease activity in ever-smokers with limited capacity for xenobiotic detoxification and ROS product elimination due to GSTM1, GSTP1 and GSTT1 polymorphisms. The combination of GSTM1 deletion and smoking and the combination of GSTP1 polymorphisms and smoking did not modify the disease activity (data not shown), but we observed an interesting interaction between GSTT1 polymorphism and smoking status. Smokers with only GSTT1 gene deletion had significantly higher mean DAS28 values and an 8.6-fold higher risk for developing high disease activity as compared to smokers with at least one intact GSTT1 gene. Although in our patient group smoking status was not associated with disease activity score or its components, we observed an effect of smoking in combination with GSTT1 deletion on disease activity that was higher than each separate effect. Also smokers with a homozygous deletion in both GSTM1 and GSTT1 genes had significantly higher disease activity as compared to smokers with both genes present. The observed effect of the combination of GSTM1 and GSTT1 genotypes on disease activity in smokers can probably be ascribed predominantly to GSTT1 genotype, since this one had an independent effect.

The association of the interaction of GSTT1-0 genotype and smoking with disease activity was mainly due to the association with increased number of tender joints. The biological mechanism behind this observation is not yet understood. Some evidence comes from a previous study that reported on a significant association of smoking status with disease activity (26). They observed a gradient of increase in SJC, TJC and VAS from never smokers to former smokers to current smokers, but no significant association of smoking with CRP was found. Based on these observations we proposed that the association of the combination of GSTT1 deletion and smoking with an increase in TJC and SJC, but not with ESR and CRP may be predominately due to the effect of smoking. Nevertheless, more evidence is needed to confirm our hypothesis.

So far, two studies investigated the association between GST genotypes and smoking in RA (23, 27). They have reported contradictory data: Criswell *et al*. observed an increased risk for RA in smokers with the GSTM1 gene present, while Mattey *et al*. observed a higher disease severity in smokers with GSTM1 deletion. In our study, the combination of smoking and genetic polymorphisms of GSTs was not significantly associated with RA severity.

In conclusion, our study is reporting on the association of GST genotype with the activity of RA. GSTT1 homozygous deletion contributed to higher disease activity in RA patients. The risk for developing high disease activity was the strongest in smokers with GSTT1-0 genotype as they had an 8.6-

fold higher risk for developing highly active disease as compared to smokers with at least one GSTT1 gene present. Further studies are needed to determine if smoking cessation has a beneficial effect on clinical outcome in RA patients with GSTT1 gene deletion.

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