

Influence of glutathione-related genes on symptoms and immunologic markers among vulcanization workers in the southern Sweden rubber industries

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Abstract

Objective The aim was to elucidate the role of genetic variants on symptoms of the eyes and airways, headache and nausea, as well as on immunologic markers, among vulcanization workers in the contemporary Swedish rubber industry. Polymorphisms in genes, which are involved in the defense against reactive oxygen species and metabolism of toxic substances present in the vulcanization fumes, were analyzed.

Methods One hundred and forty-five exposed and 117 unexposed workers were included in the study. Medical and occupational histories were obtained in structured interviews. Symptoms were recorded and immunologic markers analyzed in blood. Polymorphisms in glutathione-related genes (glutamate cysteine ligase catalytic subunit (*GCLC*)-129, glutamate cysteine ligase modifier subunit (*GCLM*)-588, glutathione S-transferase alpha 1 (*GSTA1*)-52, *GSTM1**O, *GSTP1*-105, *GSTP1*-114, and *GSTT1**O) were analyzed by Taqman-based allelic discrimination and ordinary PCR.

Results A protective effect of *GSTA1*-52 (G/A + A/A) genotype on symptoms and immunologic cells, in particular among exposed workers, was suggested. Exposed workers with *GSTT1**O had increased risk of nosebleed compared to exposed workers with *GSTT1**1. Exposed workers with *GSTP1*-105 (ile/val + val/val) had decreased levels of total immunoglobulin E (IgE) compared to exposed workers with *GSTP1*-105 ile/ile. *GCLC*-129 variant genotype demonstrated increased levels of immunologic cells among exposed workers, although statistical significance was not reached.

Conclusion Our data indicate that hereditary factors influence the susceptibility to symptoms and the immunologic response of workers in the rubber industry.

Keywords Airway symptoms · Biomarkers · Glutathione · Polymorphisms · Rubber industry

Introduction

Workers in the rubber industry are exposed to a complex mixture of toxic compounds, and have increased risks for several diseases and symptoms (Roth 1999). Elevated risks of eyes and airways symptoms, headache and nausea have recently been observed in contemporary Swedish rubber workers by our group (Jönsson et al. 2007a). However, symptoms were found in only a fraction of the exposed workers. This could partly reflect exposure to different compounds and partly be due to inter-individual differences in symptom susceptibility.

The small tripeptide glutathione (GSH) and the family of glutathione S-transferases (GSTs) are involved in the protection against reactive oxygen species, which is a key component of damage to the airways by various toxic substances found in the rubber industry (De Vocht et al. 2005; Gilmour et al. 2006; Rahman et al. 2006). Furthermore, data indicate that GSH and GSTs are involved in the metabolism of toxic compounds such as carbon disulphide (CS₂) and polycyclic aromatic hydrocarbons (PAHs), present in the rubber industry. For example, it has been shown that *GSTA1*-1, *GSTM1* and *GSTP1*-1 are involved in the detoxification of PAHs (Jernström et al. 1992; Sundberg et al. 1997), and results from our group suggest that *GSTM1* and *GSTT1* are involved in the biotransformation of CS₂ (Jönsson et al. 2007b).

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GSH-synthesizing genes, such as glutamate cysteine ligase catalytic subunit (*GCLC*) and glutamate cysteine ligase modifier subunit (*GCLM*), as well as GSTs display genetic polymorphisms that affect the enzyme function or expression levels (Strange and Fryer 1999). Thus, part of the inter-individual variation in symptom susceptibility among vulcanization workers in the rubber industry may be due to genetic variation in GSH-related genes.

The aim of this study was to elucidate the role of genetic variants on symptoms of the eyes and airways, headache and nausea, as well as on immunologic markers among vulcanization workers in the contemporary Swedish rubber industry. Polymorphisms in genes, which are involved in the defense against reactive oxygen species and metabolism of toxic substances present in the vulcanization fumes were analyzed, i.e., *GCLC*-129, *GCLM*-588, *GSTA1*-52, *GSTM1**O, *GSTP1*-105, *GSTP1*-114, and *GSTT1**O. The hypothesis was that the study subjects carrying low function genotypes would suffer from more symptoms compared to individuals carrying high function genotypes. Moreover, this genetic effect was hypothesized to be most evident among exposed workers.

Materials and methods

Study subjects

One hundred and forty-five exposed workers, who cured with sulfur or worked in the same hall as people curing with sulfur on the day of the medical examinations were included in the study. The exposed workers were employed at eight different rubber companies, which are described in detail elsewhere (Jönsson et al. 2007b). The companies were selected on the grounds of geographical accessibility and diversity of production methods. In addition, 117 unexposed workers with no occupational contact with rubber or plastic chemicals were included. They were working as assemblers of armature at an electric light fittings company ($n = 21$), as meat cutters at a butchery ($n = 20$), as operators at a dairy plant ($n = 11$), as postmen ($n = 27$) or as department store workers ($n = 38$). All study subjects were working in southern Sweden and were present at work at the time of the medical examinations. Some individual characteristics of the exposed and unexposed workers are shown in Table 1. Study subjects with Eastern Asian descent ($n = 21$) were excluded from the study, since mixed ethnicities may result in spurious associations due to differences in allelic frequency and symptom prevalence between ethnic groups. The study subjects gave their informed written consent to take part in the study and the study was approved by the Regional Ethical Committee of Lund University.

Table 1 The characteristics of the exposed and unexposed workers

	Exposed workers ($n = 145$)	Unexposed workers ($n = 117$)
Age, median (range) years	38 (19–65)	41 (20–63)
Sex (male/female)	83/83	57/60
Smoking [n (%)] ^a	52 (31)	44 (38)
Snuffing [n (%)] ^a	22 (19)	30 (18)
Atopy [n (%)]	52 (32)	31 (27)

^a Denoted smoker or snuffer if stopped < 6 months ago

Medical examination and blood analysis

In the present study, the focus was on those symptoms and immunologic markers that in a previous study by our group demonstrated statistically significant associations with exposure in the rubber industry (Jönsson et al. 2007a).

Medical and occupational histories were obtained in structured interviews, based on a questionnaire, slightly modified from Nielsen et al. (1988) and used by Littorin et al. (2000). The structured interviews were carried out by a physician, a trained specialist in occupational medicine. The same person performed all the interviews, and was aware of the exposure status, but not of the genotypes or the levels of immunologic markers at the time of the interviews. Questions dealt with symptoms, which had occurred during the past 12 months and had affected the eyes (itching, running or burning) or throat (burning or dryness). Furthermore, information on 12-month record of symptoms of hoarseness, severe dry cough, nosebleed, headache and nausea was obtained.

Venous blood samples were collected during work and handled according to the instructions from the laboratories involved. Number per liter of leukocytes, neutrophils and eosinophils, plasma concentrations of total IgG, and IgA and the concentrations in serum of total IgE, were analyzed by routine clinical methods. Atopy was defined as positive when at least one of the allergens in the Phadiatop test (which measures specific IgE against eleven common allergens) provided a response. The blood analyses were performed on blinded samples.

Polymorphisms analyzed

GCLC contains a polymorphism in the promoter at position–129. The T allele has shown lower promoter activity compared to the C allele in vitro in human endothelial cells (Koide et al. 2003). The 5′-flanking region of *GCLM* contains a polymorphism (–588C/T) in which the T allele has shown lower promoter activity compared to the C allele when exposed to oxidants (Nakamura et al. 2002). Nakamura et al. (2002), also showed that the plasma GSH levels

were significantly lower in the CT and TT genotypes than in the CC genotype.

Both *GSTM1* and *GSTT1* have non-functional null alleles, which are due to deletions of the genes (Hayes and Strange 2000). Thus, individuals who have a homozygous deletion have no enzymatic activity. These genotypes are named *GSTM1**O and *GSTT1**O, while genotypes with at least one functional allele are named *GSTM1**1 and *GSTT1**1.

GSTA1 contains a polymorphism in the promoter (−52G/A), which may cause differential gene activity as the variant allele misses a binding site for the transcription factor Sp1 (Morel et al. 2002).

Two functional polymorphisms in the coding region of *GSTP1* were also examined. The variant allele of *GSTP1*-105 encodes valine (val) instead of isoleucine (ile) at codon 105, due to a base pair exchange, where G substitutes A. The variant allele of *GSTP1*-114 encodes valine (val) instead of alanine (ala) at codon 114, due to a base pair exchange, where T substitutes C. These substitutions may affect the ability and the rate of which different toxic compounds bind to GSTP1-1 (Ali-Osman et al. 1997).

Genotyping

DNA was extracted from whole blood using QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) at the SWEGENE Resource Center for Profiling Polygenic Disease, Malmö University Hospital, Malmö, Sweden, or by a modified salting out extraction method (Miller et al. 1988).

GCLC, *GCLM*, *GSTA1* and *GSTP1* were genotyped with Taqman-based allelic discrimination on an ABI 7000 instrument (Applied Biosystems, Foster City, CA, USA) as described earlier (Custodio et al. 2004; Jönsson et al. 2007b). *GSTM1* and *GSTT1* were genotyped with ordinary PCR and subsequent agarose electrophoresis as described earlier (Pemble et al. 1994; Hou et al. 1995; Jönsson et al. 2007b).

For all analyses, positive controls for the different genotypes as well as a negative control (water instead of DNA) were included in each run. Moreover, approximately 10% of the samples were reanalyzed as a control of the method.

Statistical analysis

Genotype distribution was analyzed with χ^2 , except when the expected individual frequencies were smaller than five and then Fisher–Freeman–Halton’s test was used. Regarding *GSTM1* and *GSTT1*, where heterozygotes could not be differentiated from homozygotes (no deletion), gene frequencies found in the literature (Garte et al. 2001) were used as expected frequencies.

Genotypes were dichotomized as follows: *GCLC*-129 and *GCLM*-588 (C/C and C/T + T/T), *GSTA1*-52 (G/G and

G/A + A/A), *GSTP1*-105 (ile/ile and ile/val + val/val) and *GSTP1*-114 (ala/ala and ala/val + val/val).

The possible association between genotypes and symptoms was estimated by odds ratios (ORs) calculated using logistic regression. To estimate the differences in levels of immunologic markers between genotypes, Analysis of Variance (ANOVA) was used. The genetic influence on the symptoms and immunologic markers was first analyzed among all study subjects and possible interaction between genotype and exposure status (exposed or unexposed) was evaluated by the inclusion of an interaction term (genotype) $\times$ (exposure status). Since there was an indication of interaction, subsequent analyses were stratified for exposure status. For all analyses, two-sided significance was used. Statistical significance refers to $P \leq 0.05$ or, equivalently, to 95% CI for an OR that excludes unity or a difference that excludes zero.

A probability–probability plot suggested that the levels of immunologic markers should be transformed (natural logarithm) before use in the models. For the transformed variables, the formula $100(e^{\beta}-1)$, where β is the effect estimate, was used to estimate the percentage increase or decrease in the average level of the immunologic marker in the evaluated group compared to the reference group (Vittinghoff et al. 2005).

All models were adjusted for atopy, age, sex, snuffing and smoking. Since exposure duration showed a correlation with age ($R_s = 0.55$), exposure duration was not included in any multivariate model.

For all statistical analyses SPSS Version 13.0 (SPSS Inc., Chicago, IL, USA) was used.

Results

Genotype distribution

The genotype distributions are shown in Table 2. For *GCLC*-129, *GCLM*-588, *GSTA1*-52, *GSTP1*-105 and *GSTP1*-114 the genotype distributions were consistent with the population being in Hardy–Weinberg equilibrium, both when analyzing all study subjects together and exposed and unexposed workers separately. Furthermore, frequencies of the deletion genotypes of *GSTM1* and *GSTT1* were in accordance with the frequencies found in the literature (Garte et al. 2001), except for the frequency of *GSTM1**O among the unexposed workers, among whom the frequency was lower than that reported earlier.

Genotype and symptoms

The effects of the polymorphism *GSTA1*-52 on symptoms among exposed workers showed a consistent pattern of a

Table 2 The number and frequencies (%) of the genotypes among all study subjects, exposed workers and unexposed workers

Polymorphism (genotype)	Study subjects		
	All	Exposed	Unexposed
<i>GCLC</i> -129 (C/C)	212 (84)	115 (82)	97 (85)
(C/T)	40 (16)	23 (16)	17 (15)
(T/T)	2 (0.8)	2 (1.4)	0
<i>GCLM</i> -588 (C/C)	185 (73)	100 (71)	85 (75)
(C/T)	58 (23)	35 (25)	23 (20)
(T/T)	11 (4.3)	5 (3.6)	6 (5.3)
<i>GSTAI</i> -52 (G/G)	80 (32)	44 (31)	36 (32)
(G/A)	130 (51)	74 (53)	56 (49)
(A/A)	44 (17)	22 (16)	22 (19)
<i>GSTM1</i> *O	120 (48)	71 (51)	49 (43)
<i>GSTM1</i> *1	132 (52)	67 (49)	65 (57)
<i>GSTPI</i> -105 (ile/ile)	118 (47)	61 (44)	57 (50)
(ile/val)	117 (46)	71 (51)	46 (40)
(val/val)	19 (7.5)	8 (5.7)	11 (9.6)
<i>GSTPI</i> -114 (ala/ala)	219 (86)	116 (83)	103 (90)
(ala/val)	34 (13)	23 (16)	11 (9.6)
(val/val)	1 (0.4)	1 (0.7)	0
<i>GSTT1</i> *O	42 (17)	25 (18)	17 (15)
<i>GSTT1</i> *1	209 (83)	112 (82)	97 (85)

protective effect of the *GSTAI*-52 (G/A + A/A) genotype. Statistical significance was reached regarding nosebleed ($P = 0.014$) and headache ($P = 0.009$) and almost regarding dry cough ($P = 0.064$) (Fig. 1, Table 3). A tendency to interaction was indicated ($P < 0.1$) for exposure and *GSTAI*-52, regarding nosebleed and headache. The polymorphisms in the remaining genes showed no consistent pattern of effect (data not shown). However, *GSTT1**O showed a statistically significant increase regarding the risk

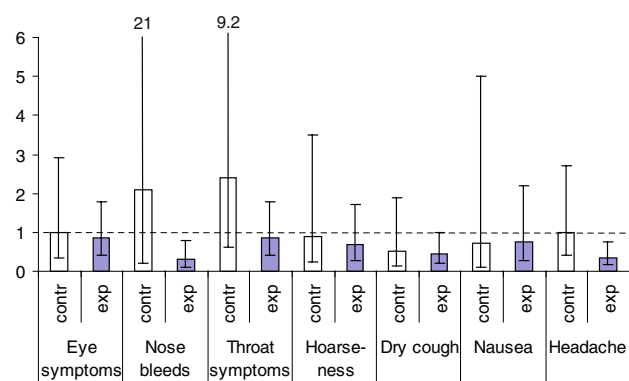


Fig. 1 The effect of *GSTAI*-52 (G/A + A/A) genotype on symptoms among unexposed (*contr*) and exposed workers (*exp*). Effect estimates are presented as odds ratios adjusted for sex, age, atopy, smoking and snuffing. Reference genotype is *GSTAI*-52 (G/G) (OR = 1.0). A 95% confidence interval is indicated. Values outside the scale of the Y-axis are indicated with Arabic numbers. Statistically significant values are presented in Table 3

of nosebleed and *GCLC*-129 was almost statistically significant regarding hoarseness (OR 2.8, 95% CI 1.0–7.6). No statistically significant interactions between exposure status and genotype on the examined symptoms were found, apart from *GSTPI*-105 regarding hoarseness (Table 3).

Genotype and immunologic markers

Exposed workers with *GSTPI*-105 (ile/val + val/val) were found to have decreased levels of total IgE (Table 4). Unexposed workers with *GSTPI*-114 (ala/val + val/val) expressed lower levels of total IgG compared to those unexposed with *GSTPI*-114 ala/ala. Furthermore unexposed workers with *GCLM*-588 (C/T + T/T) genotype had increased levels of eosinophils. The *GSTAI*-52 (G/A + A/A) genotype displayed a pattern of protective effects, in particular regarding immunologic cells, among exposed and unexposed workers (Fig. 2). Exposed workers with *GCLC*-129 variant genotype demonstrated increased levels of immunologic cells although statistical significance was not reached (leukocytes 10 (95% CI –0.76, 23); neutrophils 15 (–0.19, 31); eosinophils 17 (–7.2, 47)). None of the interactions between exposure status and genotype reached statistical significance (Table 4).

Discussion

Establishing gene-environment associations for very complex exposures such as vulcanization fumes are complicated. Nevertheless, when analyzing exposed and unexposed workers, a protective effect of the *GSTAI*-52 (G/A + A/A) genotype on the examined symptoms was found, in particular among the exposed workers. A similar, but non-significant pattern for immunologic cells was observed among both exposed and unexposed workers. Moreover, among the exposed workers, the *GCLC*-129 (C/T + T/T) genotype was associated with increased levels of leukocytes and neutrophils, and *GSTPI*-105 (ala/val + val/val) was associated with decreased levels of total IgE. A gene exposure interaction was found for *GSTPI*-105 regarding hoarseness.

GSTAI-1 has been shown to protect against oxidative stress, induced by hydrogen peroxide, in human retinal pigment epithelial cells (Liang et al. 2005), as well as against doxorubicin-associated lipid peroxidation in human lung cells (Sharma et al. 2006). *GSTAI*-1 is the predominating GST in the liver but the enzyme is also present in other tissues, such as the brain, pharynx and lung (Singhal et al. 1992; Rowe et al. 1997; <http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.446309>).

The *GSTAI*-52 (A/A) genotype has been associated with an approximately fourfold lower expression compared to the *GSTAI*-52 (G/G) genotype in liver samples (Coles et al. 2001) and also lower expression in rectum (Tijhuis et al.

Table 3 The effect of different polymorphisms on the risk of symptoms among unexposed and exposed workers

Gene (genotype)	Symptom	ORs (95 % CIs) ^a		
		Unexposed workers	Exposed workers	Interaktion, <i>P</i> -value ^{a, b}
<i>GSTA1</i> -52 (G/G)	Nosebleed	1.0	1.0	
<i>GSTA1</i> -52 (G/A + A/A)		2.1 (0.20, 21)	0.31 (0.12, 0.79)	0.082
<i>GSTA1</i> -52 (G/G)	Headache	1.0	1.0	
<i>GSTA1</i> -52 (G/A + A/A)		1.0 (0.40, 2.7)	0.35 (0.16, 0.77)	0.071
<i>GSTP1</i> -105 (ile/ile)	Hoarseness	1.0	1.0	
<i>GSTP1</i> -105 (ile/val + val/val)		3.6 (0.79, 16)	0.66 (0.28, 1.6)	0.049
<i>GSTT1</i> *1	Nosebleed	1.0	1.0	
<i>GSTT1</i> *O		–	2.9 (1.0, 8.3)	1.0

Presented are results that reached statistical significance with respect to genotype-associated risk in either exposure-strata, or in the analysis of interaction between genotype and exposure status

^a Adjusted for sex, age, atopy, smoking and snuffing

^b The interaction was estimated by a separate analysis using a term of interaction between exposure status and genotype

Table 4 The effect of different polymorphisms on the risk of changed levels of immunologic markers among unexposed and exposed workers

Gene (genotype)	Immunologic markers	Changes (95% CIs) ^a		
		Unexposed workers ^b	Exposed workers ^b	Interaktion, <i>P</i> -value ^{b, c}
<i>GCLM</i> -588 (C/C)	Eosinophils	0	0	
<i>GCLM</i> -588 (C/T + T/T)		27 (2.6, 57)	9.9 (−9.5, 33)	0.34
<i>GSTP1</i> -105 (ile/ile)	IgE	0	0	
<i>GSTP1</i> -105 (ile/val + val/val)		27 (−23, 97)	− 36 (−55, −9.3)	0.063
<i>GSTP1</i> -114 (ala/ala)	IgG	0	0	
<i>GSTP1</i> -114 (ala/val + val/val)		− 12 (−22, −0.003)	−1.7 (−9.8, 7.3)	0.23

Presented are results that reached statistical significance with respect to genotype-associated risk in either exposure-strata, or in the analysis of interaction between genotype and exposure status

^a Percentage change in the average value of the outcome in each examined group compared to the reference genotype (Vittinghoff et al. 2005)

^b Adjusted for sex, age, atopy, smoking and snuffing

^c The interaction was estimated by a separate analysis using a term of interaction between exposure status and genotype

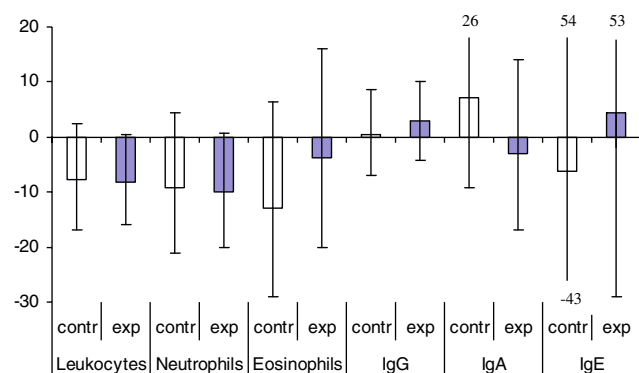


Fig. 2 The effect of *GSTA1*-52 (G/A + A/A) genotype on immunologic markers among unexposed (*contr*) and exposed workers (*exp*). Effect estimates are presented as percentage change in the average value of the outcome in each examined group compared to the reference genotype (Vittinghoff et al. 2005). Reference genotype is *GSTA1*-52 (G/G). Effect estimates are adjusted for sex, age, atopy, smoking and snuffing. A 95% confidence interval is indicated. Values outside the scale of the Y-axis are indicated with Arabic numbers. Statistically significant values are presented in Table 4

2007). However, the *GSTA1*-52 genotype did not correlate with *GSTA1*-1 expression in pancreas (Coles and Kadlubar 2003). The effect of the polymorphism on expression levels in cells from other tissues, e.g., in the airway system, has not yet been studied.

The mechanisms responsible for the fact that a protective effect was observed in participants who had the variant *GSTA1* genotype and were exposed to vulcanization fumes, are not clear and our results are difficult to explain, given the suggested protective role of the *GSTA1*-1 enzyme against oxidative stress and a lower expression level for the variant allele in liver and in rectum. One possibility is that *GSTA1*-1 is involved in biotransformation of a compound of the vulcanization fumes that generates toxic metabolites, which in turn may have deleterious effects. Previous results from our group did not show any association between urinary levels of TTCA (2-thiothiazolidine-4-carboxylic acid; biomarker of CS₂ exposure) and *GSTA1*-52 genotype (Jönsson et al. 2007b), or between urinary 1-HP levels

(1-hydroxypyrene; biomarker of PAH exposure) and *GSTA1*-52 genotype (unpublished results). Unfortunately, in those studies, no information on air levels of CS₂ or PAH was available, which may have limited the possibilities to detect associations. Another possibility would be that subjects with the *GSTA1*-52 variant genotype have a compensatory up-regulation of some other GSTs and hence a better defense against toxic compounds. It could also be that high conjugation activity of *GSTA1*-1 may cause reduction of the cellular GSH pool, which in its turn causes increased oxidative stress and subsequent damage to cells and tissues.

Increased levels of immunologic cells among vulcanization workers compared to unexposed workers have previously been observed by our group (Jönsson et al. 2007a). In the present study, the *GCLC*-129 (C/T + T/T) genotype demonstrated increased levels of leukocytes and neutrophils among exposed workers compared to the *GCLC*-129 (C/C) genotype, although statistical significance was not reached. The *GCLM*-588 (C/T + T/T) genotype showed increased levels of eosinophils, but the effect was more pronounced among unexposed workers. This finding may reflect that genetic variants resulting in lowered expression levels of glutathione are associated with a stronger inflammatory response.

Our finding of a statistically significant decrease of total IgE among exposed workers carrying *GSTP1*-105 (ile/val + val/val) is in agreement with previous findings by Fryer et al. (2000), showing a decrease in total IgE, and atopy as well as risk of asthma and bronchial hyperresponsiveness among study subjects with the *GSTP1*-105 variant genotype. In the present study, the unexposed workers carrying *GSTP1*-105 (ile/val + val/val) showed increased, although not statistically significant, levels of total IgE. A similar pattern was observed for hoarseness with increased risks among the unexposed workers and decreased risks among the exposed workers.

We found an association between the variant *GSTP1*-114 genotype and decreased levels of total IgG among unexposed workers. To our knowledge, the effect of this polymorphism on immunologic markers has not been studied before. It has been studied in relation to lung function and Vibhuti et al. (2007) found that patients, who have chronic obstructive pulmonary disease (implies an increased oxidative burden) as well as *GSTP1*-114 (ala/val + val/val) genotypes, had a significantly decreased FEV₁% as compared to individuals with *GSTP1*-114 (ala/ala). No effects on airway symptoms could be detected in our study. However, the symptoms recorded by us are less severe than in chronic obstructive pulmonary disease, which might explain the differences in result.

In earlier studies on *GSTM1* genotype and exposure, individuals with *GSTM1**O had increased sensitivity to nasal allergen challenge and decreased lung function (Gilliland et al. 2004; Romieu et al. 2004). However, we found no effect of *GSTM1* genotype in the present study.

We found an increased risk of nosebleed among exposed workers with *GSTT1**O genotype. Earlier studies have suggested that *GSTT1**O is associated with susceptibility to asthma and accelerated lung function decline in men in the general population (Coles and Kadlubar 2005; Imboden et al. 2007) but no effect on either airway symptoms or immunologic markers were found in the present study.

It was difficult to find controls at the included rubber companies, who were not occupationally exposed to rubber chemicals, but had the same socio-economic status as the exposed workers. Instead the controls in the present study were working at different companies, but those companies were selected for including employees with similar socio-economic status as the exposed workers. Furthermore, the unexposed workers were working in the same region of Sweden as the exposed workers but differences in the local environment (e.g. proximity to highways, industries, etc.) can exist.

We observed a lower frequency of *GSTM1**O among unexposed workers than what was expected from the literature (Garte et al. 2001). This is probably not caused by a methodological failure as the unexposed workers were analyzed together with the exposed workers and this deviation in expected frequency was not seen among them. In addition, there were no differences in frequencies in any group regarding *GSTT1*, which was analyzed in a similar way.

Multiple polymorphisms and symptoms/immunologic markers were considered in this study. We were aware of the problems with false positive and false negative findings, which are influenced by the significance level, statistical power and prior credibility of the associations tested (Wacholder et al. 2004). However, we did not formally adjust the significance level and some findings of our study could be the results of the large number of analyses performed.

Our data indicate that hereditary factors, in particular the *GSTA1*-52 genotype, influence the susceptibility to symptoms and immunologic responses among vulcanization workers in the rubber industry. The patterns were not clear and need further elucidation. However, at the same time, we would like to emphasize the importance of such information, since it may in the future, influence the risk assessments for rubber workers.

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