

Glutathione S-transferase *GSTP1* is a susceptibility gene for occupational asthma induced by isocyanates

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Background: Polymorphism at the π class glutathione-S-transferase locus (*GSTP1*) is associated with allergen-induced asthma and related phenotypes.

Objective: We sought to determine whether *GSTP1* polymorphism influences susceptibility to asthma induced by toluene diisocyanate (TDI).

Methods: The role of *GSTP1* was assessed in 131 workers exposed to TDI, 92 with TDI-induced asthma and 39 asymptomatic subjects. The phenotype of the disease was characterized by using detailed clinical history, lung volumes, airway responsiveness to methacholine, and airway responsiveness to TDI.

Results: In patients exposed to TDI for 10 or more years, the frequency of the *GSTP1* Val/Val genotype was lower in subjects who had asthma (odds ratio, 0.23; 95% confidence interval, 0.05-1.13; $P = .074$). Similarly, the frequency of this genotype was significantly lower in subjects with evidence of moderate-to-severe airway hyperresponsiveness to methacholine compared with the frequency in subjects with normal or mild hyperresponsiveness ($P = .033$).

Conclusion: These data suggest that homozygosity for the *GSTP1**Val allele confers protection against TDI-induced asthma and airway hyperresponsiveness. This view is supported by the finding that the protective effect increases in proportion to the duration of exposure to TDI. (J Allergy Clin Immunol 2002;109:867-72.)

Key words: Isocyanates, occupational asthma, airway hyperresponsiveness, genetic markers

Abbreviations used

AHR: Airway hyperresponsiveness
GST: Glutathione-S-transferase
GSTP1: π Class glutathione-S-transferase locus
OR: Odds ratio
ROS: Reactive oxygen species
TDI: Toluene diisocyanate

Exposure to isocyanates, such as toluene diisocyanate (TDI), in the workplace is a major cause of occupational asthma, and thus clinical symptoms of asthma develop in 5% to 15% of exposed subjects.¹ Although the mechanism by which these chemicals can induce host sensitization remains unclear, TDI-induced asthma exhibits similar functional and morphologic features to allergen-induced asthma, reflecting immune proinflammatory mechanisms underlying host sensitization to TDI.² However, unlike allergic asthma, low-molecular-weight sensitizers like TDI tend to cause asthma symptoms in nonatopic subjects and nonsmokers.³

TDI-induced asthma may reflect an interaction between an adverse work environment with host genetic factors.^{4,5} Although most genetic studies in allergic asthma have concentrated on chromosomes 11q and 5q⁶ and their association with key asthma-related phenotypes of atopy and airway hyperresponsiveness (AHR), relatively little is known of the genetic factors that are associated with predisposition to TDI-induced asthma. Thus although DQA1*0104 and DQB1*0503 confer susceptibility to TDI-induced asthma and DQB1*0501 offers protection,⁷ no other genetic markers for this type of occupational asthma have been identified.

A critical feature of TDI-induced asthma is the development of persistent airway inflammation. Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of this inflammation.⁸ Although host antioxidant defenses should detoxify ROS, individuals differ in their ability to deal with an oxidant burden; such differences are in part genetically determined.⁹ Inability to detoxify ROS perpetuates the inflammatory process, activating bronchoconstrictor mecha-

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TABLE I. Characteristics of the study population

	Asthmatic subjects (n = 92)	Control subjects (n = 39)
Age, mean $\pm$ SD (y)	41.3 $\pm$ 11.8	34.9 $\pm$ 9.0
Duration of exposure to TDI, mean $\pm$ SD (y)	17.9 $\pm$ 12.1	9.8 $\pm$ 8.4
Sex		
Male	76	35
Female	16	4
Smoking history (%)		
Never smoked	55.4	46.1
Current smoker	13.0	25.7
Exsmoker	31.6	28.2
Atopy (%)		
Nonatopic	69.2	74.4
Atopic	30.8	25.6
FEV ₁ (% predicted)*	97.3 $\pm$ 18.8	103.1 $\pm$ 13.3
FVC (% predicted)*	96.3 $\pm$ 14.4	100.4 $\pm$ 10.8
Airway responsiveness to methacholine (%)		
No-mild	46.6	79.3
Moderate-severe	53.4	20.7

*Predicted values are from reference 29.

nisms and leading to onset of asthma symptoms. We propose members of the glutathione-S-transferase (GST) superfamily as genetic markers for occupational asthma: *GST* genes are critical in the protection of cells from ROS because they use a wide variety of oxidative stress products, including lipid and DNA hydroperoxides.¹⁰

We have focused on the π class glutathione-S-transferase locus (*GSTP1*), located on chromosome 11q13, because it encodes the predominant cytosolic GST enzyme in lung epithelium,¹¹ and previous studies from our laboratory have shown a strong association between *GSTP1* genotypes and the risk of allergen-induced asthma and related phenotypes.¹² Specifically, *GSTP1 Val¹⁰⁵/Val¹⁰⁵* conferred a 9-fold lower risk of asthma than did *GSTP1 Ile¹⁰⁵/Ile¹⁰⁵*. We now examine the hypothesis that polymorphic variants in *GSTP1* are also associated with increased susceptibility to asthma induced by exposure to TDI. We have determined the prevalence of *GSTP1* genotypes in 131 individuals exposed to TDI and stratified by means of atopic status (skin prick tests), airway obstruction–airway responsiveness, and duration of exposure to TDI. Our main aim was to establish whether *GSTP1* is specific to allergic asthma or whether it is more generally involved in mediating inflammation in the airways.

METHODS

Subject recruitment

The association between *GST* genotypes and susceptibility was studied in 131 unrelated white European subjects residing in the north of Italy. All were exposed to TDI in furniture or body shops. The 92 asthmatic patients were recruited in the Department of Environmental Medicine and Public Health of the University of Padua, which was established to diagnose occupational asthma induced by

exposure to isocyanates. The 39 asymptomatic workers were employed in the same factories for at least 10 years. The demographics of the study population are shown in Table I.

Diagnosis of occupational asthma was based on the following: (1) exposure to TDI; (2) a history of wheezing, cough, dyspnea, and/or chest tightness; (3) a spirometric demonstration of a decrease in FEV₁ of at least 20% after exposure to TDI in the laboratory; and (4) the presence of AHR to methacholine inhalation challenge or the presence of airway obstruction. The asymptomatic subjects exposed for a long period to isocyanates did not perform the inhalation challenge with TDI for ethical reasons. Subjects with any other past or current disorder, either respiratory or nonrespiratory, were excluded. Atopy was defined by the presence of a personal history of allergies, seasonal rhinitis, eczema, or allergic conjunctivitis and positive skin prick test responses (skin reaction with a mean wheal diameter of ≥ 3 mm larger than that produced with a saline control) with a panel of 7 common aeroallergens. This study was carried out according to the principles of the Declaration of Helsinki, with written informed consent for inhalation challenge with methacholine and TDI, and for genetic investigation.

TDI and methacholine challenge testing

TDI and methacholine inhalation challenges were performed as previously described.¹³ An asthmatic reaction occurred when FEV₁ had decreased by greater than 20% after exposure to TDI in the laboratory. Methacholine inhalation challenge was stopped when FEV₁ had decreased by greater than 20% over FEV₁ baseline levels (PD₂₀ FEV₁). Moderate or severe AHR was defined by a PD₂₀ FEV₁ value of less than 700 μ g.

Identification of *GSTP1* genotypes

All genotyping assays were performed by workers who were unaware of the clinical status of individual subjects. Leukocyte DNA was extracted from peripheral blood collected into tubes containing ethylenediamine tetraacetic acid by using a standard phenol-chloroform procedure. Blood samples were obtained from all subjects.

Genotyping of the *GSTP1 Ile¹⁰⁵-Val¹⁰⁵* substitution was performed by using PCR performed with primers to exon 5, as previously described.^{14,15}

PCR of *GSTP1* for the determination of the *Ala¹¹⁴-Val¹¹⁴* substitution was performed essentially as described by Board et al,¹⁶ with an annealing temperature of 65°C. *Ala¹¹⁴-Val¹¹⁴* genotype data were not available for 9 subjects because DNA samples were exhausted. The *GSTP1 AC* and *BD* genotypes showed heterozygous patterns by using both of the above assays. Accordingly, we used an amplification refractory mutation system assay to unequivocally determine *GSTP1* genotype under these conditions. This assay was used as previously described.¹⁷

Statistical analysis

χ^2 Tests were used to assess between-group homogeneity (eg, positive asthma findings vs negative asthma findings). All statistical analyses were done with the Stata statistical package (version 6; Stata Corporation, College Station, Tex). In a subset of subjects, it was possible to match for age and duration of TDI exposure by comparing clusters of patients and control subjects with conditional logistic regression analysis.¹⁸ Clusters were defined by grouping subjects on the basis of age (20–29, 30–39, 40–49, 50–59, and 60–69 years) and duration of exposure (<5, 5–9, 10–14, 15–19, and 20–29 years), thereby generating 25 possible clusters. However, some clusters contained either only patients or only control subjects and were therefore excluded from the analysis. By using this approach, it was also possible to further correct for other potential confounding factors, such as sex, smoking history, and atopic status.

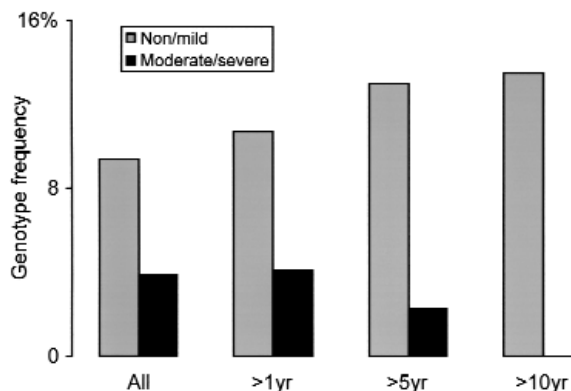


FIG 1. Frequency of the *GSTP1* Val¹⁰⁵/Val¹⁰⁵ genotype in patients with no or mild versus moderate or severe AHR according to the duration of exposure to TDI. Groups were defined as follows: Non/mild AHR (shaded bars), PD₂₀ FEV₁ methacholine greater than 700 µg; moderate/severe AHR (filled bars), PD₂₀ FEV₁ methacholine less than 700 µg.

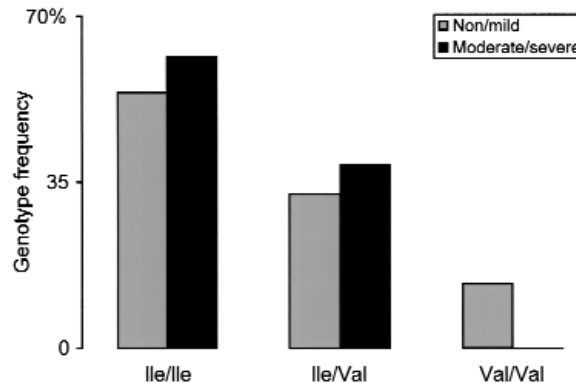


FIG 2. Association of *GSTP1* Ile¹⁰⁵-Val¹⁰⁵ genotype frequencies with AHR in subjects exposed to TDI for longer than 10 years. Groups were defined as follows: Non/mild AHR (shaded bars), PD₂₀ FEV₁ methacholine greater than 700 µg; moderate/severe AHR (filled bars), PD₂₀ FEV₁ methacholine less than 700 µg. Ile/Ile + Ile/Val versus Val/Val: *P* = .033.

RESULTS

GSTP1 genotype frequencies

Table II shows *GSTP1* genotype frequencies in the study population. Frequencies of the *GSTP1**A, *GSTP1**B, *GSTP1**C, and *GSTP1**D alleles were 0.694, 0.177, 0.069, and 0.044, respectively. *GSTP1* genotype frequencies achieved Hardy-Weinberg equilibrium and were similar to those observed in a population of atopic asthmatic subjects, atopic nonasthmatic subjects, and nonatopic asthmatic subjects from North Staffordshire, England, except that the *GSTP1**D frequency (0.005) was lower.¹⁴

Association of *GSTP1* genotypes with asthma and AHR

GSTP1 genotype frequencies in the total group stratified by the presence or absence of asthma diagnosis and AHR are also shown in Table II. In the total cohort (*n* = 131) the frequency of homozygotes for the Val¹⁰⁵ allele (*GSTP1* BB, BC, and CC genotypes combined) was lower in asthmatic subjects (6.5%) than in nonasthmatic subjects (10.3%), although this did not achieve statistical significance (*P* = .462). Similarly, frequencies of these genotypes were lower in subjects with positive AHR results (3.4%) than in subjects with negative AHR results (9.4%). Matching for age and duration of exposure and correction for atopic status, sex, and smoking history by using conditional logistic regression showed the same nonsignificant trends for both asthma (odds ratio [OR], 0.53; *P* = .468) and AHR (OR, 0.45; *P* = .406), although the number of subjects for whom matching was possible was reduced (asthma, *n* = 94; AHR, *n* = 97).

Effect of duration of exposure on *GSTP1* genotype associations with asthma and AHR

Because the association between genotype and asthma-AHR risk may depend on duration of TDI exposure,

we analyzed the data at 1 year or greater, 5 years or greater, and 10 years or greater of TDI exposure. These data showed that Val¹⁰⁵/Val¹⁰⁵ frequencies increased from 10.3% (in the total cohort) to 18.8% (in subjects exposed to TDI for ≥10 years) in nonasthmatic subjects; an inverse trend from 6.5% to 5.1% was observed in asthmatic subjects. Similar data were obtained for the association of the *GSTP1* genotype with AHR. Fig 1 shows the frequency of the *GSTP1* genotype in patients with presence or absence of AHR according to duration of exposure to TDI. This shows that the protective effect of the Val¹⁰⁵/Val¹⁰⁵ genotype is more apparent with increasing duration of exposure. Thus the frequency of this genotype increased from 9.4% to 13.5% in subjects with negative AHR results, and individuals with positive AHR results demonstrated a corresponding decrease from 3.8% to 0% (Fig 1).

Because the protective association of the Ile¹⁰⁵-Val¹⁰⁵ substitution with both AHR and asthma was strongest at 10 or more years of TDI exposure, we further analyzed the data, excluding those with less than 10 years of exposure (Table II). This showed that the frequency of *GSTP1**Val¹⁰⁵ was lower in subjects with positive AHR results compared with subjects with negative AHR results. Thus compared with other *GSTP1* genotypes combined, those homozygous for the Val¹⁰⁵ allele (*GSTP1* BB, BC, and CC) were significantly lower (0.0%) in subjects with positive AHR results than in those with negative AHR results (13.5%, *P* = .033; Fig 2). It was not possible to compare Val¹⁰⁵ homozygosity in the matched subset because of the absence of Val¹⁰⁵ homozygotes in the subjects with positive AHR results.

Similarly, homozygosity for *GSTP1* Val¹⁰⁵ was lower in those in whom asthma developed (5.1%) after 10 years of TDI exposure than in those in whom it did not develop (18.8%). Homozygosity for *GSTP1* Val¹⁰⁵ was associated with a lower risk of having asthma, although this

TABLE II. *GST* genotype frequencies according to asthma-AHR status and duration of TDI exposure in the total cohort and in subjects exposed to TDI for longer than 10 years

Genotypes	Definition of genotypes	Total group (%)	Asthma negative (%)	Asthma positive (%)	AHR negative (%)	AHR positive (%)
All subjects: <i>GSTP1</i>						
AA	<i>Ile</i> ¹⁰⁵ / <i>Ile</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Ala</i> ¹¹⁴	60 (49.2)	17 (50.0)	43 (48.9)	30 (50.9)	23 (46.9)
AB	<i>Ile</i> ¹⁰⁵ / <i>Val</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Ala</i> ¹¹⁴	31 (25.4)	6 (17.7)	25 (28.4)	16 (27.1)	13 (26.5)
AC	<i>Ile</i> ¹⁰⁵ / <i>Val</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Val</i> ¹¹⁴	10 (8.2)	3 (8.8)	7 (8.0)	4 (6.8)	5 (10.2)
AD	<i>Ile</i> ¹⁰⁵ / <i>Ile</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Val</i> ¹¹⁴	11 (9.0)	4 (11.8)	7 (8.0)	3 (5.1)	6 (12.2)
BB	<i>Val</i> ¹⁰⁵ / <i>Val</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Ala</i> ¹¹⁴	4 (3.3)	2 (5.9)	2 (2.3)	1 (1.7)	1 (2.0)
BC	<i>Val</i> ¹⁰⁵ / <i>Val</i> ¹⁰⁵ + <i>Ala</i> ¹¹⁴ / <i>Val</i> ¹¹⁴	5 (4.1)	2 (5.9)	3 (3.4)	4 (6.8)	1 (2.0)
CC	<i>Val</i> ¹⁰⁵ / <i>Val</i> ¹⁰⁵ + <i>Val</i> ¹¹⁴ / <i>Val</i> ¹¹⁴	1 (0.8)	0 (0.0)	1 (1.1)	1 (1.7)	0 (0.0)
Total		122	34	88	59	49
Subjects exposed to TDI for ≥10 y: <i>GSTP1</i>						
AA	—	—	4 (28.6)	25 (44.6)	15 (42.9)	11 (39.3)
AB	—	—	3 (21.4)	17 (30.4)	10 (28.6)	9 (32.1)
AC	—	—	1 (7.1)	4 (7.1)	2 (5.7)	3 (10.7)
AD	—	—	3 (21.4)	7 (12.5)	3 (8.6)	5 (17.9)
BB	—	—	1 (7.1)	1 (1.8)	1 (2.9)	0 (0.0)
BC	—	—	2 (14.3)	1 (1.8)	3 (8.6)	0 (0.0)
CC	—	—	0 (0.0)	1 (1.8)	1 (2.9)	0 (0.0)
Total	—	—	14	56	35	28

Status was defined as follows: *asthma positive*, subjects with TDI-induced asthma; *asthma negative*, asymptomatic subjects exposed to TDI; *AHR positive*, subjects with a PD₂₀ FEV₁ methacholine less than 700 μg; *AHR negative*, subjects with a PD₂₀ FEV₁ methacholine greater than 700 μg.

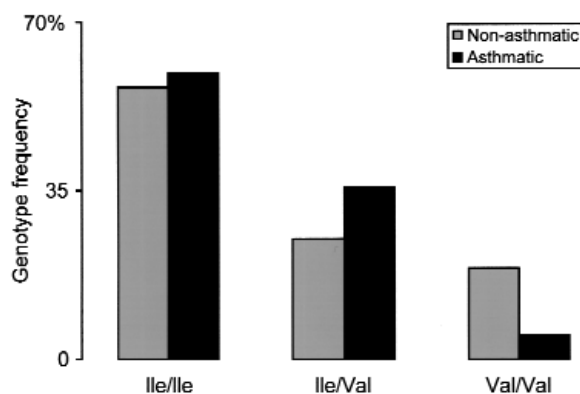


FIG 3. Association of *GSTP1* *Ile*¹⁰⁵-*Val*¹⁰⁵ genotype frequencies with asthma in subjects exposed to TDI for longer than 10 years. Groups were defined as follows: *Nonasthmatic* (shaded bars), Asymptomatic subjects exposed to TDI; *asthmatic* (filled bars), subjects exposed to TDI with documented TDI-induced asthma. *Ile/Ile* + *Ile/Val* versus *Val/Val*: *P* = .074.

just failed to achieve statistical significance (OR, 0.23; 95% confidence interval, 0.05-1.13; *P* = .074; Fig 3). In the matched subset the use of conditional regression showed that a similar OR was observed (OR, 0.35), although significance was lost (*P* = .296) as a result of the reduction in numbers of subjects (*n* = 56).

DISCUSSION

We have shown that polymorphism in *GSTP1* is associated with susceptibility to asthma induced by prolonged exposure to TDI, a commonly used occupational respiratory sensitizer. We have also demonstrated that

homozygosity for the *Val*¹⁰⁵-encoding alleles confer protection against the development of the asthma-associated phenotype AHR; this protective effect increased in proportion to the duration of TDI exposure. Our data suggest that individuals lacking this genotype may, over time, exhibit ongoing TDI-induced proinflammatory processes with consequent airway remodeling, leading to irreversible asthma symptoms.

This study confirms previous data showing a strong association of *GSTP1* genotypes with established asthma and AHR in a cohort of English patients with atopic asthma.¹² Thus rather than representing a disease-specific marker, we suggest that these associations reflect a role of *GSTP1* in modulating the development of the underlying pathology: AHR and persistent airway inflammation. Inhalation tests, bronchoalveolar lavage, induced sputum, and bronchial biopsy studies have all confirmed the presence of cellular infiltrates and proinflammatory mediators within the airways of individuals with TDI-induced asthma.¹⁹⁻²² Once established, such changes may persist, even after the individual is removed from TDI exposure.²³ The role of *GSTP1* may therefore reflect genetically determined differences in host ability to handle the inflammation-derived oxidative burden. ROS-derived products, such as lipid hydroperoxides, substrates for *GSTP1*, are essential in the mobilization of arachidonic acid and regulation of proinflammatory eicosanoid release.²⁴ Thus an inability to efficiently detoxify ROS would result in perpetuation of the inflammatory process, activate bronchoconstrictor mechanisms, and cause asthma symptoms.

A recent study of *GST* genotypes in a population of diisocyanate-exposed workers showed no significant association between *GSTP1* genotype and risk of asth-

ma.²⁵ The authors suggest that the discrepancies between our original finding of a protective effect of *GSTP1* Val¹⁰⁵/Val¹⁰⁵ in atopic asthma¹² and their data from patients with occupational asthma may reflect differences in the underlying mechanism between the 2 populations. However, the same can not be said of our current findings. An alternative explanation of the differences between the results of the current study and the data of Piirila et al²⁵ may be that we have identified a protective effect only in those patients exposed to TDI for at least 10 years. In our total population a significant association was not identified. Piirila et al also identified an increased frequency of Val¹⁰⁵/Val¹⁰⁵ in atopic workers exposed to diisocyanates, which contrasts with our previous findings of a protective effect of this genotype in atopic asthmatic subjects¹² and our lack of a statistical association in the present study ($P = .504$, data not shown). Although 2 of these studies examined diisocyanate-exposed workers, the populations differ in several regards. For example, we have focused on TDI, whereas Piirila et al²⁵ also examined subjects exposed to other diisocyanate compounds. The definitions of asthma, AHR, and atopy remain very variable between centers, and the level and nature of exposure is likely to be very different between factories.

Although there have been many studies examining genetic markers in allergic asthma, there are little data in the literature on such markers in occupational asthma. The importance of chromosome 6 is demonstrated by our previous studies showing that HLA DQA1*0104 and DQB1*0503 confer susceptibility to TDI-induced asthma, whereas DQB1*0501 is protective.⁷ Although 11q13, the chromosomal location of *GSTP1*, is a region of particular interest in studies on patients with allergic asthma, apart from the recent study described above, it has not been examined in patients with asthma induced by low-molecular-weight compounds, such as TDI.

In this study we have focused on *GSTP1* because our previous data show that this is the major *GST* isoform expressed in the lung,²⁶ and polymorphism at this locus, but not *GSTM1*, *GSTM3*, or *GSTT1*, confers protection against asthma and AHR in atopic patients.¹² The specificity of the association of polymorphism at *GSTP1*, but not other *GSTs*, is supported by our own observations. Thus in contrast to Piirila et al,²⁵ we did not identify associations between polymorphism at the *GSTM1* or *GSTT1* loci and asthma or AHR in our TDI-induced asthma cohort (data not shown). Our data also suggest that the *GSTP1* Ile¹⁰⁵-Val¹⁰⁵, rather than the Ala¹¹⁴-Val¹¹⁴, substitution mediates the protective effect.

We have shown that the association of asthma and AHR with *GSTP1* genotype is significant in subjects exposed to TDI for longer than 10 years, although the trend was apparent in those exposed for shorter periods. A longer interval between onset of exposure and development of symptoms in patients with occupational asthma appears to be associated with an IgE-mediated mechanism, whereas an IgE-independent mechanism is associated with development of symptoms after shorter exposure periods.²⁷ This may sug-

gest different pathophysiologic causes underlying disease development dependent on an interaction between host genetic profile and duration of exposure to the environmental insult. In our study the association of the *GSTP1* genotype with TDI-induced asthma appeared independent of atopy and other confounding factors because the ORs after correction for atopic status, sex, age, and smoking history in the total group (OR, 0.21) and in the matched subset (OR, 0.35) were similar to the uncorrected value (OR, 0.23). Thus although traditional markers of increased risk of IgE-dependent asthma may be important in TDI-induced asthma, the ability to handle ROS and their products may be a critical independent risk factor in determining development of airway inflammation in these patients. This view is supported by our data in atopic asthmatic patients, in whom the association of the *GSTP1* genotype with asthma and AHR appeared independent of atopic status.¹²

Although our data are statistically significant, we acknowledge that the findings presented here are preliminary because of the small number of patients, particularly those exposed to TDI for longer than 10 years. However, differences in selection of subjects may have been a confounding factor that is unlikely to account for the differences observed. Although there are dangers in overinterpretation of multiple statistical analyses, the a priori reason for studying *GSTP1* comes from our previous data in patients with allergic asthma. Accordingly, we have focused on *GSTP1* alone. However, although the magnitude of the effect of the *GSTP1* genotype (expressed as ORs) is potentially clinically relevant, the levels of significance observed are low, and therefore the study requires confirmation in a separate, larger cohort.

Because prolonged exposure to TDI may lead to permanent respiratory impairment or disability,²⁸ early diagnosis with concomitant instigation of early therapeutic measures or withdrawal from exposure measures may be the key to a favorable outcome in these high-risk subjects. Accordingly, consequent medical, social, and economic implications of TDI-induced asthma would support such intervention measures.

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