

The *GSTP1* Ile105 Val polymorphism modifies the metabolism of toluene di-isocyanate

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Background Toluene di-isocyanate (TDI) is widely used in the production of polyurethane foams and paints. As TDI causes respiratory disease in only a fraction of exposed workers, genetic factors may play a key role in disease susceptibility. Polymorphisms in TDI metabolising genes may affect elimination kinetics, resulting in differences in body retention, and in its turn differences in adverse effects.

Objectives To analyze how genotype modifies the associations between (i) TDI in air (2,4-TDI and 2,6-TDI) and its metabolites toluene diamine (TDA; 2,4-TDA and 2,6-TDA) in hydrolyzed urine; and (ii) 2,4-TDA and 2,6-TDA in hydrolyzed plasma and 2,4-TDA and 2,6-TDA in urine.

Methods Workers exposed to TDI were analyzed for 2,4-TDI and 2,6-TDI in air ($N=70$), 2,4-TDA and 2,6-TDA in hydrolyzed urine ($N=124$) and in plasma ($N=128$), and genotype: *CYP1A1**2A, *CYP1A1**2B, *GSTA1*-52, *GSTM1*O, *GSTM3*B, *GSTP1* I105V, *GSTP1* A114V, *GSTT1*O, *MPO*-463, *NAT1**3, *4, *10, *11, *14, *15, *NAT2**5, *6, *7, and *SULT1A1* R213H.

Results *GSTP1* 105 strongly modified the relationship between 2,4-TDA in plasma and in urine: ValVal carriers had

about twice as steep regression slope than IleIle carriers. A similar pattern was found for 2,6-TDA. *CYP1A1**2A, *GSTM1*, *GSTP1*, *GSTT1*, and *MPO* possibly influenced the relationship between TDA in plasma and urine.

Conclusion Our results show, for the first time, genetic modification on the human TDI metabolism. The findings suggest that *GSTP1* genotype should be considered when evaluating biomarkers of TDI exposure in urine and plasma. Moreover, the results support earlier findings of *GSTP1* 105 Val as protective against TDI-related asthma. *Pharmacogenetics and Genomics* 20:104–111 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Toluene di-isocyanates (TDI) are used worldwide in the production of polyurethane products, mainly as a mixture of the two isomers 2,4-TDI and 2,6-TDI. TDI is known to cause negative effects on the respiratory system, and TDI-related asthma is a problem in many countries [1]. Despite the medical surveillance programs at Swedish work places for isocyanate-exposed workers, workers still suffer from airway symptoms associated with TDI exposure [2].

Surveillance of TDI exposure is mainly performed by air monitoring, as imposed by the Swedish Work Environment Authority, but recently biological monitoring by the measure of 2,4-toluenediamine (TDA) and 2,6-TDA in hydrolyzed urine (short-term measure) and plasma (long-term measure) has been suggested as an alternative way to control exposure. Biological monitoring has several advantages compared to air monitoring, such as more convenient sampling, the fact that biomarkers reflect total absorbed dose, and that these measurements can reflect long-term exposure. We earlier showed that

there were strong associations between the personal air measurements of TDI and TDA biomarker levels [3], but there were inter-individual variations in biomarker levels, which could reflect differences in the metabolism of TDI. Many of the genes (see below) involved in metabolism of TDI are polymorphic. Genetic variants that result in metabolism differences influence the levels of biomarkers, and in its turn the estimation of exposure in biomonitoring. Moreover, because di-isocyanates cause asthma or airway-related symptoms in only a minor fraction of exposed workers, genetic factors involved in the metabolism of TDI have been proposed to modify the susceptibility to toxic effects [4–6].

The metabolism of TDI is not yet understood in detail, but there is evidence for several different metabolic pathways involved in the biotransformation of TDI. The different isomers 2,4-TDI and 2,6-TDI have different reactivity [7], and are possibly also metabolized partly by different pathways. For all enzymes described below, there are allelic variants with an impact on enzyme function or expression.

Isocyanates react with intracellular glutathione, either directly or by catalysis by the glutathione transferase (GST) system [8]. The formed conjugates are reactive and can react further with other compounds [9], possibly mediated by the GST system. Nonenzymatic hydrolysis converts isocyanates to their corresponding amines. However, free amines have been difficult to detect in plasma and urine after isocyanates exposure [10] and are only found in low levels compared to the hydrolysable part of the amine [11]. Thus, either the formed amines are not formed in large quantities or they are metabolized further very fast. Aromatic (and heterocyclic) amines are activated by oxidation of the primary amine by cytochrome P450 enzymes to aryl hydroxylamines. CYP1A1 as well as CYP1A2 and CYP1B1 can activate a range of arylamines to reactive intermediates [12]. Another frequent step in arylamine metabolism is the generation of acetoxy esters by *N*-acetyltransferases (NATs) [13]. *N*-acetylation of the amine group is considered as a detoxification mechanism, but *O*-acetylation causes more reactive products [14] and both activities can be catalyzed by the NAT1 or NAT2 enzymes. An additional alternative route in the biotransformation of many aromatic amines is sulfation by cytosolic sulfotransferases (SULTs; [15]), where SULT1A1 is one of the major isozymes. A peroxidase-catalyzed mechanism for the activation of arylamines (and heterocyclic) amines has also been suggested [16–18]. Myeloperoxidase (MPO) exists in neutrophils and utilizes H₂O₂ to produce cytotoxic oxidants.

It is known that TDI binds to macromolecules in the body. However, it is unknown if it is the isocyanate itself, the glutathione conjugate or a reactive amine metabolite that form the protein adducts. Genetic factors probably influence the amount adducted to macromolecules. In a study where rats were exposed to radioactively labeled TDI, most of the radioactivity in blood was found in plasma and almost this entire radioactivity was bound to macromolecules [19]. Moreover, it has been described that most of the hydrolysable TDA found in plasma of an exposed worker is bound to macromolecules and that the majority of this is bound to serum albumin [20]. In contrast, in the rat study only about 10% of the radioactivity was found in the blood. Therefore it seems probable that many other proteins than albumin are adducted with TDI. However, it is unclear if the protein adducts are involved in the pathophysiologic mechanism since isocyanate specific IgEs are seldom found, and when they are, they do not seem to be related to the airways symptoms.

The objective of this study was to characterize genetic effect modification on the metabolism of TDI, that is, the influence of different polymorphisms in TDI-metabolising genes on the associations between the short-term measures of TDI in air and TDA in urine,

and between the long-term measure TDA in plasma and TDA in urine. The different isomers 2,4-TDI (2,4-TDA) and 2,6-TDI (2,6-TDA) were, based on different reactivity and possibly different metabolic pathways, analyzed separately.

Methods

Study population description

This population has earlier been described in detail in Littorin *et al.* [2] and the characteristics of the participants are presented in Table 1. In the analyses only workers with results for genotypes were included: TDI in air *N* = 70, 2,4-TDA and 2,6-TDA in hydrolyzed urine (*N* = 124) and in plasma (*N* = 128). The median age for all investigated individuals was 36 years (range 19–63). All workers were exposed to TDI when working with molding, continuous foaming, flame lamination or low-heating or non-heating processes in the southern part of Sweden (for further details about the exposure, see [21]). The study was approved by the Regional Ethical Committee at Lund University, Sweden. Written informed consent was obtained from all participants.

Air and biological monitoring methods

Personal airborne exposure was measured during one shift with single glass-fibre filters impregnated with 2MP (MOP) and analysis of the isocyanates was performed by liquid chromatography combined with tandem mass spectrometry [21]. The airborne exposures were between 0.004 and 5.2 ppb, with a median exposure of 0.34 ppb.

Urine samples were collected as a pooled sample during the last 4 h of the shift. Further, within a 2-week period, a health examination was performed, and another urine sample was collected, as well as a blood sample. The collection of biological samples is described in detail in Sennbro *et al.* [3]. All biological samples were stored at –20°C until the day of analysis. The biomarker levels in the urinary and plasma samples were analyzed as the TDI-related diamines 2,4-TDA and 2,6-TDA after hydrolysis for 24 h in 0.3 mol/l NaOH. The diamines were extracted with toluene and derivatised with pentafluoropropionic acid anhydride, and quantified by gas chromatography and mass spectrometry. Each sample was analyzed at least twice and the precision was 7–18% [10].

Genotyping

The distributions of the genotypes are presented in Table 1. In all PCR assays negative (containing water instead of DNA) and positive (containing DNA from individuals with known genotype) controls were included. The polymorphisms analyzed are briefly mentioned below. The genotyping methods have been described in detail in Broberg *et al.* [6] and for *GSTA1*-52 in Jönsson *et al.* [22].

Table 1 Characteristics of the study population^a

	N		N		N	
	Air	%	Urine	%	Plasma	%
Sex						
Male	69	79	100	81	103	80
Female	18	21	24	19	25	20
Phadiatop						
Negative	47	67	85	72	91	72
Positive	23	33	33	28	36	28
Smoking						
Nonsmoker	31	44	50	41	52	41
Smoker	40	56	72	59	74	59
<i>CYP1A1</i> *2A						
A	60	86	106	89	113	88
B+C	10	14	13	11	15	12
<i>CYP1A1</i> *2B						
AA	66	94	115	97	123	96
AG+GG	4	6	4	3	5	4
<i>GSTA1</i> -52						
GG	22	32	36	31	40	32
AG	32	47	57	49	61	49
AA	14	21	24	21	24	19
<i>GSTM1</i>						
+	37	53	55	46	62	48
-	33	47	64	54	66	52
<i>GSTM3</i>						
AA	48	69	84	71	91	71
AB+BB	22	31	35	29	37	29
<i>GSTP1</i> 105						
II	40	57	61	51	66	52
IV	23	33	45	38	48	38
VV	7	10	13	11	14	11
<i>GSTP1</i> 114						
AA	59	84	102	86	111	87
AV	11	16	17	14	17	13
<i>GSTT1</i>						
+	60	86	97	82	106	83
-	10	14	22	18	22	17
<i>MPO</i>						
GG	52	74	89	75	94	73
GA	13	19	25	21	29	23
AA	5	7	5	4	5	4
<i>NAT1PH</i>						
IM+S	2	3	2	2	3	2
R	68	97	117	98	125	98
<i>NAT2PH</i>						
S	45	64	81	68	85	66
IM	21	30	30	25	33	26
R	4	6	8	7	10	8
<i>SULT1A1</i>						
GG	28	40	40	34	41	32
AG	36	51	68	57	75	59
AA	6	9	11	9	12	9

IM, intermediate phenotype; MPO, myeloperoxidase; R, rapid acetylator phenotype; S, slow acetylator phenotype.

^aOnly individuals with genotype data were included in the study.

Two closely linked polymorphisms in the *CYP1A1* gene were analyzed: the *MspI* polymorphism in the 3' end of *CYP1A1* (denoted *CYP1A1**2A, <http://www.cypalleles.ki.se/>) is because of a base substitution 264 base pairs downstream the poly (A) signal; and the polymorphism in the seventh exon (denoted *CYP1A1**2B), which arises from an A>G base change and results in replacement of isoleucine (Ile) by valine (Val) at residue 462 in the heme binding region of the enzyme.

The GSTs *GSTM1* and *GSTT1* were analyzed for gene deletions resulting in no enzyme (null polymorphisms). *GSTA1* was analyzed for a promoter polymorphism -52

resulting in an A to G exchange. The *GSTP1* polymorphisms analyzed result in an Ile to Val substitution at residue 105 in exon 5, and an alanine (Ala) to Val substitution at residue 114 in exon 6, respectively. When combining the genotypes at both positions of *GSTP1*, five different combinations were found out of nine possible. Owing to the low number of individuals with some combinations, the combinations were collapsed into three groups; group A: IleIle + AlaAla at position 105 and 114, respectively; group B: IleVal + AlaAla or ValVal + AlaAla; and group C: IleVal + AlaVal or ValVal + AlaVal. In *GSTM3*, the three base pair deletion polymorphism in intron 6 was analyzed.

Five different SNPs in the *NAT1* gene, 559 C > T, 560 G > A, 640 T > G, 1088 T > A and 1095 C > A, were analyzed to determine the alleles *3 (1095 C > A), *4 (wild-type), *10 (1088 T > A and 1095 C > A), *11 (640 T > G), *14 (560 G > A) and *15 (559 C > T). Individuals homozygous or heterozygous for the NAT1*10 allele and not carrying the *14 or *15 alleles were considered to have the rapid acetylator phenotype (R). Individuals homozygous or heterozygous for NAT1*3, *4 or *11 alleles and not carrying the *14 or *15 alleles were considered to have the intermediate phenotype (IM). The slow acetylator alleles NAT1*14 and *15 were rare in the study population: there were only three individuals being heterozygotes for *14 or *15 and all were considered as slow acetylators (S). The *NAT2* polymorphisms (341T > C, 590 G > A, and 857 G > A) were analyzed and the NAT2 phenotype was deduced from the genotype and individuals were classified as slow (S=carriers of two mutations), intermediate (IM=carriers of one mutation), or rapid (R=carriers of wild-type alleles only) acetylators. In *MPO*, the G-463A polymorphism in the promoter region of the *MPO* gene was determined. In sulfotransferase *SULT1A1*, the G to A transition in exon 7, leading to an Arg to His exchange at position 213, was analyzed.

Statistical methods

All genes were found to be in Hardy–Weinberg equilibrium, apart from *SULT1A1*, which displayed more variant heterozygotes than expected [6]. The Hardy–Weinberg disequilibrium was not dependent on method failure and furthermore, it did not influence the results. The genotypes of *GSTM1* and *GSTT1* were analyzed according to the inferred phenotype: present versus null. The effects of the other genes were assessed separately for each genotype apart from polymorphisms with rare variant alleles frequencies (in our material), which were grouped based on prior biological function: *CYP1A1**2A (A and B + C), *CYP1A1**2B (A/A and A/G + G/G), *GSTP1* 114 (Ala/Ala and Ala/Val, no participant was homozygous for the variant allele), *GSTM3* (AA and AB + BB), and the inferred phenotype S+IM versus R for *NAT1*.

Differences between genotypes regarding levels of TDA in plasma or in urine (samples obtained at the health examination) were examined using the Mann–Whitney test or the Kruskal–Wallis test, depending on number of genotypes. How the genotype modifies the relations between (i) TDI in air and TDA in urine, and (ii) TDA in plasma and TDA in urine, were tested using linear regression. Analysis (i) was performed with measures of TDA in urine samples collected at the same day as the air sampling, and (ii) was performed with TDA measures in the plasma and urine samples collected on the day of health examination. Stratified analyses were used to assess the beta coefficient for each genotype. Interaction was assessed in a separate analysis, with an interaction

term between genotype and exposure variable in order to account for multiplicative effect modification, by the formula: $\text{TDA in urine (ln)} = \alpha + \beta_1(\text{TDI/TDA in air/plasma}) + \beta_2(\text{genotype}) + \beta_3(\text{TDI/TDA in air/plasma (genotype)})$. In order to use linear regression analysis of this material, normally distributed residuals of the outcome variables were obtained by transformation by using the natural logarithm.

All models were analyzed both unadjusted and with adjustments for sex, age, and smoking (nonsmoker + former smoker vs. smoker), as the latter factors may influence the expression of the metabolic enzymes. For all analyses, two-sided significance was used. Statistical significance refers to $P \leq 0.05$ or, equivalently, to a 95% confidence interval for a difference that excludes the null hypothesis. We here show the unadjusted results and comment upon differences in statistical significance for adjusted analysis. We present only those results that were statistically, or borderline, significant. For all statistical analyses, SPSS v.15.0 (SPSS Inc., Chicago, Illinois, USA) was used.

Results

Direct genetic effects on biomarker levels

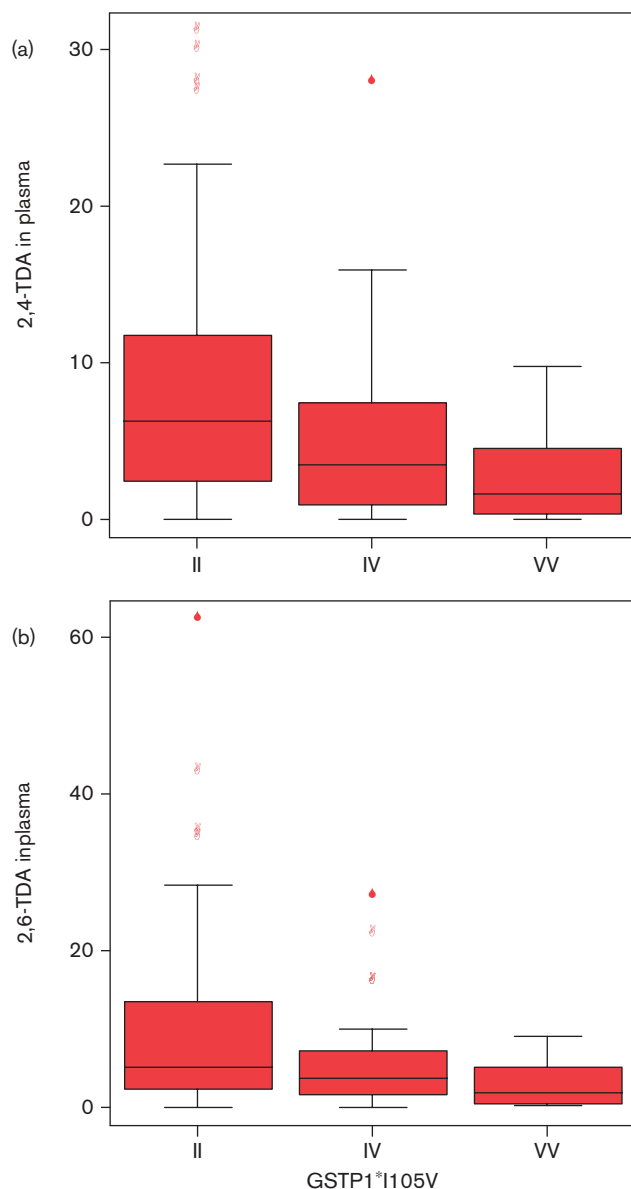
There was an allele-dosage effect of the Val-allele in *GSTP1* 105 for 2,4-TDA ($P = 0.005$) and 2,6-TDA ($P = 0.008$) in plasma, where ValVal-carriers showed the lowest levels (Fig. 1). There were no associations between air levels of 2,4-TDI and 2,6-TDI, sampled ± 14 days before health examination ($N = 70$ workers), and the different *GSTP1* 105 genotypes (data not shown). *CYP1A1**2A variant carriers showed increased levels of 2,4-TDA in plasma ($P = 0.060$) compared to homozygous wild-type carriers (median concentrations 6.4 vs. 4.3 $\mu\text{g/l}$) this could not be seen for 2,6-TDA. *GSTM1* null carriers showed significantly lower levels of 2,6-TDA (2.5 vs. 4.8 $\mu\text{g/l}$, $P = 0.011$), and of 2,4-TDA (1.5 vs. 3.0 $\mu\text{g/l}$, $P = 0.052$) in urine samples taken at the health examination.

Genetic effect modification

There were two types of analyses performed: how genotype influences the relation (i) between 2,4-TDI in air and 2,4-TDA in urine and (ii) between 2,4-TDA in plasma and 2,4-TDA in urine. The same analyses were performed for 2,6-TDI and 2,6-TDA.

For analysis (i) there was evidence of genetic effect modification for *GSTM1*: for 2,6-TDI in air versus 2,6-TDA in urine, *GSTM1* null carriers had a steeper regression coefficient ($\beta = 0.56$) than individuals with *GSTM1* gene present ($\beta = 0.34$, P value for interaction = 0.006). However, this association was dependent on one null carrier at higher TDI-exposure. For the other genotypes, there were no statistically significant associations (data not shown).

Fig. 1



Box plots displaying *GSTP1**1105V genotypes and plasma adducts of 2,4-TDA (a) and 2,6-TDA (b). TDA, toluene diamine. 'O' denotes outliers, that is, cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range. '*' denotes extreme values with values more than 3 box lengths from the upper or lower edge of the box.

For analysis (ii) the strongest finding was found for *GSTP1* 105, where an allele dosage effect was found for 2,4-TDA, as well as for 2,6-TDA, based on statistical analysis as well as visual inspection of the scatter plots (Table 2, Figs 2 and 3). *GSTP1* 105 ValVal had the steepest regression line and thus, with increasing plasma levels, higher urine levels were found relative IleVal and IleIle. The results were very similar when combining *GSTP1* 105 and *GSTP1* 114 (data not shown).

Furthermore, there were clear indications of genetic effect modification for *CYP1A1**2A, *GSTM1*, *GSTT1*, *NAT2*, and *MPO* (Table 2), showing statistically different regression lines for the genotypes. However, the visual inspection of the scatter plots revealed that a few individuals were very influential on the regression lines for those genes (data not shown). These findings were also valid when adjusting for sex, age, and smoking, apart from the effect of *GSTM1* on the relation between 2,4-TDA in plasma and in urine (Table 2, adjusted $P = 0.078$).

Discussion

Workers with the *GSTP1* 105 ValVal genotype had about half the levels of TDA in plasma compared to the IleIle carriers, whereas the IleVal carriers had intermediate levels. In addition, we found effect modification, in particular of the *GSTP1* 105 variant, on the association between biomarkers of TDI in plasma and in urine, suggesting that differences in TDI retention are associated with different genotypes. ValVal carriers showed about twice as steep regression slope of 2,4-TDA from plasma to urine, and an even steeper slope was seen for 2,6-TDA. This is, as far as we know, the first time effects of genetic variation have been shown on biomarkers of TDI.

It is not probable that the finding of genetic differences of TDI levels in plasma is because of exposure differences, as there was no difference between the three-genotype groups looking at air levels. Despite the fact that these air levels were not sampled at the same occasion and from only 70 of the workers, such a large difference in exposure would, if present, have been detected between the air levels as well.

The mechanism is not known by which the *GSTP1* 105 Val variant enzyme affects the TDI metabolism. Several in-vitro studies have shown a significantly reduced activity for 105 Val variant allozyme relative wild-type enzyme with 1-chloro-2,4-dinitrobenzene as substrate [23–25]. Those studies indicate that 105 Val alters the active site of the enzyme and, as a result, substrate specificity. On the basis of this finding, one could speculate that the 105 Val variant may be better at conjugating GSH to TDI, compared to the 105 Ile. Hypothetically, a better conjugation activity among Val carriers could result in less free TDI in plasma that could form protein adducts. An alternate explanation could be that it is the GSH-TDI conjugate that forms the protein adducts mediated by the GST system and that 105 Val has a lower activity in this reaction resulting in lower levels of protein adducts. The effect of the *GSTP1* 105 genotype was in our study obvious both for 2,4-TDA and 2,6-TDA, possibly reflecting no large differences in substrate specificity between 2,4-TDI and 2,6-TDI for the 105 Val allozyme. The strongest effect was found for *GSTP1* 105, and thus, *GSTP1* 114 appeared less

Table 2 Genetic effect modifications on the association between 2,4-TDA in plasma and 2,4-TDA in urine, and on the association between 2,6-TDA in plasma and 2,6-TDA in urine

Gene	Isomer	Genotype	β -coefficient	95% CI		<i>P</i> value*
				Lower	Upper	
<i>CYP1A1*2A</i>	2,4	A	0.21	0.18	0.25	<0.001
		B+C	0.089	0.019	0.16	
<i>CYP1A1*2A</i>	2,6	A	0.12	0.097	0.14	0.004
		B+C	0.057	0.013	0.10	
<i>CYP1A1*2B</i>	2,4	AA	0.19	0.16	0.22	0.15
		AG+GG	0.62	-0.68	1.91	
<i>CYP1A1*2B</i>	2,6	AA	0.10	0.082	0.12	0.19
		AG+GG	0.57	-1.31	2.45	
<i>GSTA1-52</i>	2,4	GG	0.22	0.17	0.28	0.47
		AG	0.18	0.14	0.23	
		AA	0.17	0.12	0.23	
<i>GSTA1-52</i>	2,6	GG	0.12	0.078	0.17	0.71
		AG	0.099	0.072	0.13	
		AA	0.10	0.059	0.15	
<i>GSTM1</i>	2,4	+	0.16	0.12	0.20	0.043
		-	0.22	0.17	0.27	
<i>GSTM1</i>	2,6	+	0.078	0.056	0.10	0.002
		-	0.14	0.11	0.18	
<i>GSTM3</i>	2,4	AA	0.21	0.17	0.24	0.067
		AB+BB	0.15	0.11	0.20	
<i>GSTM3</i>	2,6	AA	0.10	0.08	0.13	0.69
		AB+BB	0.10	0.066	0.12	
<i>GSTP1 105</i>	2,4	II	0.17	0.13	0.21	0.007
		IV	0.26	0.21	0.32	
		VV	0.35	0.19	0.51	
<i>GSTP1 105</i>	2,6	II	0.094	0.070	0.12	<0.001
		IV	0.16	0.11	0.21	
		VV	0.49	0.33	0.65	
<i>GSTP1 114</i>	2,4	AA	0.19	0.16	0.22	0.10
		AV	0.32	0.21	0.43	
<i>GSTP1 114</i>	2,6	AA	0.10	0.081	0.12	0.43
		AV	0.14	0.052	0.22	
<i>GSTT1</i>	2,4	+	0.18	0.15	0.21	0.15
		-	0.24	0.16	0.32	
<i>GSTT1</i>	2,6	+	0.094	0.072	0.12	0.026
		-	0.18	0.12	0.23	
<i>MPO</i>	2,4	GG	0.19	0.15	0.22	0.10
		AG	0.19	0.12	0.26	
		AA	0.19	0.11	0.27	
<i>MPO</i>	2,6	GG	0.13	0.10	0.16	0.019
		AG	0.072	0.043	0.10	
		AA	0.083	0.001	0.17	
<i>NAT1PH</i>	2,4	IM+S	0.18	0.15	0.22	0.69
		R	0.20	0.14	0.25	
<i>NAT1PH</i>	2,6	IM+S	0.095	0.074	0.12	0.17
		R	0.13	0.079	0.18	
<i>NAT2PH</i>	2,4	S	0.21	0.18	0.25	0.016
		IM	0.13	0.078	0.17	
		R	0.26	0.12	0.40	
<i>NAT2PH</i>	2,6	S	0.099	0.074	0.12	0.51
		IM	0.12	0.078	0.16	
		R	0.074	0.003	0.15	
<i>SULT1A1</i>	2,4	GG	0.19	0.13	0.24	0.81
		AG	0.19	0.15	0.23	
		AA	0.15	0.050	0.26	
<i>SULT1A1</i>	2,6	GG	0.12	0.084	0.16	0.61
		AG	0.096	0.072	0.12	
		AA	0.097	-0.005	0.20	

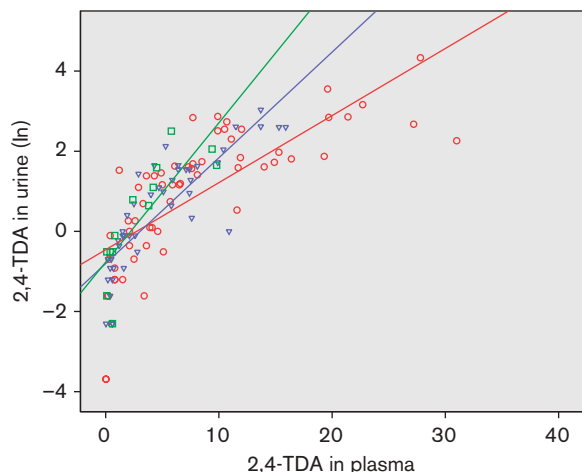
CI, confidence interval; MPO, myeloperoxidase; SULT, sulfotransferase; TDA, toluene diamine.

**P* value for interaction.

important for TDI metabolism, in line with what has been shown for other substrates of GSTP1. In contrast, for 4,4'-diphenylmethane di-isocyanate, *GSTP1* 114 may in particular influence the relation between the 4,4'-diphenylmethane di-isocyanate-related amine biomarker in plasma and urine [26].

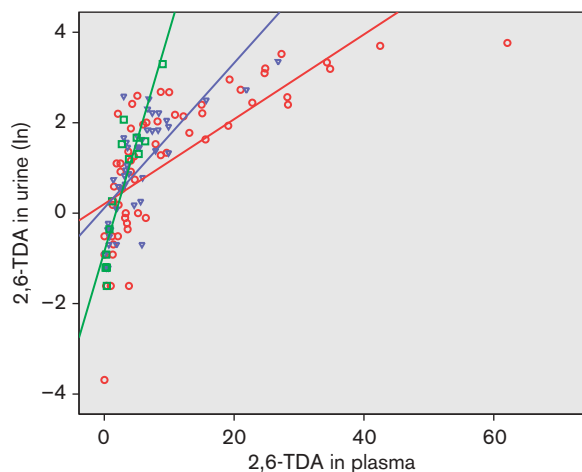
The association between plasma and urine is thought to reflect the slow phase of the TDI metabolism and elimination, whereas the association between air and urine reflects the fast phase of TDI metabolism [20]. Our finding, that the genetic effect was more pronounced between plasma and urine, indicate that the genetic

Fig. 2



Scatter plot depicting the associations between 2,4-TDA in urine and 2,4-TDA in plasma. Influence of the *GSTP1* I105V polymorphism is shown with regression lines for the different genotypes (Ile/Ile genotype is shown with ○; Ile/Val with ▽; and Val/Val with □). Ile, isoleucine; TDA, toluene diamine; Val, valine.

Fig. 3



Scatter plot depicting the associations between 2,6-TDA in urine and 2,6-TDA in plasma. Influence of the *GSTP1* I105V polymorphism is shown with regression lines for the different genotypes (Ile/Ile genotype is shown with ○; Ile/Val with ▽; and Val/Val with □). Ile, isoleucine; TDA, toluene diamine; Val, valine.

variation is more important for the slow than for the fast phase of TDI metabolism and elimination. Still, this needs to be carefully interpreted. In this type of cross-sectional study, the urine level is a sum measure of the different phases of the TDI metabolism.

In our earlier study on genetic modification on TDI-related symptoms, *GSTP1* 105 modified the effect of TDI-exposure on the symptom cough with mucus, which may represent an early indication to TDI-related asthma:

GSTP1 Val variant carriers were protected among exposed workers compared to controls where variant carriers were at increased risk [6]. Similarly, Mapp *et al.* [27] reported that the Val-allele was underrepresented among patients with TDI-related asthma. This is in line with the findings of this study, where *GSTP1* 105 Val-carriers showed lower levels of plasma protein adducts, indicating faster elimination of 2,4- and 2,6-TDA; this was also suggested by the genetic effect modification analysis. However, data are not unambiguous: Piirilä *et al.* [28] did not find a protective effect of the *GSTP1* 105 Val allele against TDI-asthma induced by isocyanates. In our earlier study, *CYP1A1* variant carriers had a pattern of increased risk of several symptoms [6]. Here, variant carriers demonstrated slower elimination from plasma to urine, but this requires confirmation, as it was based on very few individuals with variant genotypes. In our earlier study, we also saw a protective effect of *SULT1A1* on several of the symptoms, but there was no proof of genetic effect modification for *SULT1A1* on the metabolism of TDI. This is not surprising, as this protective effect was found among the controls as well, indicating an effect of *SULT1A1* irrespective of exposure. There was little indication of influence of the NAT system on TDI metabolism, and neither did NAT modify symptoms of the lower airways in our study on genetic influences on TDI-related symptoms [6]. Wikman *et al.* [29] reported increased risk for TDI-asthma in *NAT1* slow carriers, but because of few individuals with *NAT1* slow, the effect of this genotype on TDI metabolism could not be evaluated here.

The data of this study suggest that, in particular, the GST system is of importance for the metabolism of TDI. In addition to the strong effect of *GSTP1*, the importance of the GST system is also indicated by the finding of significant effect modification in individuals lacking *GSTM1*. The finding of effect modification of *CYP1A1* variants is difficult to evaluate because of few individuals with variant alleles. Our findings of influence of the GST but not of the NAT system, indicates that it is the isocyanate and not its amine that is the causative agent for the TDI-related symptoms. Still, the fact that several genotypes have been analyzed on a number of outcome variables increases the possibility of false-positive findings. Therefore, it is important to cautiously interpret the findings, in particular for the associations based on few individuals with rare genotypes. Thus, we have carefully evaluated the data by statistical analysis as well as by visual inspection.

The findings from this study suggest that *GSTP1* genotype should be taken into consideration when evaluating the biomarkers. Moreover, it is important to further investigate whether *GSTP1* Ile/Ile carriers may be at increased risk for symptoms of the lower airways at exposure levels well below (the Swedish) occupational exposure limit values.

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