

Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma

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Background: The factors contributing to the oxidant/antioxidant imbalance in asthma are incompletely understood.

Objective: To determine the factors associated with oxidative stress including asthma severity and the genotype of the antioxidant enzymes.

Methods: A total of 196 children with mild asthma, 116 children with moderate-severe asthma, and 2 healthy control groups (187 and 68 children) were included in the study. Plasma levels of malondialdehyde were measured as the indicator of oxidative stress, and reduced glutathione levels as the indicator of antioxidant defense. Children were genotyped for null variants of *glutathione S transferase (GST) T1* and *GSTM1*, and ile105val variant of *GSTP1*. Risk factors were analyzed with multivariate logistic regression.

Results: Systemic levels of malondialdehyde increased and reduced glutathione levels decreased significantly from healthy controls to patients with mild asthma and then to patients with moderate-severe asthma ($P < .001$ for each). Multivariate logistic regression identified asthma and asthma severity as independent factors associated with oxidative stress (odds ratio between 17 and 56; $P < .001$). Children with asthma with *GSTP1* val/val genotype had higher malondialdehyde and lower glutathione levels compared with other genotypes ($P = .023$ and $P = .014$, respectively). *GSTP1* val/val genotype was independently associated with asthma severity (odds ratio, 4.210; 95% CI, 1.581-11.214; $P = .004$).

Conclusion: Our study indicates the presence of a strong oxidative stress in children with asthma that increases with the severity of the disease. In this population, val/val genotype at *GSTP1* ile105val locus may be an important factor in determining the degree of oxidant injury.

Clinical implications: Children with asthma with val/val genotype at *GSTP1* ile105val locus may be good candidates for

supplemental antioxidant therapy. (J Allergy Clin Immunol 2006;118:1097-104.)

Key words: Asthma, genotype, glutathione, glutathione *S* transferase, malondialdehyde, oxidation, severity

Asthma is a disease of chronic airway inflammation.¹ The cells infiltrating the bronchial mucosa in patients with asthma produce a variety of mediators including reactive oxygen species.² Increased production of reactive oxygen species leading to an imbalance between the oxidative forces and the antioxidant defense systems favoring an oxidative injury has been implicated in the pathogenesis of asthma.^{3,4} Reactive oxygen species such as superoxide radical, hydrogen peroxide, and hydroxyl radical are capable of producing a variety of pathological changes that are highly relevant in asthma. These include lipid peroxidation, increased airway reactivity and secretions, production of chemoattractant molecules, and increased vascular permeability.^{5,6} These changes lead to increased mediator release from the epithelium resulting in recruitment of immune effector cells, which further increase the oxidative damage.⁷

The evidence for oxidative injury in asthma comes from both systemic and local studies. Locally, elevated hydrogen peroxide and nitric oxide levels have been noted in the exhaled breath of patients with asthma,⁸⁻¹⁰ and inflammatory cells in the airways such as macrophages and eosinophils were shown to produce elevated amounts of reactive oxygen species.^{11,12} A similarly increased production of reactive oxygen species was shown for eosinophils and macrophages obtained from the peripheral blood of patients with asthma.¹³⁻¹⁵ Systemically, many studies have shown marked changes in the markers of oxidation, such as glutathione and malondialdehyde. Glutathione, in its reduced form, can chemically detoxify hydrogen peroxide and is especially effective in protecting airway epithelial cells from free radical attack.^{16,17} This reaction is catalyzed by glutathione peroxidase and leads to the formation of oxidized glutathione. Thus, oxidative stress is associated with increases in the oxidized but reduction in the reduced form of glutathione. In fact, oxidized glutathione was higher in the bronchoalveolar lavage, induced sputum, and erythrocyte hemolysates of patients with asthma.¹⁸⁻²⁰ Malondialdehyde, on the other hand, is formed as a result of the action of reactive oxygen species on membrane

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Supported by Hacettepe University grant #02 02 101 020.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication February 4, 2006; revised August 4, 2006; accepted for publication August 9, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.08.012

Abbreviations used

ECSOD: Extracellular superoxide dismutase
 GST: Glutathione S transferase
 OR: Odds ratio
 SNP: Single nucleotide polymorphism

phospholipids and is an indirect measure of oxidative stress.^{21,22} We have previously shown that malondialdehyde (MDA) is increased in the sera of children with asthma compared with controls and is further elevated during an asthma exacerbation.²³

To counteract the oxidative stress, the human lung and blood are equipped with a wide array of enzymatic antioxidant defenses including glutathione S transferases (GSTs) and superoxide dismutase. GSTs (E.C. 2.5.1.18) are a superfamily of enzymes consisting of α , κ , μ , π , δ , θ , ζ , and Ω families in humans. Within this family, *GSTM1* (μ), *GSTT1* (θ), and *GSTP1* (π) are strongly expressed in the lung, are involved in antioxidant defense pathways, and have common functional variant alleles.²⁴⁻²⁷ Both *GSTM1* and *GSTT1* have 2 alleles: a wild-type allele and a variant null allele that results in the absence of functional activity.²⁸ *GSTP1*, on the other hand, has 4 alleles formed from 2 linked single nucleotide polymorphisms (SNPs) in codons 105 (ile-val) and 114 (ala-val).²⁸ Because val105 and val114 show significant linkage disequilibrium and val114 does not seem to affect the function of 105 variant, association studies have generally concentrated on the genotype at codon 105. Associations between homozygosity for the deleted *GSTM1* and *GSTT1* null alleles, *GSTP1* val allele, and allergic diseases and asthma have been previously investigated in various populations.²⁹⁻³¹

Another important enzyme in the antioxidant defense is superoxide dismutase.³² A SNP leading to arginine glycine change (Arg213Gly) in extracellular superoxide dismutase (ECSOD) changes the heparin binding affinity of the molecule and leads to decreased ECSOD activity in the extracellular space.^{33,34} This polymorphism was reported to increase significantly the risk of asthma in a Finnish population.³⁵

Even though many studies have established the presence of oxidative stress in asthma, the determinants of oxidative stress in children and especially the contribution of the genotypes of the antioxidant enzymes to the observed oxidative stress are incompletely understood. Therefore, we aimed to determine the systemic oxidative stress and its determinants in children. For this purpose, we measured systemic markers of oxidation, glutathione and MDA, in children, involving those with mild and moderate-severe asthma and healthy controls. In this cohort, we attempted to define the factors affecting the level of oxidative stress including the demographic variables; asthma diagnosis; asthma phenotypes such as eosinophil counts, total IgE, atopy, and FEV₁; asthma severity; and *GST* and *ECSOD* genotypes.

METHODS**Study population**

Patients with asthma. The patients in the asthma group have been detailed previously.^{36,37} Each child with asthma was then classified according to the severity of asthma precisely as detailed in Global Initiative for Asthma guidelines³⁸ and as detailed previously.^{36,37}

From this cohort of white children with asthma, samples of 200 children with mild asthma age 6 to 16 years who were not receiving any controller medication and who had not had any symptoms of lower or upper respiratory tract infection or asthma exacerbation within the previous 4 weeks were randomly selected. This group formed the mild asthma group. All children who had moderate-severe asthma within this cohort were included in the study to form the moderate-severe asthma group. Spirometric measurements, total IgE, and eosinophil counts were obtained, and skin testing was performed with a battery of 40 antigens including 30 aeroallergens and 8 food allergens on the upper back of the children. Reactions with an induration >3 mm that of the negative control were considered positive, and children with at least 1 positive test result were considered atopic. DNA was extracted from whole blood by standard techniques.

Healthy controls. To ascertain that the results of the comparisons between the healthy controls and children with asthma were not caused by a possible selection bias, we recruited 2 separate control populations. Both control groups were composed of white Turkish schoolchildren who responded negatively to an established and validated asthma questionnaire,³⁹ never had any diagnosis of asthma or allergic bronchitis by a physician, and never had any history of wheezing. All children underwent skin prick testing and had their total IgE measured in serum as described previously.⁴⁰

The samples for the first control group (control group 1) were obtained from a cohort belonging to a previously published study in the Ankara region.⁴⁰ All children in this control group underwent a standard hypertonic saline challenge,⁴¹ and 16 children who had no history of respiratory symptoms or asthma diagnosis but who showed a positive response (>15% fall in FEV₁) were also included in the control group.

The samples for the second control group (control group 2) were obtained from children who presented between July 2005 and May 2006 to the outpatient department of the same hospital where the children with asthma were recruited. They presented for reasons such as minor trauma or for their regular follow-up. We have made every effort to recruit all consecutive healthy children within this period. They all had normal pulmonary function tests.

All children in the asthma and control groups were genotyped for *GSTM1* and *GSTT1* (wild-type vs null) genotypes and for the presence of the SNP in codon 105 (ile105val) of *GSTP1*.

All study procedures were performed in accordance with a protocol previously approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent for the study procedures.

Study measurements

Reduced glutathione. Plasma levels of reduced glutathione were used as a measure of the systemic antioxidant defense as previously described.⁴² In this method, sulfhydryl groups of the reduced glutathione reacts with 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman reagent) and forms 5-thio-2-nitrobenzoic acid. The concentration is calculated from the OD measured at 405 nm.

Malondialdehyde. Plasma malondialdehyde level was used as an indirect measure of systemic oxidative stress using a HPLC-based method as previously described.⁴³

The person performing the reduced glutathione and malondialdehyde assays was blind to the group to which the patient belonged.

TABLE I. Characteristics of the study population

	Mild asthma (n = 196)	Moderate-severe asthma (n = 116)	Control group 1 (n = 187)	Control group 2 (n = 68)	P*	P†
Sex						
Female (%)	88 (45)	69 (60)	90 (48)	43 (63)	.04‡	.007‡
Male (%)	108 (55)	47 (40)	97 (52)	25 (37)		
Age (y)§	9.7 (8.1-11.2)	11.3 (8.5-13.2)	9.6 (9.4-9.9)	10.9 (8.5-13.1)	<.001	.001
IgE	171 (55-475)	210 (48-652)	44 (19-86)	35 (13-66)	<.001	<.001
Skin test						
Positive (%)	125 (64)	81 (70)	28 (15)	7 (10)	<.001‡	.007‡
Negative (%)	71 (36)	35 (30)	159 (85)	61 (90)		
FEV ₁ %§	97 (90-104)	79 (72-92)	111 (103-120)	98 (90-103)	<.001	<.001
Eosinophils/mm ³ §	292 (160-450)	390 (190-640)	Not available	138 (95-249)	—	<.001

*Among control group 1, mild asthma, moderate-severe asthma.

†Among control group 2, mild asthma, moderate-severe asthma.

‡By χ^2 .

§Median (interquartile range).

||By ANOVA on ranks.

Spirometry. Spirometry was performed using a dry rolling spirometer in the asthma group and control group 2 (2130 Spirometer; Sensor Medics Co, Yorba Linda, Calif) and a portable spirometer (Masterscope Version 4.1; Jaeger Toennis, Hoeberg, Germany) in control group 1. Both spirometers conformed to the specifications set forth by the American Thoracic Society.

IgE levels. IgE levels were measured in duplicate with UniCap technology in accordance with the specifications of the manufacturer (Pharmacia, Kalamazoo, Mich).

Eosinophil counts. Eosinophil counts were determined from Coulter Counter (Beckman Coulter, Inc., Fullerton, Calif) leukocyte measurements. Eosinophil count was not available in control group 1.

Genotyping

A multiplex PCR method was used to determine the wild or null genotype at *GSTM1* and *GSTT1* genes as previously described^{44,45} except that the PCR reactions for *GSTM1* and *GSTT1* were performed in separate mixtures. β -Globin gene was used as the positive control for both assays and yielded a 389-bp fragment of the *globin* gene. *GSTP1* Ile105Val genotype⁴⁶ and *ECSOD* Arg213Gly³⁵ polymorphisms were determined by PCR-RFLP according to previously described methods.

Statistical analyses

Statistical analysis was performed with Sigma Stat 2.0 (Systat Software Inc., Point Richmond, Calif) and SPSS 11.5 (SPSS, Chicago, Ill) for Windows. Hardy-Weinberg equilibrium and allele and genotype frequencies between subjects with asthma and control subjects were compared with the χ^2 statistic. All data including age, eosinophil count, IgE levels, and FEV₁ showed a nonnormal distribution; therefore, data are given as medians and interquartile ranges, and all statistical comparisons were performed by using nonparametric Mann-Whitney *U* test or ANOVA on ranks. For pairwise comparisons of the nonnormally distributed data, Mann-Whitney *U* test with Bonferroni correction was used. All comparisons were adjusted for possible confounding factors, including age and sex, and asthma severity where appropriate. A *P* value < .05 was considered significant.

Logistic regression was performed to establish the factors that were associated with oxidative stress and asthma severity. We examined the following variables: age, sex, age of onset, skin test positivity, IgE level, eosinophil counts, smoke exposure, animal ownership, family history of atopic diseases, asthma diagnosis,

asthma severity, and polymorphisms at *GSTM1*, *GSTT1*, and *GSTP1* genes. Factors that showed a significant association in the univariate analysis were included in the multivariate logistic regression to determine the variables showing an independent association. In this model, we treated the determinants of oxidative stress as dichotomous variables using a median split because various transformation techniques failed to normalize the malondialdehyde and reduced glutathione levels to allow the use of linear regression models. A 2-sided *P* value < .05 was considered significant.

RESULTS

Two hundred children with mild asthma, 118 children with moderate-severe asthma, and 187 healthy controls in control group 1 and 68 children in control group 2 were initially included in the study. The study population consisted of 196 children with mild asthma, 116 children with moderate-severe asthma (107 moderate and 9 severe), and all healthy controls who could be successfully genotyped in at least 1 locus. Characteristics of the study population are summarized in Table I. There was a significant difference among the age and the sex of the groups, resulting basically from the older age and different sex distribution of the moderate-severe asthma group.

Association among glutathione transferase genotypes, asthma diagnosis, asthma phenotypes, and asthma severity

The distribution of each of the *GSTM1*, *GSTT1*, and *GSTP1* genetic variants met the conditions of the Hardy-Weinberg equilibrium. A significant difference was observed in the distribution of *GSTP1* ile105val genotype between children with mild asthma and children with moderate-severe asthma (Table II). This finding was further supported by our logistic regression analysis, which showed that the val/val genotype of *GSTP1* was independently associated with asthma severity (Table III).

TABLE II. Distribution of *GST* genotypes in the study population*

	Mild asthma	Moderate-severe asthma	Control group 1	Control group 2	<i>P</i> [†]	<i>P</i> [‡]
<i>GSTT1</i>						
Null	38 (0.194)	26 (0.228)	41 (0.223)	7 (0.106)	NS§	NS
Wild-type	158 (0.806)	88 (0.772)	143 (0.777)	59 (0.894)		
<i>GSTM1</i>						
Null	81 (0.431)	43 (0.374)	65 (0.355)	35 (0.515)	NS	NS
Wild-type	107 (0.569)	72 (0.626)	118 (0.645)	33 (0.485)		
<i>GSTP1</i>						
ile/ile	104 (0.531)	70 (0.603)	95 (0.508)	33 (0.516)	.010	.021
ile/val	84 (0.429)	32 (0.276)	73 (0.390)	25 (0.391)		
val/val	8 (0.041)	14 (0.121)	19 (0.102)	6 (0.094)		

*Statistical comparisons are by χ^2 and are controlled for age and sex.[†]Among control group 1, mild asthma, moderate-severe asthma.[‡]Among control group 2, mild asthma, moderate-severe asthma.

§Not significant.

||Codominant model.

TABLE III. Factors affecting disease severity in children with asthma

	Univariate			Multivariate		
	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>
Age (y)	1.2	1.1-1.3	<.001	1.2	1.1-1.3	.001
Female sex	1.8	1.1-2.9	.013			>.05
Age of onset	1.2	1.1-1.3	<.001	1.2	1.0-1.3	.005
Skin test positivity	1.3	0.8-2.2	>.05			
Total IgE (kU/L)	1.0	1.0-1.0	.032			>.05
Eosinophil count	1.0	1.0-1.0	.002	1.0	1.0-1.0	.003
Family history	1.3	0.8-2.1	>.05			
Smoke exposure	1.4	0.9-2.2	>.05			
Animal ownership	2.7	0.9-7.7	>.05			
<i>GSTT1</i> null	1.2	0.7-2.1	>.05			
<i>GSTM1</i> null	0.8	0.5-1.3	>.05			
<i>GSTP1</i> val/val	3.2	1.3-8.0	.011	4.2	1.6-11.2	.004

Our analysis of the relationship between the glutathione transferase genotypes and asthma phenotypes including IgE, eosinophil numbers, and FEV₁ failed to show any association within the population with asthma.

Systemic levels of malondialdehyde and reduced glutathione

Plasma assays were basically limited by the availability of the samples and could be performed in 93% of cases in control group 1 but in all children in control group 2. There was a highly significant increase in the systemic oxidant stress (increased plasma malondialdehyde) and a similar decrease in the antioxidant defense (decreased reduced glutathione) in patients with mild asthma compared with controls, and a similarly marked difference was also observed between patients with mild and moderate-severe asthma (Fig 1, A and B). There was a very strong and highly significant correlation between plasma malondialdehyde and glutathione levels ($P < .001$; $r = -0.74$).

The effect of the genotypes of antioxidant enzymes on systemic levels of malondialdehyde and reduced glutathione

Genotyping of 50 children with asthma and 50 healthy children failed to detect any child with the arg213gly polymorphism in the *ECSOD* gene in this population and was therefore not studied any further. The relationship between *GST* genotypes and malondialdehyde and reduced glutathione levels was investigated in the whole cohort (asthma and controls), within the asthma cohort, and within the control populations, separately. The only association was within the cohort of children with asthma. No association was observed in the control population and in the whole cohort between the *GSTP1* genotypes and markers of oxidative stress (data not shown). Within the population with asthma, after stratification by asthma severity, those with the *GSTP1* val/val genotype had a stronger systemic oxidative burden with higher malondialdehyde and lower glutathione levels (Fig 2, A and B). The difference was basically attributable to the homozygosity

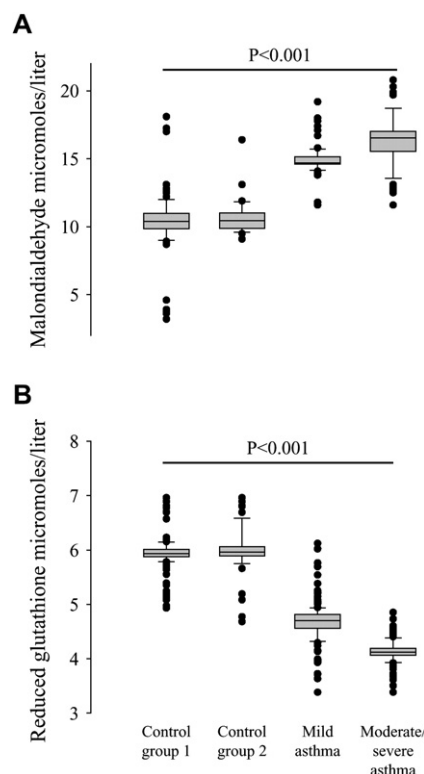


FIG 1. Malondialdehyde (A) and reduced glutathione (B) levels in the study populations. Results are expressed as medians and interquartile ranges. The differences among the 4 groups are analyzed by ANOVA on ranks. Significant pairwise differences are observed between each control group and mild asthma ($P < .001$ for each), and between mild asthma and moderate-severe asthma groups ($P < .001$). Results are controlled for age and sex.

for the val allele and was more apparent in a model in which the more frequent isoleucine allele was taken as the dominant (Fig 3, A and B). Other genotypes failed to show any association. The combination of the genotypes did not change the observed association.

Factors influencing the malondialdehyde and glutathione levels

In determining the factors influencing the malondialdehyde and reduced glutathione levels using logistic regression, we have taken 2 separate approaches. First, we have performed our analysis in the whole cohort including both subjects with asthma and healthy controls (control group 1 only). Secondly, in a separate analysis, to detect the effect of asthma severity on markers of oxidation, we have restricted our analysis to children with asthma.

Within the whole cohort, we found that the asthma diagnosis is the only variable influencing malondialdehyde levels to be above the median (14.6 mmol/L; odds ratio [OR], 18.0; 95% CI, 10.8-30.0; $P < .001$) and reduced glutathione levels to be below the median (5.43 mmol/L; OR, 56.2; 95% CI, 22.1-142.6; $P < .001$).

Within the cohort of children with asthma, disease severity emerged as the only significant determinant for malondialdehyde levels to be above the median (15.1

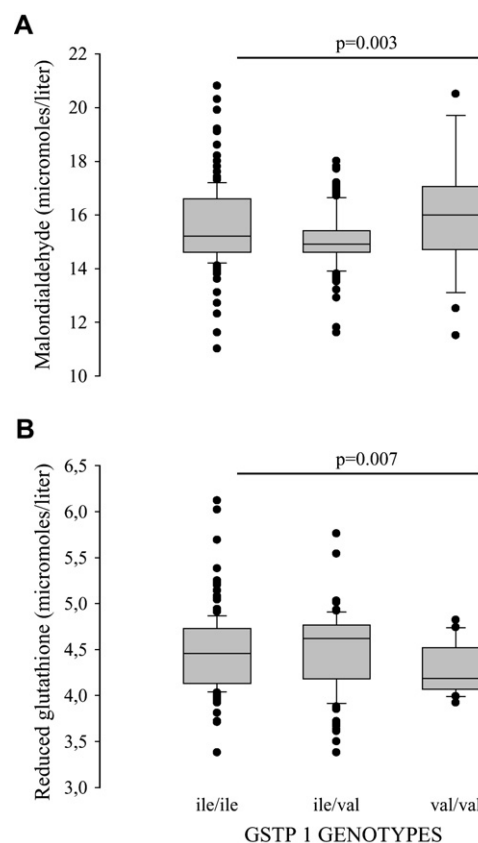


FIG 2. Malondialdehyde (A) and reduced glutathione (B) levels in children with asthma stratified by *GSTP1* ile105val (ANOVA on ranks). Results are controlled for age, sex, and asthma severity.

mmol/L; OR, 14.0; 95% CI, 7.8-25.2; $P < .001$) and for reduced glutathione levels to be below the median (4.49 mmol/L; OR, 44.7; 95% CI, 21.3-93.8; $P < .001$). In further support of this finding, logistic regression analysis, where FEV₁ was treated as continuous independent variable, showed that FEV₁ was significantly associated with malondialdehyde levels to be above the median (OR, 0.95; 95% CI, 0.94-0.97; $P < .001$) and with reduced glutathione levels to be below the median (OR, 0.93; 95% CI, 0.91-0.95; $P < .001$).

DISCUSSION

Our study indicates that asthma is associated with a strong systemic oxidative stress that increases in parallel with the severity of the disease. The sharp increase in the oxidant stress is clearly evident from the fact that there is only very little overlap in the malondialdehyde and reduced glutathione levels between the population with asthma and healthy controls. The increased oxidative burden is a result both of increased oxidative stress as evidenced by increased malondialdehyde and of the decreased antioxidant capacity as evidenced by the lowered glutathione. The strong and significant negative correlation

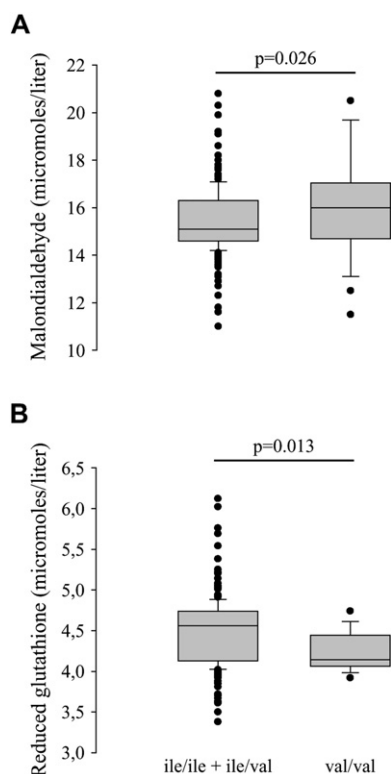


FIG 3. Malondialdehyde (**A**) and reduced glutathione (**B**) levels in children with asthma in a model in which the more frequent isoleucine allele of the *GSTP1* ile105val is taken as the dominant allele (Mann-Whitney *U* test). Results are controlled for age, sex, and asthma severity.

observed between the markers suggests that a common mechanism may lead to the observed changes in the oxidant/antioxidant system. The patients with mild asthma were not on any controller medication, whereas the majority of our patients with moderately severe asthma were on inhaled corticosteroids. Inhaled corticosteroids have been reported to decrease plasma malondialdehyde levels even after a short course of treatment.⁴⁷ Therefore, in a population with moderately severe asthma not currently on inhaled steroids, one might expect an even stronger oxidative burden.

Oxidative stress is key component of inflammation and inflammatory disorders. Host antioxidant systems are generally activated in response to the oxidant attack, but individuals have different capacities of antioxidant defense, which are in part genetically determined.⁶ One of the major foci of our study was to investigate the determinants of increased oxidative stress, including the genetic variants of antioxidant enzymes in a large cohort of children with asthma. For this purpose, we genotyped our population for the presence of *ECSOD* and *GST* polymorphisms. Genotyping of 100 children did not reveal any individual carrying the variant *ECSOD* allele. This is in keeping with other studies showing this variant to be rare or nonexistent in other populations. Juul et al,⁴⁸ for example, recently reported that *ECSOD* Arg213Gly is

quite rare in a general Danish population; 2.4% of the 9258 individuals were heterozygotes, and only 0.02% were homozygotes. In an Italian population,⁴⁹ Arg213Gly polymorphism was not found at all.

Unlike *ECSOD* Arg213Gly polymorphism, polymorphisms at the *GSTM1*, *GSTT1*, and *GSTP1* were found with considerable frequency. However, none of them turned out to be a risk factor for asthma in this population. These results are in disagreement with those of some previous studies, which suggested association between genotypes of *GSTM1* and *GSTT1* and susceptibility to asthma,^{30,50,51} but are in agreement with the results of a recent Czech study.⁵² Many factors could account for the observed discrepancies among these studies. Apparently, the racial and environmental differences among the populations might be highly significant factors. However, the results of our study also differ from a recent study that investigated *GST* gene polymorphisms in asthma in a Turkish population.³⁰ This study found disease associations between the *GSTM1* null genotype, *GSTT1* null genotype, and *GSTP1* val/val genotype in a case-control study involving adult patients with asthma. Even though age may be a factor, the exact reasons underlying the observed differences remain to be determined.

In our study, the populations from which cases and control group 1 were selected were different; therefore, it may be argued that the comparisons may be prone to selection bias. To address this issue, we recruited a second healthy population. This population was composed of children who presented to the same hospital as the children with asthma for reasons such as minor trauma or immunizations. Comparisons of genotype frequencies, malondialdehyde levels, and reduced glutathione levels between this second control group and the population with asthma produced basically similar results to control group 1. Finding consistent results substantially reduces the likelihood that these associations occurred by chance.

Functionally, in contrast with the studies by Holla et al⁵² and Gilliland et al,²⁸ who reported that *GSTM1* null variant was associated with deficit in annual growth rates for forced vital capacity and FEV₁ in children, we found that children with the *GSTP1* val/val genotype had more severe asthma compared with other genotypes (Table II). This finding is also supported by the results of multivariate logistic regression analysis of the factors influencing asthma severity, which showed that val/val genotype is independently and significantly associated with almost a 4-fold increase in the risk of more severe asthma. There is no difference in the frequency of the val/val genotype between the children with asthma and healthy children. In fact, the frequency of the val/val genotype in healthy children lies between healthy controls and moderate-severe asthma. This finding suggests that, in our cohort, the variants in the *GSTP1* gene do not cause asthma but may alter the severity of asthma through augmenting the oxidative burden in individuals who already have asthma by virtue of their genetic makeup and environmental exposures. This observation may have implications regarding the role of antioxidant treatment as an

adjunct to anti-inflammatory treatment in children with asthma.

Our study provides some evidence about how the *GSTP1* val/val genotype might be associated with more severe asthma, linking this polymorphism to increased oxidative stress. Hu et al,⁵³ using site-directed mutagenesis of the cDNA followed by bacterial expression and purification of the proteins, have previously shown that isoenzymes with valine in position 105 were more effective with the diol epoxides of polycyclic aromatic hydrocarbons but less effective with 1-chloro-2,4-dinitrobenzene than the isoforms with isoleucine 105. Our study shows that, *in vivo*, the final effect of val/val polymorphism of *GSTP1* in children with asthma is similar to its effect when it uses 1-chloro-2,4-dinitrobenzene as its substrate and is thus associated with increased oxidative stress as shown by higher malondialdehyde and lower reduced glutathione levels in children with the val/val genotype.

The major sources of oxidative stress in asthma are the inflammatory cells in the airways and in the intravascular compartment, including macrophages, eosinophils, lymphocytes, neutrophils, and monocytes.^{11-15,54} There was no association between the eosinophil counts and the oxidant stress in our study. Our study was not designed to investigate and does not provide any clues regarding the source of increased oxidative stress in children with asthma.

To minimize the risk of selection bias, we have recruited consecutive patients and a second group of controls who presented to the same hospital. For common diseases such as asthma, however, clinic-based populations are less desirable than community-based or population-based samples because they may be prone to selection bias. Therefore, it is still possible that healthy controls may not be perfect representatives of the population from which cases arose.

To conclude, our study provides strong evidence for a distorted oxidant/antioxidant balance even in mild asthma that increases in parallel with the severity of the disease. The data also suggest that in this population, *GSTP1* polymorphisms may be an important factor in determining the degree of oxidant injury in children with asthma.

We thank Professor Yildiz Saraclar for providing the DNA samples from control group 1.

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