Association of glutathione-S-transferase P1 genotypes with susceptibility to bronchial asthma in children

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Abstract

Introduction: Asthma is characterized by airway inflammation, a critical component of which is oxidative stress. We describe an association study designed to examine whether allelic variation at the glutathione-S-transferase GSTP1 locus influences expression of asthma.

Material and methods: Fifty asthmatic children, thirty-five atopic and fifteen nonatopic, with forty healthy controls were enrolled in the study. All participants were subjected to complete blood count, total serum IgE, skin prick testing and peak expiratory flow rate. Polymerase chain reaction-based assays were performed to determine GSTP1 genotypes among asthmatic and healthy control subjects. We explored whether the GSTP1 substitutions influence susceptibility to asthma and its phenotypes.

Results: Among the 90 cases, 43 cases (47.8%) had GSTP1 genotype BB, 39 cases (43.3%) had GSTP1 genotype AA, 6 cases (6.7%) had GSTP1 genotype AB, and 2 cases (2.2%) had GSTP1 genotype AD. We found no significant differences in mean values of total serum IgE and skin test positivity among different genotypes. The PEFR% predicted of the BB genotype was significantly higher than that of both AA and AB genotypes (p<0.001), with no significant differences between the latter two genotypes. The frequency of GSTP1 AA was increased significantly in the asthmatic cases (76.9%) compared to controls (23.1%), with p value <0.001. However, the frequency of GSTP1 BB was decreased in the asthmatics (32.6%) compared to controls (67.4%), with p value <0.1.

Conclusions: The results of the present study suggest that GSTP1 BB conferred almost a threefold lower risk of asthma than did GSTP1 AA.

Key words: glutathione-S-transferase P1 polymorphism, bronchial asthma.

Introduction

Asthma is a disorder with complex genetic and environmental interactions that affect the developing immune system and ultimately result in the episodic release of procontractile mediators causing susceptible individuals to wheeze [1]. Advances in asthma management are likely to depend on a better understanding of how genetic factors influence susceptibility to and outcome in this disease [2].

Although asthma is characterized by airway inflammation, a critical component of which is oxidative stress with the formation of reactive oxygen species (ROS), few data exist on genes involved in protecting against this insult [3]. Individuals differ in their ability to deal with an
oxidant burden, and such differences are in part genetically determined [4].

Inability to detoxify ROS should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms, and precipitate asthma symptoms [5]. Thus members of the glutathione-S-transferase (GST) supergene family are proposed to be attractive candidates, for linkage to asthma, as enzymes encoded by members of the mu, theta, and pi class GST gene families are critical in the protection of cells from ROS because they can utilize as substrates a wide variety of products of oxidative stress [6]. Thus, the enzymes encoded by these GST gene classes preferentially utilize different ROS products. For example, quinone metabolites of catecholamines (dopachrome) are utilized by mu GST (but not by GSTP1 or GSTT1), GSTT1 utilizes oxidized lipid and DNA, and GSTP1 catalyzes the detoxification of base propenals that arise from DNA oxidation. Mu and theta (but not pi) GST demonstrate activity toward a phospholipid hydroperoxide [7]. These GSTs may also influence the synthesis of eicosanoids (critical mediators in the asthmatic response) via modulation of ROS levels [8]. The biochemical significance of these polymorphisms is indicated by data showing that particular genotypes are associated with an increased susceptibility to and/or poor outcome in several inflammatory pathologies including systemic lupus erythematosus [9] and rheumatoid arthritis (RA) [10].

Glutathione S-transferase P1 (GSTP1), the abundant isofrom of glutathione S-transferases (GSTs) in lung epithelium, plays an important role in cellular protection against oxidative stress and toxic foreign chemicals [11]. It has been suggested that polymorphisms in the GSTP1 gene are associated with asthma and related phenotypes. The alleles of GSTP1 that encode the Ile105→Val105 substitution are associated with the asthma phenotypes of atopy and bronchial hyper-responsiveness [12]. However, a further polymorphic site (Ala114→Val114) has been identified that results in the following alleles: GSTP1 A (wild-type Ile105→Ile105), GSTP1 B (Val105→Ala114), GSTP1 C (Val105→Val114) and GSTP1 D (Ile105→Val114) [13].

Because full identification of GSTP1 alleles may identify stronger links with asthma phenotypes, in this study we will use an amplification refractory mutation system (ARMS) assay that allows identification of GSTP1 genotypes. We will also explore whether the GSTP1 polymorphism is associated with asthma and related phenotypes including atopic status as well as airway obstruction.

The aim of this work is to describe an association study designed to examine whether allelic variation at the glutathione-S-transferase GSTP1 locus influences expression of asthma.

Material and methods

This study included 50 asthmatic children, 14 (28%) females and 36 (72%) males, aged 4-14 years. They were chosen among the attendants of the outpatient clinic of allergy and immunology, Cairo University Paediatric Hospital. Asthma was diagnosed based on the Global Initiative for Asthma (GINA) guidelines [14].

Clinical examination

All cases and controls were subjected to detailed history and thorough clinical examination.

The asthmatic cases were clinically classified into asthmatic atopic (35 patients, 70%) and asthmatic nonatopic (15 patients, 30%). A positive skin reaction (mean wheal diameter of at least 3 mm more than with saline control) in response to at least one of the tested allergens and serum IgE levels greater than 100 IU/ml were used to define atopic status, together with personal history. 40 normal age and sex matched healthy children were enrolled in this work as controls.

Laboratory investigations included

Complete blood count, total serum IgE determination by enzyme linked immunosorbent assay (ELISA) using kit manufactured by Biosource Europe SA, peak expiratory flow rate (PEFR) using peak flow meter, and determination of GSTP1 genotypes. Allergy skin prick testing was performed in all cases using a panel of common environmental allergens including house dust, house dust mite, multiple pollens, mixed moulds, feather, milk, eggs, wool, grass mix, cockroach, cat and dog furs, as well as positive and negative controls. Clinical parameters of recruited subjects are shown in Table I.

Genotyping

DNA was extracted from peripheral blood of all subjects using QIAGEN DNA extraction mini kit (Catalog No. 51104). 20 μl of QIAGEN protease were pipetted into the bottom of a 1.5 ml microcentrifuge tube. 200 μl of whole blood were added to the microcentrifuge tube. 200 μl of the buffer AL were added to the sample and mixed by pulse vortexing for 15 seconds, followed by incubation at 56°C for 10 minutes. 200 μl of absolute ethanol were added to the sample and mixed again by pulse vortexing for 15 seconds. This mixture was carefully applied to the QIA amp spin column and centrifuged at 8000 rpm for 1 minute. 500 μl of the buffer AW were added and centrifuged for 1 minute at 8000 rpm. 500 μl of the buffer AW2 were added to the QIA amp spin column and centrifuged at full speed 14,000 rpm for 3 minutes. The QIA amp spin column was placed in a new 1.5 ml microcentrifuge tube and 200 μl of the buffer AE or distilled water was
added. Incubation at room temperature for 5 minutes and then centrifugation for 1 minute at 8000 rpm was performed. The DNA was then stored at –20°C.

Identification of GSTP1 Ile<sup>105</sup>→Val<sup>105</sup> and Ala<sup>114</sup>→Val<sup>114</sup> substitutions using an amplification refractory mutation system polymerase chain reaction assay

The polymorphic detection of GSTP1 gene was genotyped using the multiplex PCR approach described by Zhong et al. [15]. The PCR primers used were as follows: a forward primer upstream of the codon 105 substitution (5'-ACCCCAGGGCTCTATGGGAA-3'). Two reverse primers: primer A (Ala<sup>114</sup> specific), 5'-TCACATACTCATCTTGCCGA-3'; primer B (Val<sup>114</sup> specific), 5'-TCACATACTCATCTTGCCGA-3'. For each DNA sample two polymerase chain reactions (PCRs) were performed, amplifying a 998 base pair fragment. The first included the forward primer and the reverse primer A and the second included the forward primer and the reverse primer B.

All reactions were performed in a total volume of 25 μl containing 12.5 μTaq master mix, 25 pmole/μl of the forward primer and either of the two reverse primers, 1 μl of the extracted DNA, and the total volume was completed by distilled water. A PCR reaction mixture for each of the DNA samples was prepared under a laminar flow cabinet. The PCR reaction tubes were placed in the DNA thermal cycler (Perkin-Elmer-9600), which was programmed for the following conditions: 94°C for 4 minutes, 30 cycles of denaturation (94°C, 1 minute), primer annealing (62°C, 1 minute) and elongation (72°C, 2 minute). The samples were then analyzed by electrophoresis on a 2% agarose gel and visualized by UV transillumination. ARMS PCR products were then digested with Bsm AI to determine the cis/trans configuration of the Ile<sup>105</sup>→Val<sup>105</sup> encoding allele. Products gave fragments of 343, 322, 260 and 73 base pairs with Ile<sup>105</sup>/Val<sup>105</sup>; and all six fragments in heterozygotes Ile<sup>105</sup>/Val<sup>105</sup>.

Statistical analysis

The Pearson χ<sup>2</sup> test and unpaired Student t-test were applied to analyze the difference between two groups. Multiple logistic regression models were used to control for potential confounders, and to evaluate the associations between the risks of asthma and GSTP1 genetic polymorphisms. Odds ratios (ORs) with 95% confidence intervals (CIs) are shown. Statistical significance was set at a p value of <0.05 based on a two-sided calculation.

Results

Comparison of clinical parameters of the study groups

As shown in Table I, no significant age or sex differences were found between the different clinical groups. Mean values of total serum IgE levels were significantly higher in the asthmatic compared to the nonasthmatic and the control group (p<0.001). Also there was a statistically significant difference in the mean value of PEFR% predicted between the control group and the asthmatic group (p<0.001). The

| Table I. Comparison between clinical parameters of recruited subjects |
|-----------------|----------------|----------------|
|                  | Atopic asthmatics | Nonatopic asthmatics | Controls |
| Sex                  |                   |                   |           |
| male No (%)             | 25 (71.4)         | 10 (26.7%)        | 9 (22.5) |
| female No (%)           | 11 (73.3)         | 4 (26.7%)         |           |
| Age (years)             | 7.20±2.83         | 8.73±3.13         | 7.43±1.95 |
| IgE level (IU/ml)       | 515.20±687.25     | 176.08±132.78     | 79.13±10.20 |
| PEFR% predicted         | 72.50±12.30       | 69.13±13.40       | 94.96±7.50 |
| Eosinophilic count/mm<sup>3</sup> | 529.80±308.34  | 483.00±304.66    | 204.00±71.63 |
| No of skin test positivity | 3.06±1.06        | 0.00             | –         |

Values are means ±SD
eosinophilic count was significantly lower among the control group compared to both the atopic and nonatopic asthmatics (p<0.001), but the latter two groups did not differ significantly.

**GSTP1 genotypes in the study subjects**

All asthmatic and control subjects were subjected to ARMS PCR for identification of GSTP1 genotypes; 43 cases (47.8%) had GSTP1 genotype BB, 39 cases (43.3%) had GSTP1 genotype AA, six cases (6.7%) had GSTP1 genotype AB and two cases (2.2%) had GSTP1 genotype AD. Figure 1 shows GSTP1 gene banding patterns of the gel electrophoresis.

**Association of GSTP1 genotypes with atopic phenotypes and airway obstruction**

Comparison of the different GSTP1 genotypic groups in all subjects as regards mean values of total serum IgE level, PEFR% predicted, and skin test positivity are presented in Table II. The mean total serum IgE levels were 218.0±247.7 IU/ml for the AA genotype, 232.2±319.6 IU/ml for the AB genotype, 197.5±268.4 IU/ml for the BB genotype. We found no significant differences in mean values of total serum IgE among different genotypes. The PEFR% predicted for the GSTP1 genotype AA was 75.2±15.4%, 74.9±18.8% for genotype AB, and 88.6±13.2% for the BB genotype. The PEFR% predicted of the BB genotype was significantly higher than that of both AA and AB genotypes (p<0.001), with no significant differences between the latter two genotypes (Figure 2). The number of cases with positive skin test in the AA genotype group was 22 (56%), 3 (50%) in the AB genotype, and 25 (58%) for the BB genotype. No significant association was found between GSTP1 genotypes and skin test positivity.

**Association of GSTP1 genotypes with asthma**

Table III shows the association of GSTP1 genotype with the presence of asthma. The frequency of GSTP1 AA was increased significantly in the asthmatic cases (76.9%) compared to controls (23.1%), with p value <0.001. However, the frequency of GSTP1 BB was decreased in the asthmatics (32.6%) compared to controls (67.4%), with p value

### Table II. Comparison between total serum IgE level, PEFR% predicted and skin test positivity among different GSTP1 genotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GSTP1 genotypes</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n=39)</td>
<td>AB (n=6)</td>
</tr>
<tr>
<td>IgE level (IU/ml) Mean±SD</td>
<td>218.0±247.7</td>
<td>232.2±319.6</td>
</tr>
<tr>
<td>PEFR % pred Mean±SD</td>
<td>75.2±15.4</td>
<td>74.9±18.8</td>
</tr>
<tr>
<td>Skin test positive No (%)</td>
<td>22 (56)</td>
<td>3 (50)</td>
</tr>
</tbody>
</table>

*PEFR% pred – peak expiratory flow rate percent predicted, NS – not significant*
<0.01. Regarding the AB and AD genotypes, we could not statistically compare between groups because of the small number of cases.

Figure 3 shows the comparison between asthmatic cases and controls as regards different GSTP1 genotypes. 30 out of 50 asthmatic cases (60%) had GSTP1 genotype AA, 14 cases (28%) had GSTP1 genotype BB, 4 cases (8%) had genotype AB and only 2 cases (4%) had genotype AD. On the other hand, among 40 control children, 29 cases (72.5%) had GSTP1 genotype BB, 9 subjects (22.5%) had GSTP1 genotype AA, and only 2 subjects (5%) had GSTP1 genotype AB. The percentage of subjects with AA genotype is significantly higher in the asthmatics compared to the control group (p<0.001). However, the percentage of subjects with BB genotype is significantly lower in the asthmatics as compared to controls (p<0.01).

Risk estimation for each genotype in the asthmatic and control groups using the unconditional logistic regression analysis, when the GSTP1 BB genotype was defined as the reference, is shown in Table IV. The GSTP1 AA genotype showed an odds ratio (OR) of 2.92 (95% confidence interval [CI], 1.589-5.376, p<0.001). Thus, cases with GSTP1 genotype AA have almost threefold greater risk of developing asthma when compared with BB genotype.

### Frequencies of GSTP1 in the study groups

The frequencies of GSTP1 genotypes in the three different clinical groups including atopic asthmatics, nonatopic asthmatics and control groups are shown in Figure 4. Among the GSTP1 genotype AA, 23 (59.0%) were atopic asthmatics, 7 cases (17.9%)...
were nonatopic asthmatics and 9 controls (23.1%); while among the GSTP1 genotype BB, 8 cases (18.6%) were atopic asthmatics, 6 cases (14.0%) were nonatopic asthmatics and 29 controls (67.4%). The GSTP1 genotype AB included 3 cases (50.0%) of atopic asthmatics, one case (16.7%) of nonatopic asthmatic and 2 control cases (33.3%). Lastly, GSTP1 genotype AD had one case (50%) of atopic asthmatic and one case (50%) of nonatopic asthmatic. Significant differences were found between the atopic asthmatics (59%) and both the nonatopic asthmatics (17.9%, p<0.001) and controls (23.1%, p<0.001) in the frequencies of GSTP1 genotype AA. Also there were statistically significant differences in the frequencies of the BB genotype between the control group (67.4%) and both the atopic asthmatics (18.6%, p=0.008) and nonatopic asthmatics (14%, p<0.001); however, no significant difference was found between the last two groups.

Significant differences were found between the atopic asthmatics and both the nonatopic asthmatics (p<0.001) and controls (p<0.001) in the frequencies of GSTP1 genotype AA. There were statistically significant differences in the frequencies of the BB genotype between the control group and both the atopic asthmatics (p=0.008) and nonatopic asthmatics (p<0.001).

**Discussion**

Previous studies have shown a link between asthma and markers on chromosome 11q13 [3, 4, 16]. Although candidate genes for susceptibility to asthma have been identified, they are too distant from these markers to fully account for this linkage [17]. In this study we found associations between genotypes of GSTP1 and both asthma and airway obstruction in Egyptian children.

Polymorphisms of the glutathione-S-transferase (GST) genes are known risk factors for some environmentally-related diseases [18]. The loci encoding the glutathione-S-transferase (GST) enzymes comprise a large supergene family located on at least seven chromosomes. The main function of the GST enzymes has traditionally been considered to be the detoxification of reactive oxygen species (ROS) products by glutathione conjugation [19]. GSTP1 is of particular interest in asthma, because chromosome 11q13 is associated with its clinical phenotypes: atopy and BHR. Several candidates (high affinity IgE receptor, clara cell secretory protein) have been identified, although the encoding genes do not account for the strength of the linkage to this region [4]. We proposed that GSTP1 is another candidate gene in this region. Thus, the efficiency of detoxification of ROS products determined by polymorphism in GSTP1 may influence the development and/or severity of airway obstruction and asthma.

In this study we examined whether allelic variation at the glutathione-S-transferase GSTP1 locus influences expression of airway obstruction and asthma. In our study all asthmatic and control cases were subjected to ARMS PCR for identification of all GSTP1 genotypes. Out of the 90 subjects included in the study, 47.8% of these children had GSTP1 genotype BB, 43.3% had GSTP1 genotype AA, 6.7% had GSTP1 genotype AB, and 2.2% had GSTP1 genotype AD. However, in a group of 191 Northern European adults, Hemmingsen et al. in 2001 [20] reported that 3.7% of these subjects had BB genotype, 42.4% had AA genotype, 34.6% had AB genotype, and 11% had AD genotype. These differences in the results may be related to racial variations between the study groups.

In the present study there were no significant associations between GSTP1 genotypes and either IgE level or skin test positivity. On the other hand, the mean value of PEFR% predicted was significantly higher in subjects with BB genotype compared with that of both AA and AB genotypes, indicating a protective effect of the BB genotype. Similarly, Hemmingsen et al. [20] found no association of GSTP1 genotypes and either skin test positivity or IgE level. They also stated that the frequency of GSTP1 AA increased with severity of airway obstruction, whereas BB and BC frequencies displayed a reverse trend, while Fryer et al. [21] stated that subjects with GSTP1 Val105/Val105 have 10-fold lower risk of exhibiting atopy defined by skin test positivity and IgE level. Carroll et al. [22] showed that GSTP1 genotypes were important determinants of lung function in childhood, and that GSTP1 Val105/Val105 was associated with significantly higher forced expiratory volume in 1 second, and thus Val105 is protective against asthma and is present in the B and C alleles. Also, Gilliland et al. [23] stated that children with asthma who were homozygotes for the GSTP1 Val105 allele had substantially larger deficits in FVC, FEV1 and maximal mid-expiratory flow.

In our study, significant differences were observed between asthmatic cases and controls as regards both GSTP1 genotype AA and genotype BB. The incidence of AA genotype was significantly higher among asthmatic cases compared with controls, while the incidence of genotype BB was significantly lower among asthmatic cases as compared with controls. Also we found that the incidence of GSTP1 genotype BB was significantly lower in both atopic and nonatopic asthmatic groups compared with the control group. These results suggest a significant association between GSTP1 Ile105Val polymorphism and susceptibility to asthma and that the GSTP1 Val105/Val105 genotype may be protective against developing this disease. Also, when the GSTP1 BB genotype was defined as the reference, the GSTP1 AA genotype showed an odds ratio of 2.92 (95% CI,
GSTP1 Val105/Val105 was significantly lower in polymorphism is strongly associated with asthma supported by Fryer et al. [21], who found that GSTP1 polymorphism is strongly associated with asthma and related phenotypes, and that the frequency of GSTP1 Val105/Val105 was significantly lower in asthmatic than in control subjects. Also, Lee et al. [25] found that children who were homozygous for Ile105 locus had a significantly increased risk of physician-diagnosed asthma, while in another study by Lee et al. [26] when all participants were homozygous at the Ala114 locus, they found that only a marginally significant association existed between the frequency of homozygosity at the Ile105 locus and asthma when air pollution was not considered. In contrast to our results, Nickel and his colleagues [27] did not find a significant association of GSTP1 polymorphisms with bronchial asthma or airway hyper-responsiveness in German children.

In the present study, by comparing both asthmatic groups, the incidence of genotype AA was significantly higher in atopic asthmatics than in nonatopic asthmatics. This finding reflects that GSTP1 Ile105/Ile105 genotype is likely to be a risk factor for the development of atopic asthma. Differently, Tamer et al. [18] stated that subjects with the GSTP1 homozygous Val105/Val105 genotype had a 3.55-fold increased risk of having atopic asthma compared to nonatopic asthma. However, Aynacioglu et al. [24] showed that the distribution of GSTP1 Ile105/Val105 genotypes and the frequency of GSTP1 Val105/Val105 homozygotes (3.7 vs. 3.9%) were not significantly different between atopic and nonatopic asthmatics. The data reported here also show that there were no significant differences between atopic and nonatopic cases in respect of the frequencies of GSTP1 BB genotype. In contrast to our results, Spiteri et al. [12] confirmed that the frequency of the GSTP1 Val105/Val105 genotype is reduced in atopic subjects compared with nonatopic subjects. In conclusion, we have identified an association between polymorphism in GSTP1 and asthma and that the frequency of GSTP1 AA was increased in patients with established asthma. The data reported here also show that the frequencies of GSTP1 BB were reduced in asthmatic persons, thus indicating a protective effect.

As the number of children with some genotypes was small in our study, the association of different GSTP1 genotypes and the atopic status and its phenotypes should be assessed on a larger number of cases. We believe that our data must be considered as preliminary, because asthma is a complex polygenic disease. Individual polymorphisms may have small functional consequences; thus additional genetic polymorphisms in other genes are required to alter function significantly. Further research in this field will certainly elucidate the role of other genes, gene-gene and gene-environment interactions in asthma that will be important to prevent and/or offer a better therapy for asthma.

Conclusions

Our findings suggest that GSTP1 genotype AA is associated with the established asthma and that GSTP1 genotype BB confers a protective effect.

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References


