GSTA1*-69C/T and GSTO2*N142D as asthma- and allergy-related risk factors in Italian adult patients

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SUMMARY

1. Asthma and allergies are characterized by variable and subjective symptoms influenced by many genes, molecular mechanisms and environmental factors. The presence of inflammation and oxidative stress in the airways are important biochemical features of asthma and respiratory allergies. Glutathione S-transferase (GSTs) enzymes play an important role in cellular protection against inflammation, and functional genetic polymorphisms in GST genes show a significant association with asthma and allergy risk. Specifically, our previous study on asthmatic children highlighted GSTA1 and GSTO2 as novel susceptibility loci for asthma.

2. In the present study we focused our attention on GSTA1*-69C/T (rs3957357) and GSTO2*N142D (rs156697) polymorphisms to confirm our previous results in an independent adult study population and to clarify whether GSTA1 and GSTO2 gene polymorphisms are involved in a non-discriminative pathway towards asthma and respiratory allergy.

3. To accomplish this, we recruited 103 patients with respiratory allergies, 199 patients with asthma and 200 healthy controls. Genomic DNA extracted from buccal cells was screened for GSTA1*-69C/T and GSTO2*N142D single nucleotide polymorphisms.

4. The GSTA1*-69T and GSTO2*D142 variants are both associated with a significantly increased risk of asthma, whereas only GSTA1*-69C/T is significantly associated with allergies. These outcomes confirm the involvement of GSTO2 loci in asthma and suggest that GSTA1 is a common risk factor for asthma and allergies.

Key words: genetic factors, glutathione S-transferases, GSTA1, GSTO2, respiratory diseases, single nucleotide polymorphisms.

INTRODUCTION

Asthma is a common chronic inflammation of the respiratory tracts; the cause and the nature of its pathogenesis have not been well established as yet. However, it is known that asthma is a complex multigenic disorder determined by the complicated interaction of genetics with the environment. To date, there is not a clear definition of the asthma phenotype and, for this reason, researchers have focused their attention on several characteristics that can be measured objectively, such as atopy, airway hyperresponsiveness and allergen sensitization. Rackeman first reported the distinction of two clinical patterns of asthma: a form in which extrinsic factors of the disease were triggering the symptoms (allergic asthma) and another in which no peculiar triggering factor could be found (non-allergic asthma). Ever since the first description of non-allergic asthma, there has been debate about the relationship of the disease to atopy. Non-allergic asthma could represent a form of autoimmunity, or auto-allergy, triggered by infection because a respiratory influenza-like illness often precedes onset. The existence of two distinct asthma phenotypes suggests that their respective genetic predispositions could be different. However, although several genes have been shown to be linked to asthma, no relationship to the allergy was reported. Although a host allergy is a recognized risk factor for airway inflammation, atopy alone cannot cause asthma. Indeed, results of different epidemiological studies have consistently shown that allergies and asthma often coexist in the same patient. Moreover, allergies are a risk factor for asthma and, despite the severe impact on patients and society as a whole, respiratory allergies are neglected and/or under-recognized. Therefore, some genes may be common for asthma and allergies and some genes may be disease-specific markers.

The discovery of specific markers for asthmatic and allergic phenotypes would help greatly in recognizing the different entities of these respiratory syndromes. At the clinical level, the discovery of these markers would also help facilitate the prevention and treatment of the diseases in individuals. The presence of inflammation in the airways is an important common feature of asthma and respiratory allergies. In particular, attention is mainly focused on oxidative stress, a key component of inflammation. In recent years a number of genome-wide association studies (GWAS) in asthma or allergy phenotypes have described novel and interesting gene polymorphisms, but have also set the role of some anti-oxidant genes previously described as being involved.
in this scenario. However, the genetic component involved in respiratory disease development or symptom worsening has not been completely clarified. Indeed, the GWAS approach may underestimate the genomic and environmental contexts, leaving uncovered loci involved in gene–environment interaction, such as detoxification genes.

Several studies have demonstrated that some changes in the function of the xenobiotics detoxification system result in an increased susceptibility to asthma development or symptom worsening. Recently, interest has focused on genes encoding the glutathione S-transferases (GSTs), the principal Phase II enzyme superfamily. For GST enzymes, functional polymorphisms have been shown to be risk factors for common diseases and for xenobiotics-related susceptibility. Regarding GST genes and respiratory phenotypes, most of the studies have analysed the association of GSTM1, GSTP1 and GSTT1 gene polymorphisms to disease risk, with contrasting results. Recent meta-analyses have highlighted that the genetic associations of GSTM1, GSTP1 and GSTT1 to asthma are not statistically significant. In addition, high heterogeneity is present among the studies.

This outcome is probably due not only to publication bias and sample size, but also to the extreme gene–environment interactions present in the asthmatic phenotypes and to the genetic differences present among human populations. Our previous case-control study recently associated two functional polymorphisms of GSTA1 and GSTO2 enzymes (GSTA1*-69C/T and GSTO2*N142D) with asthma susceptibility in Italian children. Although no other genetic association studies have investigated these variants and none of the GWAS have found these polymorphisms to be significant, some evidence seems to confirm our association hypothesis. In 2007, the Framingham Heart genome-wide association study hypothesized a role for GSTO2 as a credible candidate for a gene associated with pulmonary function. Sohn et al. highlighted that GSTA1 is upregulated in asthmatic airways, suggesting that it plays an important role in protecting the airways from oxidative stress. In order to confirm the role of GSTA1 and GSTO2 as susceptibility loci for asthma and to understand their involvement in allergies, we investigated GSTA1*-69C/T and GSTO2*N142D polymorphisms in three groups: asthmatics, allergy patients and healthy controls.

**METHODS**

**Study population**

The participants were recruited from the ‘San Giovanni Calibita’ Fatebenefratelli Hospital in Rome, Italy. The sample consisted of 103 patients with respiratory allergies (i.e. rhinitis, conjunctivitis, sinusitis), 199 patients with asthma (148 atopic and 51 non-atopic) and 200 healthy controls living in the same geographical area. The allergy patients were recruited from the Clinical Pathology Department, whereas both the asthmatic and healthy controls were recruited from the Clinical Pathophysiology Centre. Respiratory allergy diagnosis was based on a current physician’s diagnosis, positivity to Prick and Rast tests and the use of anti-allergic medication. To exclude an asthmatic component in allergic patients, we selected allergic patients without bronchial hyperresponsiveness. As reported previously, asthma was diagnosed in accordance with Global Initiative on Asthma criteria:

(i) a current physician diagnosis of asthma; (ii) symptoms; and (iii) the use of anti-asthma medication. Ethnicity-matched controls were selected from non-asthmatic, non-atopic, healthy individuals with normal lung function who were visiting Fatebenefratelli Hospital for an annual check-up.

**Genotyping procedures**

Buccal cells were collected with an oral swab from each participant. Written informed consent was obtained from all individuals and the study was approved by the hospital’s ethics committee. DNA from buccal cells was obtained using the phenol–chloroform–isoamylic alcohol method. The GSTA1*-69C/T and GSTO2*N142D polymorphisms were detected as reported previously. To ensure the reliability of the results, approximately 15% of the samples were randomly selected and analysed independently by a second researcher using the same protocol; in all cases the outcome was concordant.

**Statistical analyses**

The χ²-test and Student’s t-test were used for the analysis of categorical and quantitative variables, as appropriate. The results of the genotyping procedures were used to verify Hardy–Weinberg equilibrium. Because we investigated two genetic markers in control and patient groups, the standard significance level for genetic association analysis has been adjusted to α = 0.05/2 = 0.025, according to the application of Bonferroni correction for multiple testing. Power analysis was performed using the Power for Genetic Association version 2.0. The minimum detectable effect with odd ratios (ORs) was calculated in a codominant genetic model, based on an α of 0.025, an asthma prevalence of 6% and an allergy prevalence of 30% in the general population. With this sample size, we had 80% power to detect an OR of 1.57 for asthma association and an OR of 1.77 for allergy association if the risk allele frequency was higher than 25%. The ORs and 95% Confidence Intervals (CIs) were calculated to evaluate the association between GST variants and asthma and GST variants and respiratory allergic disease. To estimate the ORs, different genetic models were considered: codominant (each genotype has a different disease risk), dominant (one copy of the allele is sufficient to increase the disease risk), recessive (two copies of the allele are necessary to increase the disease risk) and log-additive (r-fold increased risk for one copy of the allele, r² increased risk for two copies of the allele). To evaluate the best genetic model, the Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used.

**RESULTS**

Demographic and clinical characteristics of the groups investigated are given in Table 1. No significant differences were found between asthmatic patients and healthy controls in terms of age distribution, sex ratio or any of the other characteristics analysed. Conversely, significant differences were found for age and body mass index (BMI) distribution among allergy patients and healthy controls (P < 0.001). Data regarding the allelic and the genotype frequencies are reported in Table 2. All genotype frequencies in our controls were within the ranges reported previously in Italy.
Asthma and respiratory allergies are complex diseases characterized by variable and subjective symptoms that are influenced by many genes and molecular mechanisms. The results of several studies have suggested the existence of different subphenotypes.\(^2^,27\) However, more studies are needed to adequately define and characterize these respiratory diseases. In this scenario, environmental factors also play a fundamental role: asthma and allergies are, in fact, believed to be a result of the combined effects of genetic and environmental factors.\(^2\) Asthma and allergy phenotypes have been studied extensively, and association studies are performed. In these studies, several candidate genes were directly or indirectly implicated, but it has been reported that the prevalence of these candidate genes varies considerably by ethnicity.\(^2^0^,26\)

The GST genes are important in the protection of cells from reactive oxygen species (ROS) and they can also affect the synthesis of proinflammatory eicosanoids via modulation of ROS levels.\(^8\)

Our outcome suggests that GSTA1*-69C/T and GSTO2* N142D are functional variants that affect the detoxification role of encoded enzymes, increasing the risk of respiratory disease development. Regarding asthma, our data confirm the association and strongly suggest a role in genetic predisposition for both variants investigated. Conversely, only GSTA1*-69C/T seems to be involved in respiratory allergy predisposition. The function of GSTA1 supports our results: GSTA1 is widely expressed in

### DISCUSSION

the genotype frequencies were in Hardy–Weinberg equilibrium in the patient and control cohorts. The ORs and 95% CIs calculated for different genetic models are given in Table 3. Regarding asthmatic versus control comparisons, both the polymorphisms studied showed an association with asthma risk. Regarding GSTA1, the OR analysis highlighted significant results for the codominant model (CT genotype: OR 2.74, 95% CI 1.72–4.35; TT genotype OR 3.08, 95% CI 1.41–6.72; P < 0.001), dominant model (OR 2.79; 95% CI 1.79–4.35; P < 0.001) and log-additive model (OR 2.10; 95% CI 1.48–2.97; P < 0.001). To detect the best model for our data, we used two standard model selection criteria: the AIC and BIC. The dominant model achieved the best scores for AIC and BIC. Regarding GSTO2, the OR analysis revealed significant outcomes for the codominant model (ND genotype: OR 1.20, 95% CI 0.76–1.89; DD genotype: OR 4.50, 95% CI 1.96–10.33; P < 0.001), recessive model (OR 4.10, 95% CI 1.85–9.10; P < 0.001) and log-additive model (OR 1.69, 95% CI 1.21–2.36; P = 0.002). The recessive model achieved the best scores for AIC and BIC.
several tissues (i.e. kidney, lung, small intestine, prostate, testis, adrenal gland, pancreas and trachea) and it is particularly expressed in the liver; this enzyme plays a fundamental role in the cellular detoxification of several phase I-activated derivatives of environmental pollutants or products of oxidative stress.\textsuperscript{10,33} The GSTA1*69C/T polymorphism is a single nucleotide polymorphism (SNP) localized in the proximal promoter and it is linked with other SNPs. It has been suggested that it may play a role in the development of asthma and allergies or the exacerbation of symptoms, because subjects with the −69T allele in the GSTA1 genotype exhibit lower GSTA1 expression.\textsuperscript{31} In this way, a deficiency in detoxifying air pollutants or allergens could play a role in the risk of atopic disease development. Regarding GSTO2, the encoding enzyme plays an important role in terms of response to oxidative stress through its dehydroascorbate reductase activity.\textsuperscript{32} This function is important for the oxidant–antioxidant imbalance present in lung tissue.\textsuperscript{33} Therefore, a decrease in anti-oxidant capacity due to reduced GSTO2 activity may trigger increased oxidative stress that could lead to a pulmonary diminution of its function. Wilk \textit{et al.}\textsuperscript{24} in their GWAS on lung function have identified a number of novel gene regions associated with pulmonary function, in which the GSTO2*N142D polymorphism is included. Furthermore, this variant has shown significant association with forced expiratory volume in the first second (FEV\textsubscript{1}) and forced vital capacity (FVC) phenotypes.\textsuperscript{34} It has been demonstrated that GSTO2 may exhibit expression in bronchial epithelial cells. In fact the GSTO2*D142 variant showed a minor percentage of expression with respect to the wild-type.\textsuperscript{24} Association studies on genes involved in lung function measurements and asthma may help identify lung-specific mechanisms in asthma development. These findings could help explain why GSTO2 seems to be linked only to asthma and not to allergies. Our hypothesis is that GSTO2 may be not involved in general inflammatory mechanisms, such as eosinophilia, that play a role in atopic diseases.

In conclusion, our investigation confirms the role of \textit{GSTA1} and \textit{GSTO2} loci in asthma risk and permits us to hypothesize that \textit{GSTA1} is a common susceptibility locus for asthma and allergies, whereas \textit{GSTO2} is an asthma-specific locus. The results obtained in this association study allowed us to open new directions of inquiry into the study of GST genes in respiratory diseases. In particular, further studies on \textit{GSTA} and \textit{GSTO} genes may uncover important gene–gene and gene–environment interactions in the pathogenesis of asthma and allergies.

**ACKNOWLEDGEMENTS**

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**DISCLOSURE**

The authors declare no conflicts of interest.

**REFERENCES**


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**Table 3** Association of \textit{GSTA1} and \textit{GSTO2} variants with asthma and allergy

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic versus control</th>
<th></th>
<th>Allergic versus control</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>AIC</td>
<td>BIC</td>
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<tr>
<td>\textit{GSTA1}^{*}69C/T (rs3957357)</td>
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<tr>
<td>Codominant</td>
<td>2.74 (1.72–4.35)</td>
<td>&lt; 0.001</td>
<td>477</td>
<td>516</td>
</tr>
<tr>
<td>−69C/−69T</td>
<td>3.08 (1.41–6.72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>2.79 (1.79–4.35)</td>
<td>&lt; 0.001</td>
<td>475</td>
<td>510</td>
</tr>
<tr>
<td>Recessive</td>
<td>1.83 (0.87–3.83)</td>
<td>0.110</td>
<td>494</td>
<td>529</td>
</tr>
<tr>
<td>Log-additive</td>
<td>2.10 (1.48–2.97)</td>
<td>&lt; 0.001</td>
<td>478</td>
<td>513</td>
</tr>
<tr>
<td>\textit{GSTO2}^{*}N142D (rs156697)</td>
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<tr>
<td>Codominant</td>
<td>1.20 (0.76–1.89)</td>
<td>&lt; 0.001</td>
<td>483</td>
<td>521</td>
</tr>
<tr>
<td>D142/D142</td>
<td>4.50 (1.96–10.33)</td>
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<tr>
<td>Dominant</td>
<td>1.51 (0.98–2.33)</td>
<td>0.062</td>
<td>492</td>
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<tr>
<td>Recessive</td>
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<td>&lt; 0.001</td>
<td>481</td>
<td>516</td>
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<tr>
<td>Log-additive</td>
<td>1.69 (1.21–2.36)</td>
<td>0.002</td>
<td>485</td>
<td>520</td>
</tr>
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</table>

\textit{P}-values and odds ratios (OR) adjusted for confounding variables (i.e. age, sex, body mass index, active and passive smoking and occupational risk) are reported.

Association analysis was performed considering different genetic models (i.e. codominant, dominant, recessive and log-additive).

Significance was set at \( P = 0.025 \) (two-tailed after Bonferroni’s adjustment).


