

Lack of Association Between *GSTM1*, *GSTP1*, and *GSTT1* Gene Polymorphisms and Asthma in Adult Patients From Rome, Central Italy

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■ Abstract

Background: Asthma is a complex multifactorial disease that is not yet fully understood. Oxidative stress due to an imbalance between the oxidative forces and the antioxidant defense systems has been implicated in asthma pathogenesis. However, much debate still surrounds the key genetic factors involved in the development of this disease. Candidate genes include the glutathione S-transferases (GSTs). In particular, mu, pi, and theta classes of GSTs play an important role in regulating inflammatory responses. However, few and contradictory data are available on the association between asthma development and GST gene polymorphisms (*GSTM1*, *GSTP1*, and *GSTT1*).

Objective: To investigate whether *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms are associated with asthma development.

Methods: We recruited 200 unrelated healthy individuals and 199 asthmatic patients from Rome in Central Italy. Genotyping of *GSTM1* and *GSTT1* genes was performed by a multiplex polymerase chain reaction (PCR) while the *GSTP1* polymorphism (rs1695) was determined using PCR-restriction fragment length polymorphism analysis.

Results: Our results suggest that the GST polymorphisms analyzed are not associated with asthma, confirming the uncertain role of GST genes in the development of asthma.

Conclusions: Oxidative stress is certainly involved in the development of asthma, and GSTs may therefore influence asthma risk, although, as our results show, their role in pathogenesis remains to be elucidated. Future studies should focus on the interactions of GST genes with the environment and other antioxidant genes to shed light on the role of GSTs in asthma.

Key words: Asthma. Glutathione S-transferases. Gene-candidate study. Adult patients. Oxidative stress.

■ Resumen

Introducción: El asma bronquial es una compleja enfermedad multifactorial no bien conocida. El estrés oxidativo debido al desbalance entre las fuerzas de oxidación y los sistemas de defensa frente a la misma se han relacionado con la patogénesis de la misma, sin embargo existe un debate sobre los factores genéticos involucrados en el desarrollo de la enfermedad. Los genes candidatos incluyen a las glutatión S-transferasas S. En particular las clases mu, pi y theta de GSTs juegan un papel importante en la regulación de la respuesta inflamatoria. Existen pocos y contradictorios datos sobre la asociación entre el desarrollo de asma y polimorfismos de los genes GST (*GSTM1*, *GSTP1*, y *GSTT1*). En este estudio se investiga la posible asociación entre polimorfismos *GSTM1*, *GSTP1*, y *GSTT1* y el asma bronquial.

Métodos: Se reclutaron 200 individuos sanos no relacionados y 199 pacientes asmáticos procedentes de Roma. Se realizó en todos ellos la genotipificación de los genes *GSTM1* y *GSTT1* mediante la reacción en cadena de la polimerasa (PCR) y el polimorfismo de *GSTP1* (rs1695) mediante análisis del polimorfismo por restricción de la longitud del fragmento por PCR.

Resultados: Los resultados sugieren que no hay una asociación entre los polimorfismos de GST y el desarrollo de asma bronquial.

Conclusiones: Se concluye que el estrés oxidativo está ciertamente involucrado en el desarrollo de asma bronquial, los GSTs influyen en el riesgo de asma, aunque su papel en la patogénesis no se ha podido determinar. Hacen falta más estudios que traten de evaluar las posibles interacciones de los genes GST con el ambiente y otros genes antioxidantes.

Palabras clave: Asma. Glutatión S-transferasas. Estudio de genes candidatos. Pacientes adultos. Estrés oxidativo.

Introduction

Asthma is a disorder of the airways characterized by several symptoms such as airflow obstruction, airway inflammation, and hyperresponsiveness. These symptoms, however, can in some cases resolve, either spontaneously or as a result of treatment [1]. A clinical diagnosis is made on the basis of several criteria such as physical findings, medical history, and exclusion of diagnoses that mimic asthma [2]. The study of genetic factors involved in complex pathologies such as asthma is arduous, not only because of human genetic variability, or incomplete penetrance, but also because, in complex disease studies, the importance and strength of gene to gene and gene to environment interactions need to be considered [3]. The gene to environment interaction is a mechanism where genes define the potential and limitations of an organism's reaction to environmental conditions. The effects of genetic and environmental components overlap, and in several cases may be dependent on each other. Thus, a certain phenotype or disease may be caused by a genetic factor, by an environmental condition, or by a combination of the two.

Several studies have highlighted that oxidative stress damages pulmonary function and might act as a key player in the worsening of asthma symptoms [4]. The damage caused by oxidative injury leads to an increase in airway reactivity and/or secretions, and also influences the production of chemoattractants and increases vascular permeability. All these factors exacerbate inflammation, which is a hallmark of asthma. Several candidate genes have been implicated in the development of atopy and asthma. In particular, those involved in oxidative stress responses are candidates for asthma. The prevalence of candidate gene polymorphisms varies considerably worldwide, and accordingly, ethnicity should be considered as a factor that might act on and influence asthma development. Furthermore, previous data based on intra- and inter-population frequency differences suggest that the association between a given genetic polymorphism and asthma cannot be extrapolated from one ethnic group to another [5]. Phase II detoxification enzymes, particularly classes of glutathione S-transferases (GSTs), play an important role in inflammatory responses triggered by xenobiotic or reactive oxygen compounds [6]. GSTs are characterized by a different localization in cells and are known to belong to the cytosolic, the mitochondrial, or the membrane-bound microsomal families. Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta classes characterize the cytosolic family [7]. GST genes are polymorphic and research by several groups in recent years has focused on evaluating the association between certain allelic variants and several complex diseases and explaining their role. The inability of GST variant enzymes to detoxify reactive oxygen species (ROS) contributes to the activation of the inflammatory process, bronchoconstriction, and the exacerbation of asthma symptoms. An increasing risk for asthmatic disease development and an increase in individual susceptibility to the pro-allergic effects of xenobiotics have been demonstrated to be linked to functional polymorphisms of GST enzymes [6]. In particular, *GSTM1* and *GSTT1* null polymorphisms and a single nucleotide polymorphism (SNP) of *GSTP1* (rs1695; Ile105Val) may influence the pathogenesis

of respiratory diseases. Numerous studies have documented associations between genes implicated in the oxidative stress response and respiratory phenotypes, but the data suggest that they may not be consistent across ethnic groups owing to differences in intra- and inter-ethnic allele frequencies [8]. To date, few studies on the association between these genes and the risk of asthma and related phenotypes have been conducted in the Italian population [9,10]. Due to the paucity of data in this field, we undertook a descriptive study to evaluate the association between *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and asthma in a sample of individuals from Rome, Italy.

Materials and Methods

The participants were recruited in Rome, Central Italy. The sample consisted of 199 patients with asthma (mean age [SD], 50.9 [1.4] years) and 200 healthy controls (mean age, [SD] 53.9 [1.2] years) living in the same geographical area. Both patients and controls were recruited from the clinical pathophysiology center of "San Giovanni Calibita" Fatebenefratelli Hospital of Rome during a routine health care visit. Asthma diagnosis was based on the following criteria: (1) a current diagnosis by a physician, (2) symptoms, and (3) use of anti-asthma medication according to the Global Initiative on Asthma criteria [11]. None of the controls had a history of pulmonary disease or atopy. Information on demographic characteristics, medical history, smoking habits, and occupational risk were obtained in face-to-face interviews with medical staff using a structured questionnaire. All the interviews were conducted in accordance with Italian law, which guarantees individual privacy. We distinguished between active smoking (smoker, ex-smoker, and nonsmoker) and passive smoking (exposed, not exposed). For risk at work we considered occupational exposure to chemical substances, irritating material, or toxic dust. Buccal cells were collected with an oral swab from each participant. The samples were stored at room temperature until they were returned to the laboratory, where they were stored at 4°C before processing. 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: isoamyl alcohol method [12]. Genotyping of *GSTM1* and *GSTT1* was performed by a multiplex polymerase chain reaction (PCR) reaction [13]. The PCR products were analyzed on 1.5% agarose gels stained with ethidium bromide (10 mg/mL). A fragment of 273 base pairs (bp) indicated the presence of *GSTM1*, a fragment of 480 bp indicated the presence of *GSTT1*, and a fragment of 312 bp indicated the positive internal control, CYP4501A1. This technique makes it possible to discriminate a null phenotype (gene deletion in homozygous status) from a positive phenotype (wild genotype in homozygous and heterozygous status). The *GSTP1* Ile105Val polymorphism was analyzed by PCR-restriction fragment length polymorphism analysis [14]. The PCR products were digested for 24 hours at 37°C in 20 µL of 2 U BsmAI (New England Biolab). The digestion products for *GSTP1**I105V revealed the presence of 3 different patterns: the A/A wild-type homozygote (Ile/Ile) showed a 176-bp fragment;

the G/G polymorphic homozygote (Val/Val) showed the expected 91- and 85-bp fragments; and the A/G heterozygote (Ile/Val) showed 176-, 91-, and 85-bp fragments. To ensure the reliability of the results, approximately 15% of the samples were randomly selected and genotyped independently by a second researcher using the same protocol. In all cases the results coincided.

Statistical Analysis

All data analyses were carried out using the statistical software SPSS 15.0. Descriptive analyses were performed using *t* tests for continuous variables (age and body mass index [BMI]), whereas the χ^2 and Fisher exact tests were used for categorical variables (sex, smoking habits, and genotype distribution). The results of genotyping procedures were used to verify the Hardy–Weinberg equilibrium. Significance levels for 2-sided tests of the main effects were set at $\alpha=.05$. Logistic regression was used to calculate adjusted odds ratios (ORs) and 95% CIs for the association between GST gene polymorphisms and asthma.

Results

We analyzed 199 unrelated asthmatic adults, approximately 74% of whom were atopic, and 200 unrelated healthy controls. Table 1 summarizes the characteristics of both groups. No significant differences were found between asthmatic patients and healthy controls for age distribution, sex ratio, or any of the other characteristics analyzed.

Data regarding the genotype frequencies of the *GSTP1* Ile105Val polymorphism and the *GSTM1* and *GSTT1* homozygous deletions are shown in Table 2. *GSTP1* genotype frequencies were in Hardy–Weinberg equilibrium. The genotype frequencies of GST polymorphisms in our study population were within the ranges reported previously in Italy and in other European populations [15].

No significant differences in the genotype distributions of the *GSTM1*, *GSTP1* or *GSTT1* genes were found between asthmatics and healthy controls. X2 tests and adjusted ORs did not show any associations between genetic polymorphisms and asthma.

Table 1. Characteristics of the Study Population^a

	Asthmatics	Controls	Statistical Test
Mean (SD) age, y	52.4 (1.2)	54.9 (1.0)	<i>t</i> =1.60 P=.110
Sex			
Male (%)	82 (41)	89 (44)	$\chi^2=0.32$ P=.57
Female (%)	117 (59)	111 (56)	
Mean (SD) BMI, kg/m ²	25.3 (0.3)	25.0 (0.2)	<i>t</i> =0.83 P=.405
Atopy			
Atopic	148 (74)	–	
Nonatopic	51 (26)	200 (100)	
Active smoking			
Smoker	41 (21)	41 (21)	$\chi^2=6.65$ p=0.036*
Ex-smoker	42 (21)	64 (32)	
Nonsmoker	116 (58)	95 (47)	
Passive smoking			
Exposed	78 (39)	61 (31)	$\chi^2=2.95$ p=0.085
Occupational risk	62 (33)	48 (24)	$\chi^2=2.12$ p=0.137

Abbreviations: BMI, body mass index.

^aData are expressed as no. (%) of patients unless otherwise indicated.

Table 2. Genotype Distribution and Odds Ratios (ORs) for Asthmatics and Controls^a

	Asthmatics	Controls	P Value	Crude OR (95% CI)	Adjusted OR (95% CI) ^b
<i>GSTM1</i>					
Null	109 (55)	101 (51)	.451	1.19 (0.80-1.76)	0.95 (0.65-1.40)
Positive	90 (45)	99 (49)			
<i>GSTP1</i> *I105V (rs1695)					
I105/I105	82 (41)	96 (48)	.370	1.32 (0.88-1.96) ^c	1.21 (0.54-2.70) ^c
I105/V105	99 (50)	87 (43)			
V105/V105	18 (9)	17 (9)			
<i>GSTT1</i>					
Null	72 (36)	59 (30)	.189	1.36 (0.89-2.06)	1.08 (0.59-1.55)
Positive	127 (64)	141 (70)			

^aData are expressed as no. (%) of patients unless otherwise indicated.

^bOdds ratios and 95% CIs adjusted for age, sex, body mass index, active smoking, passive smoking, and occupational risk.

^cDominant genetic model (wt/mut + mut/mut vs wt/wt);

^dRecessive genetic model (mut/mut vs wt/mut + wt/wt).

Discussion

The GST superfamily of enzymes is involved in xenobiotic biotransformation and in protection against ROS, using a wide range of oxidative stress metabolites as substrates [5]. Inflammation is a key component of asthma, and previous studies have reported that oxidative stress is implicated in inflammation processes [4]. Furthermore, a defect in ROS detoxification processes may influence the development of asthma or the worsening of symptoms [4]. Several studies have previously demonstrated a significant association between individuals with reduced or no *GSTM1*, *GSTP1*, or *GSTT1* activity and the risk of developing lung disease [16,17], but a recent meta-analysis has shown the extreme heterogeneity of associations between the GST genotype and asthma risk [18]. The results of our work suggest the absence of an association between *GSTM1*, *GSTT1*, *GSTP1* and asthma in adults and do not sustain the hypothesis that these genotypes are involved in increasing risk. The lack of association could be due to other interactions between host factors (not only gene polymorphisms) and the environment.

Respiratory diseases, such as asthma and its intermediate phenotypes, are the result of interactions between multiple genetic and environmental factors. The existence of a link between certain GST allelic variants and asthma remains to be demonstrated and clarified. The potential involvement of GSTs in modulating oxidative stress, activating certain substrates, and producing lipid mediators of arachidonic acid offers an interesting basis for explaining how these enzymes might contribute to proinflammatory and anti-inflammatory processes. Several factors influence asthma development and the exacerbation of symptoms. The mechanisms whereby both genetic and environmental factors influence not only the development but also the differentiation of several asthma phenotypes are complex and interactive. Furthermore several factors linked to an increased risk for asthma development are not themselves true causal factors. The differences highlighted in the prevalence of asthma in different ethnic groups reflect and highlight genetic variances with a significant coverage of environmental conditions. The growing prevalence of asthma seems to be linked to different factors. The rapid change in asthma prevalence is not linked to genetic changes in populations because these mechanisms are too slow to explain this scenario. Data on the role of the effects of environmental exposures on the risk of asthma might emerge from cross-sectional studies that are not influenced by temporal changes. The effect of environmental exposures and interactions between genetic factors and environmental conditions are still a matter of debate. Few studies, in fact, have considered the possible interaction between genetic factors and the environment.

In this scenario, the heterogeneity of the results regarding GST genes and asthma may be due, not only to publication bias and sample size, but also to extreme gene-environment interactions present in the asthmatic phenotypes and to genetic differences between human populations.

In conclusion, *GSTM1*, *GSTP1*, and *GSTT1* polymorphisms were not associated with the development of asthma in a sample of patients from Rome. Oxidative stress is certainly

implicated in the pathogenesis of asthma and GST enzymes may therefore exert either a direct or indirect influence on asthma risk. Our results, however, confirm the uncertain role of GST genes in the development of asthma. Future studies with large samples should focus on interactions between GST genes and environmental oxidative exposure and other genes involved in antioxidant pathways in order to explain the role of GST gene polymorphisms in asthmatic phenotypes.

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