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Polymorphism of *Glutathione S-Transferase M1* and *T1* Genes and Susceptibility to Childhood Asthma: A Study from North India

Srivastava DSL[1]*, Mittal K[1], Jindal P[1] and Kumar A[2]

[1]*Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak-124001, Haryana, India*

[2]*Center of Medical Biotechnology, M.D. University, Rohtak, Haryana, India*

Abstract

Objectives: Glutathione S-transferases (GSTs) enzymes play an important role in the xenobiotic biotransformation of endogenous or exogenous toxicants and thought to protect the airways from oxidative stress, inflammation, and genotoxicity. Polymorphisms in the GST genes may lead to an increased imbalance in antioxidant systems and may influence the pathogenesis of asthma. We examined the association of the GST gene polymorphism to ascertain whether high-risk genotypes of *GSTM1*/*GSTT1* could influence the susceptibility to childhood asthma in the North Indian population.

Methods: The study constituted of 100 childhood asthmatic cases and 180 age-matched controls. The *GSTT1* and *GSTM1* null genotypes were identified by multiplex PCR in peripheral blood DNA samples. Statistical analysis was done by using SPSS 20.0 software.

Results: No association was seen either the null genotype of the *GSTM1* or *GSTTT1* ($P > 0.05$); however, in atopy patients significant association were observed with null genotype of *GSTT1* (OR=2.67; $P<0.05$) for risk of child hood asthma. In combined analysis of GSTM1/*GSTT1*, presence of null genotypes of *GSTM1* &*GSTT1* further append the risk of childhood asthma (OR=3.92; $P<0.05$) compared to positive genotypes of GSTM1 and *GSTT1*. When compared among gender, *GSTT1* null genotype was found to be statistically significant in male (OR=2.71; $P<0.005$) as compared to female childhood asthmatic patients.

Conclusion: In conclusion, null genotype of *GSTT1* exhibits significant association for the risk of childhood asthma, especially in disease predisposition and initiation.

Keywords: Childhood asthma • Glutathione S-Transferases • Gene polymorphisms • Atopy • Odd ratios

Introduction

The burden of asthma worldwide is immense with more than 300 million individuals are suffering from it and about a tenth part of those who are living in India [1]. It is a complex respiratory disease characterized by variety of clinical manifestations, such as inflammation, reversible airway obstruction, and airway hyper-responsiveness and manifest by paroxysmal dyspnoea, wheezing, or cough [2,3]. Although the exact pathogenesis of asthma is still unclear, there are evidences suggest that environmental, oxidative stress, genetic predisposition, familial, infectious, and immunological factors may influence asthma either individually or in concert [4-6]. Recent studies have reported that genetic polymorphism in drug metabolizing enzymes play an important role in the xenobiotic biotransformation of endogenous or exogenous toxicants and might be associated with asthma risk [3,4,7-9].

The glutathione S-transferases (GSTs) area supergene family of phase II drug-metabolizing enzymes, play a significant role in reactive oxygen species (ROS) mediated cell protection, xenobiotic metabolisms, and regulating inflammatory responses stimulated by xenobiotic compounds [10,11]. The evidence from studies suggest that genetic predisposition may also alter the capability of the airway to protect itself against inhaled environmental toxicants such as allergens, air pollution, smoking, drugs (beta blocker, ACE inhibitors, acetomenophen), cold air stress and certain viruses [4,6,12-18]. Identification of susceptibility genes may highlight the link between childhood asthma and oxidative stress, inflammation, and immunity [5,13]. Among the genes affecting asthma, GSTs play an important role in the functioning of antioxidant defenses by repairing the damage caused by reactive oxygen species [9,13]. GSTT1 and GSTM1 genes are expressed in the respiratory tract of patient and null genotype has been found to abolish enzymes activity and increases the susceptibility to oxidative stress [9,11,15-17]. It has been reported that functional consequences of the GSTM1 and the GSTT1 null genotype frequencies can vary considerably by nationality and ethnicity [17].

Recent studies have shown that the presence of null genotype of GSTM1 and GSTT1 enzymes are associated with increased susceptibility to several diseases including asthma, psoriasis, and other immune diseases [7,11,17]. Although, associations between null genotypes of GSTT1/ GSTM1 genes and risk of childhood asthma examined in various ethnic groups have shown contradictory results, some show significant association other do not but there is no report from India [16,19-25]. Therefore, the present study was undertaken to ascertain whether null genotypes of GSTM1/GSTT1 could influence the susceptibility to childhood asthma in the North Indian population. We also aimed to assess whether null genotype could affect the grade of childhood asthma, or with atopy of the disease.

Materials and Methods

Sample size for the study was calculated by using the Epi Info 7 software. Approval for the study was obtained from the institutional review board. The consent form from the participants was obtained after explaining the aims and objectives of the study. The study group consisted of 100 childhood asthma patients, and 180 age and sex matched normal healthy individuals as controls. The ethnic origin of the cases and controls were

***Address for Correspondence:** Dr. Daya Shankar Lal Srivastava (PhD), Department of Biochemistry, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak - 124001, Haryana, India, E-mail: dshankarpgi@yahoo.com*

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similar. The inclusion criteria for controls were the absence of any prior history of asthma or other diseases. Child hood asthma patients for the study was recruited from the outpatient department (OPD) of the Pediatric Department, Pt. B.D. Sharma PGIMS, Rohtak who were diagnosed based on GINA guidelines were selected [18]. Disease history was including the onset, duration, severity of symptoms based on frequency of day and night symptoms and family history of the disease. Only patients with physician-diagnosed asthma in the age group of 5 to 14 years were selected. However, patients with other causes of wheezing like cold and flu, foreign body inhalation, vocal cord dysfunction, tuberculosis, CHF, acute bronchitis, cystic fibrosis, or any other related diseases were excluded from the study. In the staging of disease, 52.94% of patient were mild persistent, followed by moderate persistent (34.31%), intermittent asthma (7.84%) and severe persistent (5%) respectively.

DNA extraction and genotyping

Two milliliters (ml) of blood of blood was collected in EDTA vials from controls and patients. Genomic DNA was extracted from blood lymphocytes using the proteinase K and phenol chloroform extraction procedure [26]. Isolated DNA (100–150 ng) was amplified in a total volume of 25 ml reaction mixture containing 20 pmol of each of the following primers: GSTM1;Forward, 5'-GAACTCCCTGAAAAGCTAAAGC-3' and Reverse, 5'-GTTGGGCT-CAAATATACGGTGG-3'; GSTT1; Forward, 5'-TTCCTTACTGGTCCTCAC ATCTC-3' and Reverse, 5'-TCACGGGATCATGGCCAG CA-3'. Exon 7 of CYP1A1 genes was co-amplified and used as an internal control using following primers: Forward, 5'-GAACTGCCACTTCAGCTGTCT-3' and Reverse, 5'-CAGCTGCATTTGGAAGTGCTC-3'. The multiplex PCR method was used to detect the presence or absence of GSTT1 and GSTM1 genes in the genomic DNA samples, simultaneously in the same tube as described previously [17]. Each set of reaction included both positive and negative controls. The multiplex PCR method will be used to detect the presence or absence of the GSTT1 and GSTM1 genes in the genomic DNA samples, simultaneously in the same tube. Electrophoreses of PCR products were done in 2% agarose gels and visualized by ethidium bromide staining. DNA from samples positive for GSTM1 and GSTT1 genotypes yielded bands of 215 bp and 480 bp whereas internal positive control (CYP1A1) PCR product corresponded to 312 bp (Figure 1).

Statistical analysis

Statistical analysis was performed using SPSS software version 20.0 (Chicago). Descriptive measures such as mean and standard deviation were applied for normally distributed variables and t-test for comparison between groups. Binary logistic regression model (BLRM) assessed differences in genotype prevalence and association between cases and controls. Multivariate analysis, Chi-square test, correlation coefficient, odds ratio (OR), and its 95% confidence interval (CI) were used to describe the strength of association. A P-value of<0.05 was considered to be statistically significant.

Results

Present case-control study was age and sex matched and the median of age (years) in control was 10 (7.25-15) and in the case was 9 (6-14) and there was non-significant differences were observed (P-value=0.092). During the study, the results of 5% of the samples were checked randomly. Data input and process was double-tracked and adopting logic check.

Table 1 represents the frequencies of GSTM1 and GSTT1 genotypes by case-control status for the risk of childhood asthma. Of the 100 patients with childhood asthma, the frequency distribution of the null genotype of GSTM1 and GSTT1 was 34.0% and 26.0% respectively while among 180 control samples, the frequency of GSTM1 and GSTT1 null genotype was 30.0% and 17.3% respectively (Table 1). In the present study, a trend of association was observed between GSTT1 null genotype and childhood asthma but statistically it was non-significant as compared to control (P-value=0.082; OR=1.69). Similarly, no association was observed with the null genotype of GSTM1 as compared to control for the childhood asthma risk (P-value=0.49; OR=1.20).

A combination of the two high-risk genotypes (null genotypes of GSTM1/GSTT1) was also compared to the non-risk genotypes (positive genotypes of GSTM1/GSTT1) for the risk of childhood asthma. The odd ratio for childhood asthma in two high-risk genotypes was 3.92-fold higher than the non-risk genotypes (P<0.001) (Table 2).

The association between gene interaction and family history with

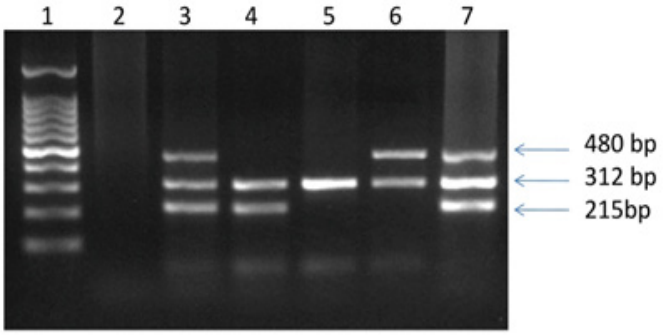

Figure 1. Lane 1 is 100 bp ladders, lane 2 negative controls, lane 3 positive control for GSTM1 and GSTT1 genes. Lane 4 depicts positive genotype of GSTM1 whereas lane 6 for GSTT1. However, lane 7 depicts positive for GSTM1 and GSTT1 genotypes whereas lane 5 for both null.

Table 1. Distribution of GSTM1 and GSTT1 genotypes among controls and pediatric asthma patients.

Genes	Controls (%) n = 180	Patients (%) n = 100	2 tailed P-value	OR (95% CI)
GSTM1 Genotypes				
GSTM1 Positive	126 (70.0)	66 (66.0)		1.0 (Ref.)
GSTM1 Null	54 (30.0)	34 (34.0)	0.490	1.20 (0.71- 2.03)
GSTT1 Genotypes				
GSTT1 Positive	149 (82.7)	26 (74.0)		1.0 (Ref.)
GSTT1 Null	31 (17.3)	74 (26.0)	0.082	1.69 (0.94- 3.04)

Table 2. Combined distribution of GSTM1and GSTT1 genotypes in controls and pediatric asthma patients.

GSTM1 and GSTT1 combined genotypes	Controls (%) n = 180	Patients (%) n = 100	2-Tailed P-value	OR (95% CI)
Both positive	102 (56.7)	52 (52.0)		1.0 (Ref.)
GSTM1 null and GSTT1 positive	48 (26.7)	22 (22.0)	0.761	0.89 (0.49 -1.65)
GSTM1 positive and GSTT1 null	24 (13.3)	14 (14.0)	0.700	1.14 (0.59 - 2.39)
Both null	6 (3.3)	12 (12.0)	0.009	3.92 (1.39 -11.05)

and without Atopy of the disease was summarized in Table 3. Our data analysis demonstrated significantly higher risk in family history with atopy of the disease (OR=2.67; P<0.001) for null genotype of GSTT1 whereas it was non-significant for GSTM1 null genotype with atopy of the disease as compared to control (Table 3).

The gender-wise frequency distribution of GSTM1 and GSTT1 null genotypes in patients and controls is summarized in Table 4. In the present study, higher frequencies of null genotype of GSTT1 for cases (33.33%) were observed than the controls (15.94%) among males. In statistical analysis, we observed significantly higher risk in males (2.7 fold) than the females for GSTT1 null genotype towards susceptibility of childhood asthma in our population (OR=2.71; P<0.05). However, no association could be established with null genotypes of GSTM1 among males or females for the disease (P > 0.05) (Table 4).

To explore the association between GSTT1/GSTM1 genotype with grade of the disease, we further the categorized severity of disease into three categories: a) mild, b) moderate and c) intermittent and severe persistent. There was no correlation was observed between GSTM1 or GSTT1 genotype and grade of the disease (P > 0.05) with respect to progression of the disease (P >0.05) (Table 5).

Discussion

In the present study, no association was observed with the null genotype of GSTM1 for childhood asthma risk (P-value=0.49; OR=1.20). Concord to our findings, no association were observed with null genotype of GSTM1 for asthma risk from studies done in Egyptian, Russian and Taiwanese; however, studies from Bulgarian, Tunisian and Turkish and American, demonstrated significant association [16,19,21,25,27-29]. Furthermore, we observed an association trend between GSTT1 null genotype and childhood asthma risk but statistically it was non-significant. Our study was validated by previous reports done in Bulgarian, Hong Kong, Egyptian and West Siberian population; however, contrast to our significant association were reported in Tunisian, Turkish, Russian and European population reported [16,21-24,27,28]. We have also observed no association between either with GSTM1 or GSTT1 genotype and the grade of disease (Table 5). In contrast to our data, a study from Southampton showed the association of the GSTT1 null genotype with an increased severity score in patients with brachial asthma but it was non-significant with null genotype [13]. In a recent meta-analysis done by Piacentini et al. [9], showed non-significant association for asthma risk GSTM1 with null genotype of GSTM1 (pooled OR=1.12, 95%, CI=0.99–1.26; P=0.072) but increased risk were observed for GSTT1 null genotype (OR of 1.33; 95% CI 1.10–1.60; P=0.003). Another meta-analysis done by Liang et al. (2013) showed a significant association for both the GSTM1 (null vs. present, OR=1.45; P<0.001) and GSTT1 polymorphism (OR=1.79; P<0.001) in the overall population. However, in subgroup analysis by ethnicity revealed no association for null genotype of GSTM1 in Asia and Russia but it was significant with GSTT1 null genotype [30]. Therefore, it appears that the association of the null genotype of GSTM1 and GSTT1 genes with childhood asthma risk varies greatly among in different ethnic group.

In combined analysis, we found the risk of development of asthma was 3.92-fold higher in those persons who have null genotypes of GSTM1/GSTT1 (two high-risk genotypes) compared to positive genotypes of GSTM1 and GSTT1 (P<0.001) (Table 2). Various studies have shown that patients who possess the null genotypes of GSTT1 and GSTM1 have a higher risk for developing the diseases such as psoriasis, and asthma as compared to those who possess positive alleles of GSTM1/GSTT1 gene [17,27]. Our finding was also in consistent with study done by Vavilin et al. in Russian and European Children which show 5 fold higher risk of asthma in children those having null genotypes of GSTM1 and GSTT1 as compared to positive genotypes of GSTM1 and GSTT1 [27]. The present study showed an increased risk of childhood asthma for the GSTM1 and GSTT1 null genotypes, and suggests that gene-gene interaction may play important role in the predisposition of childhood asthma in North Indian population.

We also determined the strength of association between gene interaction (GSTM1/GSTT1) and family history with & without atopy of the disease and

Table 3. Distribution of GSTs genotypes with family history of atopy of the disease.

Gene		Genotypes Positive	Null	2 tailed P-value	OR (95% CI)
GSTM1	Controls	126 (70.0)	54 (30.0%)		1.0 (Ref.)
	Family history without atopy of the disease	48 (66.7)	24 (33.3)	0.651	1.16 (0.65 -2.03)
	Family history with atopy of the disease	18 (64.3)	10 (35.7)	0.518	1.30 (0.562 -2.99)
GSTT1	Controls	149 (82.7)	31 (17.3%)		1.0 (Ref.)
	Family history without atopy of the disease	56 (77.7)	16 (22.3)	0.357	1.37 (0.698 -2.71)
	Family history with atopy of the disease	18 (64.3)	10 (35.7)	0.022	2.67 (1.12 - 6.34)

Table 4. Gender-wise (male/female) frequency distribution of GSTM1 and GSTT1 null genotypes among control and pediatric asthma patient.

Gene	Gender	Genotype	Controls (%) n = 180	Patients (%) n = 100	2 tailed P-value	OR (95% CI)
GSTM1	Male	Positive	94 (69.1)	42 (65.6)		1.0 (Ref.)
		Null	42 (28.9)	22 (34.4)	0.621	1.17 (0.62 -2.21)
	Female	Positive	32 (72.7)	24 (66.6)		1.0 (Ref.)
		Null	12 (27.3)	12 (33.4)	0.556	1.33 (0.51 -3.48)
GSTT1	Male	Positive	114 (83.8)	42 (65.6)		1.0 (Ref.)
		Null	22 (16.2)	22 (34.4)	0.003	2.71 (1.36 - 5.40)
	Female	Positive	34 (77.3)	32 (94.0)		1.0 (Ref.)
		Null	10 (22.3)	4 (6.0)	0.173	0.43 (0.12 -1.49)

Table 5. Association between stage of diseases and GSTs genotypes.

Gene	Disease Stage Group	Genotypes		2 tailed P-value	OR (95% CI)
		Positive	Null		
GSTM1	Intermittent and Mild stage of patients	41 (68.2)	20 (32.8)	1.0 (Ref.)	
	Moderate and Severe stage of patients	25 (64.1)	14 (35.9)	0.748	1.15 (0.49 -2.67)
GSTT1	Intermittent and Mild stage of patients	45 (73.8)	16 (26.2)	1.0 (Ref.)	
	Moderate and Severe stage of patients	28 (71.8)	11 (28.2)	0.828	1.11 (0.45 -2.72)

observed that odd ratios for null genotypes of GSTT1 were significantly higher in atopy of the disease (OR=2.67; P<0.001) as compared to non-atopic patients whereas no association were observed for null genotypes of GSTM1 either in atopic or non-atopic patients for the risk of childhood asthma (Table 3). Similar to our results, Tamer et al. in Turkish (OR=3.83; 95%, CI=1-24-11.78) and by Hanene et al. in the Tunisian population have also observed significant higher risk for GSTT1 null genotype in atopic patients (P=0.008) but a study in Hong Kong population was found to be protective with null genotypes of GSTM1 (OR=0.55; P=0.017) for the development of asthma with atopy of disease [16,22,28]. It suggests that ethnicity can be considered as one of the most important reasons for these differences and it may be due to different genetic backgrounds, and environmental exposures.

In the present study, we found a 34% frequency of GSTM1 null genotypeand20% for null genotype of GSTT1. The genotypic frequency of GSTM1 in our population lies in similar ranges as previously reported from Indian, African, and Southern Asian populations. However, studies from Caucasian, European, Eastern, and Southern Eastern Asians show higher frequency as compared to our population. Similarly, GSTT1 null genotype frequency observed in our population concord to previous data of Caucasian, European, Southern Asian, and Indian but it was contradictory from African, Eastern and Southern Eastern Asian populations [17].

On comparing the gene polymorphisms among males and females, a significant difference (P<0.05) was observed for GSTT1 null genotype among males only. GSTT1 null genotype was 33.33% in childhood asthma and it was significantly higher as compared to control (15.94%). Previous study has also shown that male children have higher risk than the females for susceptibility of asthma in Egyptian population [25]. Result obtained in our study suggests that males who possess null genotypes of GSTT1 may have a higher risk (OR=3.53) for childhood asthma as compared to females (OR=2.57) in the north Indian population (Table 4).

GSTs are phase II xenobiotic-metabolizing enzymes active in detoxification of a wide variety of potentially toxic electrophiles by conjugating them with glutathione and followed by their metabolism. They are also involved in the deactivation of oxidative metabolites of exogenous or endogenous toxic agents (i.e., allergens, industrial chemicals, dietary compound, tobacco or tobacco-like products, drugs, ozone, and other environmental toxicants) that are probably associated with asthma risk [6,12-14,29]. There is evidence suggesting that GSTM1/GSTT1 genes might interact with air pollution and tobacco smoke exposures and result in exacerbating respiratory symptoms and decreasing lung function in asthmatic individuals, thereby showing association with increasing the initiation and progression of the disease [14,20,31,32]. The association with null genotypes of GSTs observed in our study suggests that the inactive form of GSTs enzymes results in reduced detoxification of endogenous/or exogenous toxicants leading to the development of childhood asthma in our population.

This is the first genetic study in the Indian population exploring the interaction between GSTM1/GSTT1 genotype alone or in combination with family history of atopic patients and susceptibility to childhood asthma. To evaluate the interaction between genetic and environmental factors, an adequately large sample size is needed. Although, a small effect cannot be excluded and it is possible that these genes or with other detoxifying genes i.e., GSTP1, mEH, NATs, act on airway disease through interaction with environmental exposures. This is similar to what we have observed with our study. We have examined only two detoxifying genes and further study may be warranted to explore the involvement of other antioxidants and detoxification pathway genes that may be associated alone or in combined analysis in large epidemiological studies.

Conclusion

Our findings indicate that null genotypes of GSTT1 are significantly associated with childhood asthma risk in family history with atopy of disease. Moreover, the presence of both high-risk genotypes of GST genes further augments the risk of childhood asthma in the North Indian population. However, no association was observed for null genotypes of GSTM1 or GSTT1 alone with child hood asthma or with the stage of disease.

Authors' Contributions

Dr. DSLS and Professor KM conceived, designed, and conducted the study; they also wrote the manuscript. Dr PJ helped in performing the experiments whereas Dr. AK has helped in editing the manuscript. DSLS will act as guarantor for this paper. The authors are highly thankful to Vice-Chancellor, Pt. B. D. Sharma U.H.S. Rohtak for scientific encouragement and financial support.

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Nil.

Conflict of Interest

There is no conflict of interest.

Ethical Approval

Approval for the study was obtained from the institutional ethical committee of Pt BD Sharma Post Graduate Institute of Medical Sciences Rohtak in month of December 2018. The informed written consent from all the participants in the study was obtained after explaining the aims and objectives of the study.

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