

# Genetic polymorphisms of glutathione S-transferase mu1 (GSTM1) and theta1(GSTT1) and bronchial asthma susceptibility in Ukrainian population

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## Abstract

Asthma is a chronic inflammatory disease, which involves a variety of different mediators, including reactive oxygen species (ROS). Previous studies have suggested that glutathione S-transferase (GST) genotypes may play a role in determining susceptibility to bronchial asthma (BA), though the data are often conflicting. In this study we investigated GSTT1 and GSTM1 status in relation to BA in asthmatic patients in Ukraine. To evaluate the role of GSTT1 and GSTM1 genotypes in susceptibility to BA, we conducted a case-control study of 95 cases of asthmatic patients and 253 population-based controls in Kiev city, Ukraine. All patients and control group were interviewed for information on lifestyle risk factors and DNA extracted from blood samples was used for genotyping. GSTT1 and GSTM1 genotypes were identified by multiplex polymerases chain reaction. The frequencies for the GSTT1 null genotypes were 26.31% and 14.12%, and for the GSTM1 null genotypes 41.50% and 50.59% among cases and controls, respectively. The GSTT1 null genotype was associated with an increased Chi-square for BA ( $\chi^2=6.12$ ,  $P=0.013$ ), but no relationship between BA and the GSTM1 null genotype was observed. Its possibility that genetic polymorphism in detoxifying enzymes may have a role in individuals susceptibility to BA.

**Keywords:** Bronchial asthma (BA); Genetic polymorphism; Glutathione S-transferase T1 and M1.

## INTRODUCTION

The atopic diseases, such as bronchial asthma, atopic

dermatitis and allergic rhinoconjunctivitis, were rare a few decades ago, but constitute today an increasingly severe public health problem.

In the last decade bronchial asthma (BA) has become one of most frequent occurring pathology. Asthma has become an epidemic, affecting 155 million individuals in the world. Only in the United States, asthma affects 14 million persons. It is the most common chronic disease of childhood, affecting an estimated 4.8 million children. People with asthma collectively have more than 100 million day of restricted activity and 470000 hospitalizations annually. More than 5000 patients die of asthma each year in the United States, and about 60000 persons in the world (Adams and Marano.,1995 and Lefant *et al.*, 1997). These rates have increased or remained stable over the past years (Parnia *et al.*, 2002).

The cause of asthma and the nature of the basic pathogenesis have not been established. However, asthma is clearly not a single genetic abnormality, but rather a complex multigenic disorder with a strong environmental contribution. Meanwhile, recent evidence indicates that asthma is a chronic inflammatory disease and not simply due to smooth muscle contraction. Inflammation is present in virtually all asthmatic airways and is the proximate cause of the hyperreactivity and airflow limitation found in asthma. On the basis of this knowledge, recent guidelines for asthma care advocate therapies targeted toward decreasing inflammation (Szeffler and Pedersen, 2003). An increase in airway inflammation follows exposure to inducers such as allergens, viruses, exercise, or irritant inhaled. Increased inflammation leads to exacerbation of BA characterized by dyspnea, wheezing, coughing

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and chest tightness. Chronic airway inflammation in asthma involves a complex interaction of cells and mediators.

One of the assumed mechanisms, which underlie chronic inflammation process, is oxidative stress, which can arise in influence of the adverse environmental factors on susceptible human organism. Individuals susceptible to various environmental carcinogens and mutagens could have greatly heightened genotoxic responses to exposures that induce little or no response in nonsusceptible individuals (Albertini *et al.*, 1996 and Li *et al.*, 2003).

In nature, oxygen-rich environment of the lungs, the toxic effects of oxidants are carefully balanced by several antioxidant defense systems supporting normal cellular function. An increase in exogenous or endogenous oxidative stress or decrease in antioxidant capacity can lead to tissue damage and dysfunction.

Many epidemiological researches testify this due to deterioration of environmental condition owing to anthropogenesis intervention. But that is obvious, what not all people exposed to the harmful influences are affected by BA. The reason morbidity of BA is the individual sensitivity to influences of the environmental factors, that's why there is an interest upraise towards the processes of detoxification of xenobiotics. Almost all lung diseases are connected with inflammation processes, many of which arise owing to influence toxic levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). On the basis of this concept lays revealing a high level ROS and oxidative-modified proteins in respiratory ways of asthmatic patients (Calhoun *et al.*, 1992; Fahy *et al.*, 1993; Antezak *et al.*, 1997; Postma *et al.*, 1998; Sanders, 1999 and Hunt *et al.*, 2000).

The high levels ROS in the lung of asthmatic patients are produced by active inflammatory cells (macrophages, eosinophils, neutrophils, etc.). The increase in the production of ROS by neutrophils in the asthmatic patients is related to the degree of hyperresponsiveness of respiratory ways. Neutrophils and mononuclear cells from asthmatic patients generates more amount of superoxide ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) as compared to cells of matched healthy subjects. This activity correlates with metacholine-induced airway hyperresponsiveness (Calhoun *et al.*, 1992; Fahy *et al.*, 1993; Antezak *et al.*, 1997 and Postma *et al.*, 1998).

Recently it has been shown that some changes in the functions of system detoxification of xenobiotics result in the increased susceptibility organism to harmful influences and, as a consequence, increases risk of

factors occurrence of some diseases, including BA and cancer.

One of defense systems against the damaging effects of oxidative stress is the glutathione-S-transferases (GSTs) which catalyze the nucleophilic addition of thiol of glutathione (GSH) to electrophilic accepters of various substrates. This conjugation may result in the formation of compounds with greater water-solubility but less biological activity and these can easily be excreted in urine. The enzyme of GST not only participate in reactions of biotransformation of xenobiotics, but also in metabolism of wide number of endogenous substrates (e.g. serotonin, dopamine, leukotriene), which playing an important role in regulation of bronchospasm and inflammation reaction (Jakob, 1978; Janerik and Gunilla, 1997).

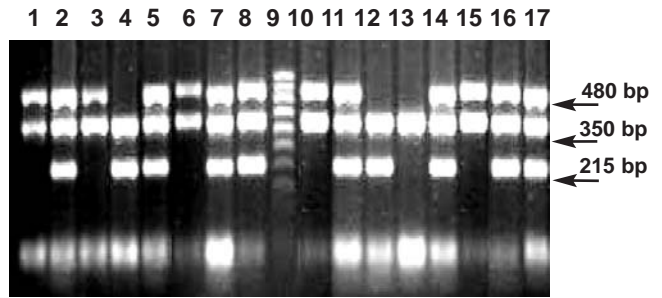
Taken together, it might be speculated, that the genes GSTs play an important role in predisposition to BA and will modify its clinical phenotype. However, the role of genes GSTs in etiology and pathology of BA remains unclear and the results received by other researchers, are controversial. To test the hypothesis that common genetic variants null genotypes for GSTT1 and GSTM1 affect susceptibility to asthma, the given research is carried out.

## MATERIALS AND METHODS

Survey was conducted on 95 adult patients BA of comprising of 28 men (29.48%), and 67 women (70.52%) aged from 18 to 70 years (average age  $44.68 \pm 1.22$  years). The age the diseases began ranged from 16 to 55 years. Controls consisted of 253 individuals of which 76 were men (29.50%) and 177 women (70.50%) aged from 17 up to 78 years (average age  $39.13 \pm 2.11$  years). All patients as well as controls were from Kiev city, Ukraine. All the asthmatic subjects had specialist physician-diagnosed asthma with i) recurrent breathlessness and chest tightness requiring ongoing treatment, ii) physician documented wheeze, iii) documented labile airflow obstruction with variability in serial peak expiratory flow rates greater than 15%, and were regular attendees at a specialist asthma clinic in the pulmonology institute in Kiev, Ukraine. All subjects were interviewed using a special questionnaire, which allowed taking into account the presence or absence symptoms of asthma and other diseases, adverse influences of environmental factors in relation with the residence, lifestyle risk the patients and their parents. All participants gave written informed consent.

**The clinical survey included:** clinic-genealogical research, routine clinical methods, estimation of lung function with the spirometry, routine analyses of blood, urine and sputum . The control group included 253 persons without BA. The basic criteria of selection of control group were the absence of BA in them and of their relatives (1<sup>st</sup> and 2<sup>nd</sup> degree of relationship) as well as absence of relationship between the individuals.

**Sample analysis:** DNA was extracted from leukocytes of peripheral blood samples from individuals of both the groups as per supplier's instruction of commercial Kit- system «DNA-sorb- B». Multiplex PCR was used to simultaneously amplify GSTT1 and GATM1, as described in article by Arand and his coworkers (1996). The GSTT1 primers were FOR 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and REW 5'-TCA CCG GAT CAT GGC CAG CA-3'. The GSTM1 primers were FOR 5'-GAA CTC CCT GAA AAG CTAA AGC-3' and REW 5'-GTT GGG CTC AAA TAT ACGG TGG-3'. The result amplified DNA fragments were separated by electrophoresis on a 1.5% agarose gel containing 0.4 µg ethidium bromide/ml and were subsequently visualized by UV detection. Albumin gene was used as internal positive control. The albumin primers were FOR 5'-GCC CTC TGC TAA CAA GTC CTAC-3' and 5'-GCC CTA AAA AGA AAA TCG CCA ATC-3' REW. Amplification reaction was carried out in amplificator GeneAmp2700. The Presence of amplified GSTT1 and GSTM1 product indicate to normal allele "+", which was defined by presence on electrophoregram, a product amplified by the size of 215 bp fragment for GSTM1 and a fragment 480 bp for GSTT1. The absence of amplified GSTT1 and GSTM1 product (in the presence of the albumin PCR product) indicated the null-genotype "-", and the present of amplified GSTT1 and GSTM1 indicated the functional genotype "+" for each (Fig. 1).



**Figure 1.** Multiplex PCR for detection of homozygous null alleles of glutathione-S-transferase mu 1(GSTM1) and theta 1(GSTT1). PCR products of 215 bp and 480 bp corresponds to the normal presence of the allele for the GSTM1 and GSTT1 genes, respectively. The 350 bp PCR fragment is an albumin gene fragment (control). Lane 9 is 50 bp DNA ladder. Lanes 1,3,6,10 and 15 show the results from individuals with the homozygous deletion in the GSTM1 allele, lanes 4 and 12 shows the results from individuals with the homozygous deletion in the GSTT1 allele and lane 13 shows a combined deletion of the GSTM1 and GSTT1 allele.

**Statistical analysis:** Chi-square test ( $\chi^2$ ) was used to examine differences between group of the patients and control group. The null hypothesis was rejected at  $P < 0.05$ .

## RESULTS

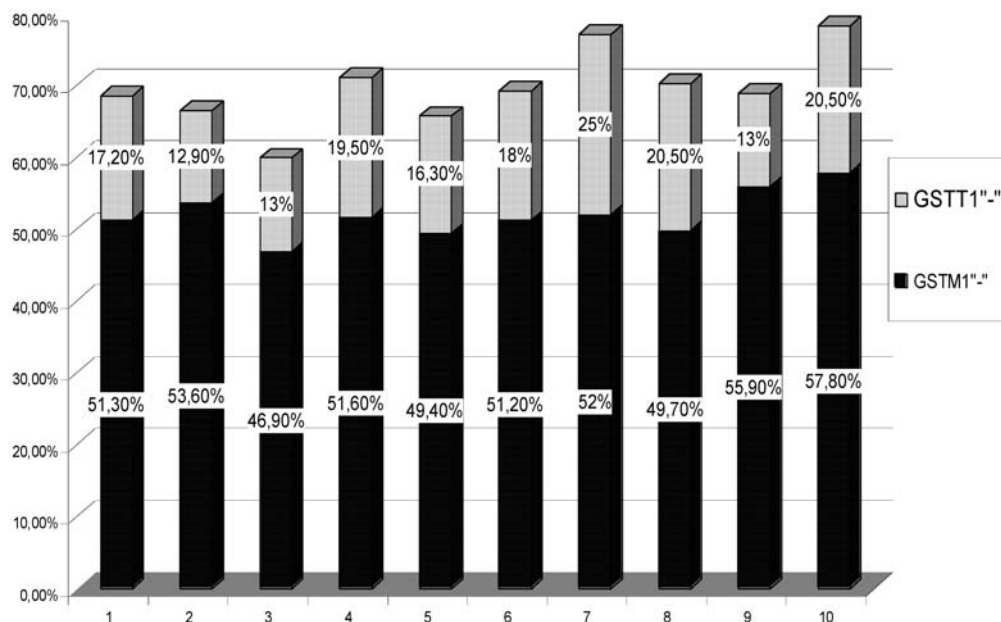
Table 1 shows the frequency of the GSTT1 and GSTM1 genotypes in control group and asthmatic patients. The distributions for gender and age among cases and controls were not statistically different.

The frequencies of distribution of genotypes GSTT1 "-" and GSTM1 "-" in control group have made 14.22 % and 50.59 %, respectively. The allele distribution was within the range of previously reported allele frequency of GSTM1 and GSTT1 null alleles in other white populations (Fig. 2).

**Table1.** The Frequency of GSTT1 and GSTT1 genotypes in control group and group of patients.

Group	Total	GSTM1				GSTT1			
		GSTM1 "-"		GSTM1 "+"		GSTT1 "-"		GSTT1 "+"	
		n	%	n	%	n	%	n	%
<b>Control</b>	n=253	128	50.59	125	49.41	36	14.12	217	85.88
<b>BA</b>	n=95	39	41.05	56	58.95	25	26.32*	70	73.68

Data are presented as n and n (%). \*significantly different at the level of  $P < 0.05$ .



**Figure 2.** Geographic distribution of GSTM1“-” (dark color) and GSTT1“-” null (white color) genotypes in Caucasian population: 1- Canada, 2- Denmark, 3- Finland, 4- Germany, 5- Italy, 6- Slovakia, 7- Slovenia, 8- Spain, 9- Sweden and 10- United Kingdom (Garte *et al.*, 2001).

The frequency of GSTT1“-” genotype in asthmatic patients was more as compared to healthy subjects (26.31%; 14.22%). The significance of  $\chi^2$  ( $\chi^2=6.17$ ,  $P=0.013$ ), testify the association of a genotype GSTT1“-” with predisposition to BA in the adult patients BA in Ukraine. In contrast, there is no significant difference ( $\chi^2=2.15$ ,  $P=0.142$ ) between the frequency of the null genotype GSTM1“-” in asthmatic patients (41.05%) and controls (50.59%).

## DISCUSSION

Many researchers have tried to established links between polymorphic expression of different GSTs and BA in different ethnic population and the results have been conflicting. One reason for the discrepancies could be the fact that most studies were conducted in different populations. The another hypothesis to explain these differents in results is that because the multiple GST enzymes have a broad substrate overlap, a decrease in the expression level of one GST may be compensated for by increased expression of another. Thus, the expression patterns for multiple relevant GST enzymes may be more closely associated with risk than the expression of each individual gene. Consequently, studies that do not control for expression of all relevant genes may generate data that are

difficult to interpret. For example, in one study the frequency of genes GSTM1“-” and GSTT1“-” in the asthmatic patients and healthy children is carried out in Novosibirsk (Russia) (Vavilin *et al.*, 2000). On the basis of the received data was concluded that: the null-genotype of GSTT1 and GSTM1 are associated with predisposition to BA, and in the children, which were exposed to influence of smoking, GSTM1“-” genotype is associated with earlier development of BA, and GSTT1“-” with severity of BA.

The same researchers in Novosibirsk (2002) studied the influence of age and sex on predisposition to BA in the children with various genotypes GSTM1 and GSTT1, NAT2. But this time, they have shown that homozygous deletion GSTM1“-” does not demonstrate significant association with predisposition to BA, whereas the genotype GSTT1“-” is significantly associated with predisposition to BA and it is more expressed in the boys than in the girls (Makarova and Vavilin *et al.*, 2002).

The one of the research conducted in Germany on 3054 children, founded that, in children lacking the GSTM1 allele who were exposed to current environmental tobacco smoke (ETS) the risk for current asthma (or 5.5, 95% CI 1.6 to 18.6) and asthma symptoms such as wheeze ever (or 2.8, 95% CI 1.3 to 6.0), current wheezing (or 4.7, 95% CI 1.8 to 12.6) and shortness of breath (or 8.9, 95% CI 2.1 to 38.4) was higher



than in GSTM1 positive individuals without ETS exposure. Hints of an interaction between ETS exposure and GSTM1 deficiency were identified. In utero smoke exposure in GSTT1 deficient children was associated with significant decrements in lung function compared with GSTT1 positive children not exposed to ETS (Kabesch *et al.*, 2004). However, Kabesch and his coworkers (Kabesch *et al.*, 2003), showed that genotype GSTT1 “-” is not associated with increase in developing BA, but in children, which were exposed to influence of smoking, the genotype GSTM1 “-” increases the risk of developing BA.

Concerning BA in adult, the study of Ivashenko and Baranov at St.-Petersburg (2001) on 39 adult patients, have established, that the frequency of homozygous deletion genes GSTM1 “-” and GSTT1 “-” in a population is significantly lower (37.8 %, 16.3 %) than in the asthmatic patients (82.1 %, 3.7 %). The other research is carried out in 112 patients with atopic BA (Gembitskaya *et al.*, 2003) also verifies the association between the BA and null-genotype of genes GSTM1 “-” and GSTT1 “-”, and more earlier beginning and severity of BA in the patients till 30 years old is associated with the presence of the combination of these two null-genotype genes in these subjects (Gembitskaya *et al.*, 2003).

But Freidin and his coworkers (2003) could not confirm the results of above two researches in the asthmatic patients in Tomsk (Russia).

As it is visible from above, the different researchers on the study of association BA with genes GSTs have received controversial results. In our opinion it will be explained as follows:

1- Till now there is no standard definition of BA, by which asthma can be diagnosed with 100 % sensitivity and 100 % specificity. It is therefore not guaranteed that false-positive and false-negative classifications are excluded, that results in complication of selecting homogeneous groups of the patients.

2- As BA is a multifactorial disease, in which there is the complex interrelations of the environmental factors and genes participate, that results in the heterogeneity (genotypically and phenotypically) of BA. In the researches the interrelation a gene-gene, gene-environment and environmental factors among themselves was not taken into account.

3- The different researches used different study design and different sample size.

4- The frequency of homozygous deletion of genes GSTs differs between various races and populations.

The frequencies of polymorphic GSTs genes in control populations have been reported to be different in various ethnic groups. In addition, intra-ethnic differences have been established (Garte *et al.*, 2001). The frequencies of GSTM1 and GSTT1 null genotypes range from 42% to 60% and 13% to 26%, respectively, in Caucasians (Garte *et al.*, 2001). Among Asians, a significant difference between Japanese and other Asians was observed for both GSTM1 “-” and GSTT1 “-”, with Japanese showing lower frequencies of both deletions (Table 2).

**Table 2.** Geographical distribution of GSTs gene alleles in Asian populations.

	GSTM1	GSTT1
Japan	0.479(639)	0.353(167)
Korea	0.521(165)	0.515(165)
Singapore	0.562(244)	0.519(243)

Numbers in parentheses denote the number of subjects tested (Garte *et al.*, 2001).

5- The processes of transformation of xenobiotics occur in two phases. Enzyme of the phase I connect to xenobiotics to produces intermediate electrophilic compounds, which comes under the action of enzyme of second phase (II) which results in production of non-toxic water-soluble derivative that are deduced from organism. A consequence of chemical updating of molecule xenobiotics can become:

a) decrease of the toxicity properties; b) amplification of the toxicity properties; c) change of character toxicity and d) initiation of the toxification process.

These finally results in the process of transformation (toxification/detoxification) depending upon the interaction of enzymes in I and II phase. The study of separate genes of biotransformation and those participating in the process, is not giving us correct and full information to estimate the condition and capacity of process transformation in organism.

In conclusion it can be said that null-allele of GSTM1 “-” genotype does not significantly demonstrate association with predisposition to BA in the adult patients in Ukraine, whereas genotype GSTT1 “-” is significantly associated with predisposition to BA. Thus, this study suggests that polymorphism of the detoxification GSTT1 are associated with the development of BA. Since BA is a complex and multifactorial disease, single elements that increase the risk for its development may be difficult to identify. Identification of these polymorphisms (and others)

may permit earlier identification of individual with an increased for the development of BA.

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