

Short communication

Polymorphisms of the GSTM1 and GSTT1 genes in patients with allergic diseases in the Czech population

Background: Allergic diseases belong to the most common chronic disorders affecting mankind and their prevalence in population is increasing. Several studies have indicated that oxidative stress impairs pulmonary function and makes existing asthma worse. Members of the glutathione-S-transferase (GST) superfamily of genes are important in protection of cells from reactive oxygen species.

Aims of the study: Relationships among allergic diseases including asthma and variations in the GST mu (GSTM1) and GST theta (GSTT1) genes were investigated in 1006 Caucasian subjects.

Methods: The multiplex polymerase chain reaction protocol was used for simultaneous amplification of both genes for molecular analysis. Genotype frequencies among patients and controls were assessed and the associations of the genotypes with intermediary phenotypes of allergy were statistically determined.

Results: The frequencies of GST null genotypes did not differ significantly between patients with allergic diseases (or asthma alone) and healthy controls. However, when compared with patients homozygous or heterozygous for GSTM1 functional allele, asthmatics carrying both GSTM1 null alleles displayed significantly worse lung function, assessed by forced expiratory volume in 1 s/forced vital capacity (FEV₁/FVC) ratio (Tiffenau index), ($P < 0.01$).

Conclusions: Genetic polymorphisms of the GSTM1 and GSTT1 genes, both individually and in combination, were not associated with the development of allergic diseases including asthma in the Czech population, the GSTM1 gene variability, however, may influence lung functions in our asthmatics.

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Allergy and asthma are complex common diseases caused by combinations of genetic and environmental factors. Although allergy, asthma, and atopy [predisposition for producing immunoglobulin E (IgE)] frequently coexist, there are important differences in clinical disease expression, reflecting different immunologic and genetic mechanisms. Several studies have indicated that oxidative stress impairs pulmonary function and can make existing asthma worse (1). The members of the glutathione-S-transferase (GST) superfamily are important in protection of cells from reactive oxygen species (ROS) because of their ability to utilize substrates of a wide range of products of oxidative stress. GSTs are separated into the following classes: alpha, mu (GSTM), pi, theta (GSTT), sigma, and kappa. The most important polymorphism encodes for a partial gene deletion in GSTM1 locus on chromosome 1p13.3 (GSTM1 null genotype) resulting in the complete absence of GSTM1 enzyme

activity. The frequency of the GSTM1 null genotype ranges from 23% to 62% in various populations and is approximately 50% in the Caucasians (2). At the GSTT1 locus on chromosome 22q11.2, the GSTT1 null genotype represents a partial gene deletion and is associated with the absence of functional activity of the GSTT1 enzyme. The frequency of the GSTT1 null genotype ranges from 16% to 64% in various populations over the world, being approximately 20% in the Caucasians (2). As a part of a study examining relations between the polymorphisms of candidate genes and asthma or other allergic diseases, we recently screened the Czech population for both polymorphisms in the GSTM1 and GSTT1 genes.

Methods

This population-based, case-control study formed a total sample of 1006 unrelated Czech subjects living in the South Moravia region. All subjects were selected using a detailed questionnaire considering lifetime symptoms suggestive of asthma, rhinoconjunctivitis, and atopic eczema/dermatitis syndrome (AEDS) extended with additional questions regarding symptoms and therapy as well as other

Abbreviations: AEDS, atopic eczema/dermatitis syndrome; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GST, glutathione-S-transferase; IgE, immunoglobulin E; PCR, polymerase chain reaction; ROS, reactive oxygen species.

diseases. A total of 675 patients with clinically manifested IgE-mediated allergic diseases [asthma and/or rhinitis and/or dermatitis, 335 men and 340 women, aged 27.9 ± 13.5 years (mean $\pm$ SD)] were studied. A total subsample of 306 patients with IgE-mediated asthma (170 men and 136 women) was included in our study. Asthma diagnosis was based on the following criteria: (i) current physician diagnosis of asthma, (ii) symptoms, and (iii) use of antiasthma medication according to the Global Initiative on Asthma (GINA) criteria (3). Asthmatics were subclassified according to asthma severity into the four categories according the GINA criteria (3): (i) intermittent ($n = 71$), (ii) mild persistent ($n = 120$), (iii) moderate persistent ($n = 46$), and (iii) severe persistent (no such case); in 69 remaining patients, severity of asthma was not determined. The diagnosis of allergic rhinitis was based on coordination between a typical history of allergic symptoms of either perennial or seasonal rhinitis and diagnostic test for IgE-mediated allergy. The AEDS was defined according to the diagnostic criteria for atopic eczema. Atopy was recognized by specific IgE (> 0.35 kU/l) and/or a positive skin prick test to one or more allergens as described previously (4). Pulmonary function, assessed by spirometric measures of forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC), was analyzed in the subgroup of asthmatic patients. The control group consisted of 331 healthy subjects (172 men and 159 women), aged 36.5 ± 16.2 years who met the following criteria: (i) no prior or current physician diagnosis of asthma and/or rhinitis and/or dermatitis, (ii) no history of wheezing, shortness of breath and other symptoms of allergic diseases, (iii) no use of antiasthma medications, and (iv) no parenteral history of atopic diseases. The study was approved by the Committee for Ethics of the Medical Faculty, Masaryk University Brno and informed consent was obtained from all participants, in line with the Helsinki declaration before inclusion in the study.

Genomic DNA for polymerase chain reaction (PCR) was isolated from peripheral blood leukocytes by a standard method. The PCR conditions for determining GSTM1 and GSTT1 genotypes were the same as those reported previously (5).

Comparisons were made between genotype frequencies in the disease and control populations. Chi-square analysis was used to test for deviation of genotype distribution from Hardy–Weinberg equilibrium and for a comparison of differences in genotype combinations among groups. Comparisons of quantitative parameters (total serum IgE levels, selected specific IgE levels, and lung functions) corresponding to particular genotype groups of the GSTM1 and GSTT1 polymorphisms were performed by Kruskal–Wallis ANOVA. For all statistical analyses programme package Statistica v 6.0 (Statsoft Inc., Tulsa, OK) was used.

Results

Table 1 shows selected clinical characteristics of the study population. Mean age of control subjects was significantly higher than those of cases, however, contrary to the opposite situation (i.e. younger controls in which the possibility of diseases development can not be ruled out) this should not seriously distort our following findings. Smoking was significantly less common in the allergic group; on the contrary total IgE levels were significantly higher in allergic patients.

Genotype frequencies of both GST polymorphisms in the case and control groups are presented in Table 2. The genotype distribution neither deviate from Hardy–Weinberg equilibrium in any of the groups nor did it differ

Table 1. Clinical characteristics of the study subjects

Characteristics	Allergic patients ($n = 675$)	Controls ($n = 331$)
Age (years $\pm$ SD)	27.9 ± 13.5	36.5 ± 16.2
Sex (females/males)	335/340	172/159
Smoking no/yes (%)	83.4/16.6	71.2/28.8
Serum IgE (IU/ml)	242.8 ± 420.8	81.0 ± 144.5
Only asthma (%)	37 (5.5)	0
Only rhinitis (%)	287 (42.5)	0
Only AEDS (%)	23 (3.4)	0
Asthma and rhinitis (%)	150 (22.2)	0
Asthma and AEDS (%)	20 (3.0)	0
Rhinitis and AEDS (%)	58 (8.6)	0
Asthma, rhinitis and AEDS (%)	100 (14.8)	0
FVC (% predicted)*	96.3 ± 13.2	ND
FEV ₁ (% predicted)*	95.1 ± 13.7	ND
FEV ₁ /FVC (%)*	80.7 ± 29.9	ND
Inhaled steroids no/yes (%)	55.7/44.3	ND

Data are expressed as means $\pm$ SD unless stated otherwise.

IgE, immunoglobulin E; AEDS, atopic eczema/dermatitis syndrome; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ND, not determined.

*Spirometry was completed in asthmatics only.

between patients and controls. The frequencies of the GSTM1 null genotype were 0.502, 0.564, and 0.542 in the healthy control subjects, allergic patients, and asthmatic subgroup respectively. The frequencies of the null genotype of GSTT1 were 0.221, 0.170, and 0.193 in control, allergic, and asthmatic groups respectively. The frequencies of the GST null genotypes did not differ significantly between the patients with allergic diseases (or asthma alone) and healthy controls. To investigate whether the profile of GST genotypes might be associated with the risk of allergy and/or asthma, we examined both genotypes in combination. The distributions of the null genotypes of both GSTM1 and GSTT1 in patients with allergic diseases (0.095) and/or asthma (0.114) were not different from that of the control group (0.094). However, when compared with patients homozygous or heterozygous for GSTM1 functional allele, the asthmatics carrying both GSTM1 null alleles displayed significantly worse lung function, assessed by ratio FEV₁/FVC (Tiffenau index) [$85.4 \pm 26\%$ (mean $\pm$ SD) vs $75.2 \pm 25\%$; $P < 0.01$ by Kruskal–Wallis ANOVA]. Finally, although comparison of genotype frequencies of the GSTM1 and GSTT1 between the group of patients with persistent (mild-to-moderate) vs intermittent asthma showed no significant differences, a trend towards higher frequencies of the GSTM1 null genotype was marginally significant in the group of patients with inhaled steroid treatment compared with those without this therapy (54.7% vs 45.34% , $P = 0.071$ respectively).

Discussion

It was suggested that inability to detoxify ROS because of null genotypes of GSTM1 and GSTT1 should perpetuate

Table 2. GSTM1 and GSTT1 genotype frequencies between allergic, only asthmatic and control subjects

Genotype	GSTM1		GSTT1		GSTM1 and GSTT1 combination		
	Wild	Null	Wild	Null	Both wild	Either null	Both null
Allergic patients	294 (43.6)	381 (56.4)	560 (83.0)	115 (17.0)	243 (36.0)	368 (54.5)	64 (9.5)
Only asthmatics	140 (45.8)	166 (54.2)	247 (80.7)	59 (19.3)	116 (37.9)	155 (50.7)	35 (11.4)
Controls	165 (49.8)	166 (50.2)	258 (77.9)	73 (22.1)	123 (37.2)	177 (53.5)	31 (9.4)

Wild genotype – patients homozygous or heterozygous for the glutathione-S-transferase mu (GSTM1) functional allele.

the inflammatory reaction in the asthmatics. In this study, we evaluated the difference in polymorphic genotypes of GSTM1 and GSTT1 genes in quite large groups of patients with allergic diseases (or IgE-mediated asthma alone) and healthy subjects. The genotype frequencies of both genes in our study population were within the ranges reported previously in the Czech (6) and also in other Caucasian white control populations (2). However, our data showed no differences in the frequencies of the polymorphic genotypes of the GSTM1 and GSTT1, both individually and in combination among all three groups. These results are in disagreement with those of previous studies, which suggested association between genotypes of GSTM1 and GSTT1 and susceptibility to asthma (7–9). On the contrary, our results concerning worse lung function in the asthmatics with both GSTM1 null alleles comply with findings of Gilliland et al. (10) who reported that GSTM1 null variant was associated with deficit in annual growth rates for FVC and FEV₁ in children.

The search for genetic contributions to complex conditions such as allergy and asthma is difficult because of genetic heterogeneity, incomplete penetrance, gene–gene, and gene–environmental interactions. Ethnic differences in the prevalence of asthma are very difficult to separate

from environmental factors. Gene frequencies are different among various racial groups, which may explain the differences in association between gene polymorphisms and asthma in the different studies. In addition, polymorphisms of each gene may impart only a small relative risk of asthma and the coincidence of many polymorphisms and their interaction with environmental factors may be different in various populations.

In conclusion, genetic polymorphisms of the GSTM1 and GSTT1 genes, both individually and in combination, were not associated with the development of allergic diseases in the Czech population but the GSTM1 gene variation may influence lung functions in our asthmatics.

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