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SHORT COMMUNICATION

The relationship between polymorphisms in the glutamate cysteine ligase gene and asthma susceptibility

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Summary
The present study was designed to investigate an association of common -588C/T and -23G/T polymorphisms within glutamate cysteine ligase modifier subunit gene with susceptibility to bronchial asthma. A total of 435 ethnically Russian subjects were recruited in this study, including 221 patients with asthma and 214 sex and age matched healthy subjects. As previously reported, the -588C/T and -23G/T polymorphisms were completely linked. The -588TT/-23TT genotype was found to be associated with decreased risk of allergic asthma after adjustment for age, gender and smoking status using multivariate logistic regression analysis (OR = 0.33 95% CI 0.15–0.70, $p = 0.036$). However, the -588CT/-23GT genotype was associated with increased risk of non-allergic asthma (OR = 2.03 95% CI 1.05–3.90, $p = 0.06$). This is a first study reporting the association between genetic variations in the glutamate cysteine ligase gene and susceptibility to bronchial asthma.

Introduction

An accumulating body of evidence indicates that imbalances in oxidant–antioxidant systems may play a role in the pathogenesis of bronchial asthma.[1–3] As oxidative stress may be involved in mechanisms causing asthma, polymorphic genes encoding for antioxidant defense enzymes may be putative candidates for genetic susceptibility to the disease. Despite the importance of this question, existing data on the impact of genetic polymorphisms of these enzymes on the development of asthma are surprisingly limited.[4–6] In the present study, we have investigated, for the first time,

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whether common polymorphisms -588C/T and -23G/T in the 5′-flanking region of glutamate cysteine ligase modifier (*GCLM*) subunit gene, the rate-limiting enzyme for glutathione (GSH) synthesis, are associated with genetic susceptibility to bronchial asthma.

Methods

A total of 435 subjects were recruited into this study, including 221 patients with asthma (97 males and 124 females) and 214 healthy subjects (92 males and 122 females). All study subjects were of Russian origin from Central Russia. The mean age of the asthmatics was 44.7 years (range: 16–69), the mean age of the healthy subjects was 41.3 years (range: 17–84). The study groups did not differ in percent of smokers (29.9% in patients vs. 26.6% in controls, $p > 0.05$). The study was approved by an Ethical Review Committee of Kursk State Medical University and the subjects who were recruited gave informed consent. All asthmatics were recruited by the Department of Pulmonology in Kursk Regional Clinical Hospital during 2003–04. All patients were diagnosed with asthma by the presence of characteristic symptoms, reversibility of airway obstruction or airway hyperresponsiveness to methacholine. Skin prick testing and total serum IgE levels were determined in all study participants. Asthmatics who showed either negative skin prick test (<5 mm) or a normal total IgE (<0.35 IU) were considered as patients with non-allergic asthma. DNA samples from study subjects were analyzed for -588C/T (rs41303970) and -23G/T (rs743119) polymorphisms in the *GCLM* gene by polymerase chain reaction followed by restriction fragment length polymorphism analyses as reported by Nakamura et al.[7] The genotyping results were scored by two independent investigators who did not know whether the sample was from a case patient or a control. Statistical calculations were performed with Statistica for Windows 6.0 (StatSoft Inc., Tulsa, OK, USA).

Results

The observed genotype frequencies of the *GCLM* gene in healthy group were in Hardy–Weinberg equilibrium. A significant deviation of *GCLM* genotype frequencies from Hardy–Weinberg equilibrium was observed in asthmatics ($p < 0.001$). The genotype distribution and allele frequencies for -588C/T and -23G/T polymorphisms of the *GCLM* gene

Table 1 Distribution of alleles and genotypes of polymorphisms -588C/T and -23G/T of the *GCLM* gene in patients with bronchial asthma and healthy subjects.

GCLM allele and genotype frequencies	Asthmatics *n* (%)†	Controls ($n = 214$) *n* (%)†	χ^2 (*p*)‡	OR (95% CI)§
Asthma, entire group (n = 221)				
Allele frequencies				
-588C/-23G allele	0.765	0.743	0.55	0.89
-588T/-23T allele	0.235	0.257	(0.46)	(0.65–1.21)
Genotype frequencies				
-588CC/-23GG genotype	120 (54.3)	120 (56.1)	0.14 (0.71)	1.04 (0.68–1.59)
-588CT/-23GT genotype	98 (44.3)	78 (36.4)	2.81 (0.09)	1.30 (0.84–2.01)
-588TT/-23TT genotype	3 (1.4)	16 (7.5)	8.34 (0.0081)*	0.33 (0.15–0.70)
Allergic asthma (n = 161)				
Allele frequencies				
-588C/-23G allele	0.776	0.743	1.12	0.83
-588T/-23T allele	0.224	0.257	(0.29)	(0.59–1.17)
Genotype frequencies				
-588CC/-23GG genotype	92 (57.1)	120 (56.1)	0.04 (0.84)	1.27 (0.79–2.04)
-588CT/-23GT genotype	66 (41.0)	78 (36.4)	0.80 (0.37)	1.04 (0.64–1.68)
-588TT/-23TT genotype	3 (1.9)	16 (7.5)	4.91 (0.036)*	0.40 (0.19–0.87)
Non-allergic asthma (n = 57)				
Allele frequencies				
-588C/-23G allele	0.728	0.743	0.10	1.08
-588T/-23T allele	0.272	0.257	(0.75)	(0.68–1.72)
Genotype frequencies				
-588CC/-23GG genotype	26 (45.6)	120 (56.1)	1.98 (0.16)	0.72 (0.38–1.38)
-588CT/-23GT genotype	31 (54.4)	78 (36.4)	6.02 (0.06)*	2.03 (1.05–3.90)
-588TT/-23TT genotype	0 (0.0)	16 (7.5)	3.28 (0.14)*	0.10 (0.01–1.74)

†Absolute number and percentage of individuals with particular genotype.
‡χ^2 statistics with Yates' correction and *p*-values (d.f. = 1).
§Odds ratio adjusted for age, gender and smoking status with 95% confidence intervals.
**p*-Values with the Bonferroni correction for multiple comparisons.

are listed in Table 1. As can be seen from Table 1, -588C/T and -23G/T polymorphisms were completely linked. The frequency of -588TT/-23TT genotype was greater among healthy subjects (7.5%) than among asthmatics (1.4%). This association remained significant after adjustment for age, gender and smoking status using multivariate logistic regression analysis (OR = 0.33 95% CI 0.15–0.70). Interestingly, a -588TT/-23TT genotype was only found to be associated with decreased risk of allergic asthma (OR = 0.40 95% CI 0.19–0.87). The frequency of -588CT/-23GT genotype was higher in patients with non-allergic asthma than in healthy controls (OR = 2.03 95% CI 1.05–3.90). However, the latter did not reach statistical significance after the Bonferroni correction for multiple comparisons ($p = 0.06$).

Discussion

Glutathione is known as major and naturally occurring antioxidant, which plays a key role in the control of pro-inflammatory processes and immune modulation in the lungs, and it has a predominant role in the regulation of intracellular redox state and protects airway cells from oxidative stress.[8,9] When airway cells are exposed to oxidative stress induced by cigarette smoke/air particulates, GSH synthesis is increased through upregulation of *GCLM* gene expression, providing a protective mechanism against free radical-mediated lung injury and inflammation.[8,9]

In the present study, we found that -588C/T and -23G/T polymorphisms in the *GCLM* gene may be a part of the genetic predisposition to asthma. A -588CT/-23GT genotype was found to be associated with increased risk of non-allergic asthma. It is known[7] that variant -588T allele but not -23T allele suppresses oxidant-induced upregulation of *GCLM* gene expression and is associated with lower plasma GSH levels. Thus, the -588T allele may possibly weaken the intracellular production of GSH in response to oxidative stress, leading to the increase in susceptibility to the oxidant-induced lung injury that is thought to occur as part of the pathogenesis of asthma. In contrast, we observed that a -588TT/-23TT genotype was associated with decreased risk of allergic asthma. Unfortunately, no data on the *GCLM* gene expression profile in carriers of -588TT genotype are available in order to explain a possible reason of this association.

In conclusion, we have identified, for the first time, that -588C/T and -23G/T polymorphisms in the 5′-flanking region of *GCLM* gene are associated with asthmatic phenotype, suggesting that such polymorphisms represent novel genetic markers able to identify subjects prone to the development of asthma. However, further investigations are required to confirm the contribution of these polymorphisms to the risk of asthma and to assess the relationships between *GCLM* genotypes and *GCLM* expression/activity. A better understanding of the genetic mechanisms contributing to an imbalance between oxidants and antioxidants in the lungs will provide more effective genotype-based therapeutic and prophylactic strategies, and will extend access to new drug targets for therapy of asthma in the future.

Conflict of interest

None declared.

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