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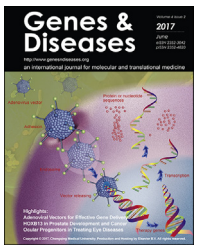

SHORT COMMUNICATION

Possible association of a distinct combined Glutathione-S-transferase members with allergic asthma patients in Pakistan

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Abstract Allergic asthma is a diverse chronic respiratory disease characterized by the inflammation of the lower airway disease affecting many people around the world with rising morbidity and mortality. Association between asthma and certain demographic features was studied in relation to genotype from 244 allergic individuals of local population. Skin prick test was used to confirm asthma. Genetic polymorphism in Glutathione-S-transferases (GSTs) was studied using multiplex PCR based method and IgE level by ELISA. Pollen and dust were the major causative aeroallergens (26%), which were associated to higher IgE levels ($P < 0.05$). Smoking was found to be significantly associated with asthma in only males ($P = 0.004$). A low prevalence of null genotype of both GSTM1 and GSTT1 genes was observed in the patients (4.34%) compared to control group (14%). No association of combined GSTM1 and GSTT1 null genotype was found with the asthma in local population. GSTM1+ and GSTT− genotype had higher risk (OR = 1.3681, $P = 0.001$) for development of asthma. There was a significant association of asthma with combined genotype of GSTM1+ and GSTT− when data was analyzed on gender basis in males ($P = 0.006$) and highly significant in age range of 26–40 years ($P = 0.001$). Combined $GSTM^{+}$ and $GSTT^{-}$ genotype was found to be risk factor for asthma in addition to family history in male patients. However a data with large patient size and different ethnic distribution may reveal the exact etiology.

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Introduction

Asthma is a disease characterized by the airway hyper responsiveness, inflammation along with fundamental structural changes to airways[1] which can lead to irreversible airway remodeling as well as intractable limitation.[2] Bronchial asthma is a severe multifactorial disease.[3] Prevalence of asthma has increased in the past few decades resulting in a significant socioeconomic impact.[4] Asthma is known to affect 35% of the children and 10% of the adults worldwide, therefore it is considered to be an increasing public health concern.[5] According to the Global Burden of Asthma report (2012) by Global Initiative for Asthma – GINA, 4.3% of the total Pakistani population (over six million people), are affected by asthma. The statistics also revealed that among asthmatic patients 32% children and 51% adults experience rigorous attacks of wheeze, cough, tightness or breathlessness.[6]

Atopy is a genetic predisposition for the development of an immunoglobulin E (IgE) induced response to the allergens. Genetic or host susceptibility to various allergens or atopy may lead to the development of asthma, and various studies show a significant relationship between allergens and asthma.[5] Atopic asthma is generally coupled with raised circulating levels of IgE.[7] But the increased level of total serum IgE is considered to be a risk factor for asthma even in nonallergic individuals. IgE molecules have been found to play a crucial role in allergic respiratory diseases and possibly cause chronic airway inflammation in asthma through activation of effector cells via high-affinity (FcεRI) or low-affinity (FcεRII) IgE receptors.[8]

It is beyond any doubt that environmental as well as genetic factors are the major contributors of allergic disease.[9] It is evident from many studies that the immune system's ability to interact with the environment is influenced by genetic predisposition.[10] In asthma a genetic predisposition may also change the capability of the respiratory airway from protecting itself against the inhaled allergens or toxic substances from the environment. This may lead to diminishing the barrier function of the airway by airway inflammation and airway epithelium damage.[11]

Overall 40–60% asthma risk is contributed by genetic factors. Many candidate genes have been identified which are related to the occurrence of atopy and asthma. The glutathione transferases (GSTs) are ubiquitously found in nature. The human GST family can be grouped into 7 distinct classes of catalytically active enzymes namely Alpha, Mu, Theta, Pi, Omega, Sigma, and Zeta.[12] It is possible that genetic alterations of GST enzymes alter the ability of the airways to deal with toxic substances and manipulate airway inflammation and lung development. Thus, GSTM1 and GSTT1 genes have been suggested as candidate genes for asthma.[13] The purpose of this work is to determine the possible association between a) IgE status with polymorphism of Glutathione-S-transferase (GSTM1 and GSTT1) gene in Pakistani population and 2) demographic and clinical parameters with polymorphism of GSTM1 and GSTT1 genes in the blood of asthmatic and allergic patients.

Materials and methods

Study subjects

Study was approved from the ethical committees of university and hospital. Informed consent was signed from all the participating individuals. Healthy individuals with no history of any sort of allergy or chronic illness with negative skin prick test were randomly selected as controls (n = 232). The study included 244 unrelated asthmatic patients from twin cities (Islamabad/Rawalpindi) of Pakistan that were confirmed by specialist doctors. All patients were recruited from Allergy Centre of National Institute of Health (NIH), Islamabad. Blood samples were taken from clinically stable asthmatic patients with positive SPT. Patients having food allergies were excluded from the data. None of the participants had received antihistamine and corticosteroids in 3 weeks prior to clinical evaluation. Questionnaires were filled for each patient as well as control group for the analysis of the data of various demographic features. This data was used to determine the association of Glutathione-S-transferase M1 and T1 gene polymorphisms with atopic asthma.

Genomic DNA from human blood samples was extracted in two consecutive days by phenol-chloroform method described by Masood et al[14] The IgE levels of controls and patients were determined from serum by using commercial kit method (MicroLISA, AMGENIX Intl. Inc., USA), a value of $\geq$100 IU/ml was considered as positive value for allergic asthma.

Multiplex PCR for GSTT1 (F: 5′ GGCGAGAGAGCAAGACTCAG 3′; R: 5′ GGCAGCATAAGCAGGACTTC 3′) and GSTM1 (F: 5′ GAACTCCCTGAAAAGCTAAAGC 3′; R: 5′ GTTGGGCTCAAATATACGGTGG 3′) was carried out to amplify fragments of 385 bp and 219 bp respectively. β-globin was used as an internal control (F: 5′ CAACTTCATCCACGTTCACC 3′; R: 5′ GAAGAGCCAAGGACAGGTAC 3′). Multiplex PCR was performed by using 50 ng of the genomic DNA in 20 μl reaction mixture and ready-to-use Mastermix (Solisbiodyne FIRE Pol 5x). The contents were mixed and placed in a thermocycler with conditions as follows; First of all, preheating of template DNA at 95 °C for 5 min which was followed by 35 cycles of amplification. Each cycle consisted of 3 steps: denaturation at 95 °C for 1 min, primer hybridization at 57.5 °C for 50 s and extension at 72 °C for 1 min. Then, the last step was of final extension at 72 °C for 10 min. Amplified products were subjected to 2% agarose gel electrophoresis stained with ethidium bromide and visualized in gel document analyser.

The collected data was coded and typed onto computer files using SPSS Statistics version 19.0. Descriptive statistics including frequency, percentages, arithmetic mean (X), standard deviation (SD) were used to describe asthma patients and healthy individuals. The study also used analytic measures such as student *t*-test, Chi-sqaure test (χ^2 test), and non-parametric tests. The level of significance selected for this study was 0.05. Odd ratios were also calculated through online available calculator (Med Calc. Version 13.0.6.0).