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Tumor Necrosis Factor-α (-308G>A) Gene Polymorphism and Its Association with Asthma and Atopy Status

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ABSTRACT

Asthma is one of the most prevalent chronic lung diseases that afflict genetically predisposed individuals. Certain cytokine gene polymorphisms have been associated with asthma. Tumor necrosis factor-alpha (TNF-α) is a potent inflammatory cytokine that can modulate nonspecific inflammation to influence asthma. This study aimed to define the relationship between the *TNF* gene polymorphism at position -308 and asthma susceptibility, as well as atopic and nonatopic asthma.

Using polymerase chain reaction with sequence-specific primers, we investigated genotype frequencies and alleles of a polymorphic gene coding for TNF-α in 86 pediatric patients with asthma and 470 healthy controls of the same race. Seventy-four patients underwent a skin prick test.

The homozygous AA variant (-308, rs1800629) was the most common genotype among patients, accounting for 63.3% of all cases. In contrast, homozygous GG (-308) was significantly less prevalent in the patient group compared to the control group. *TNF* A (-308) allele frequency was 85.5% among asthma patients and 16.6% among healthy controls. The genotype and allele frequencies of *TNF* (-308 A>G, rs1800629) did not differ between atopic and nonatopic asthma.

In conclusion, *TNF* (-308) AA and AG genotypes are associated with asthma susceptibility in Iranian children, although there was no significant difference in polymorphism between atopic and nonatopic asthma and no difference in asthma severity groups.

Keywords: Asthma; Atopy; Gene polymorphism; PCR-SSP; Single nucleotide polymorphism; Tumor necrosis factor-alpha

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INTRODUCTION

Asthma is one of the most prevalent chronic inflammatory respiratory diseases characterized by airway hypersensitivity, inflammation, and reversible airway obstruction. Asthma is a polygenic disease with a complex interaction between genetic and environmental factors. Asthma pathogenesis involves immune dysregulation along with connections to atopy and allergen sensitization. The genes controlling the development and regulation of the immune response contribute to asthma pathogenesis. A large number of genes have been linked to asthma susceptibility and pathogenesis, involving more than 100 loci. Cytokines secreted by innate immune cells are crucial for the regulation of innate and adaptive immune systems.[1,2] Gene polymorphisms that affect cytokine production and immune response regulation are found in genes that confer asthma susceptibility.[3-6] In both atopic and nonatopic asthma, allergen-induced helper T cell type 2 (Th2) inflammation plays a predominant role. Nonetheless, additional pathways, such as Th1 inflammation, have been linked to the development of asthma. Elevated levels of the Th1-derived cytokines tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in adult and pediatric patients with severe asthma suggest that neutrophils may play a role in the pathophysiology of asthma.[7-10]

The gene encoding the potent inflammatory cytokine TNF-α (*TNF*) is located on the short arm of chromosome 6. TNF-α is primarily produced by macrophages and mast cells. It induces mucous cell metaplasia, increases airway contraction and hyperresponsiveness, which may result from the recruitment and activation of eosinophils and neutrophils in the airway, and increases cytokines, such as interleukin (IL)-5, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, intercellular adhesion molecule 1 (ICAM1), chemokines (such as eotaxin, monocyte chemotactic protein-1 [MCP1], and RANTES), neuropeptide, and mucin.[8-11] Thus, TNF-α stimulates inflammatory cell recruitment, directly influences the smooth muscles of the airways, or initiates a chain reaction of inflammation through the release of mediators, such as enhanced sensitization and increased histamine release. High TNF-α levels are closely correlated with severe asthma, asthma complications, and corticosteroid-dependent asthma, according to a number of lines of research. Also, TNF-α stimulates the production of glycosaminoglycans in human lung fibroblasts and causes eosinophils to produce matrix metalloproteinases, which explains the additional remodeling action of TNF-α in asthma.[11,12]

The classification of asthma as atopic or nonatopic is based on the atopy status, defined as any positive skin prick test ≥3mm to common allergens. Past studies have found no difference between atopic and nonatopic asthma in the production of chemokines, eotaxins, RANTES, MCP3, MCP4, Th1 and Th2 cytokines, and cytokine receptors in the bronchial epithelium and submucosa.[13] In the industrialized world, approximately 50% of asthma cases are attributable to atopy. However, this percentage is much lower in developing countries.[14-16] In both atopic and nonatopic asthma, there is an increase in the secretion of Th1 and Th2 cytokines as well as cytokine receptors.[17,18] Numerous studies have evaluated the association between gene polymorphism and the risk of asthma in various populations, with the majority of these studies focusing on rs1800629 (-308 G>A). However, the results of these studies have been inconsistent, and fewer studies have examined the effects of this polymorphism on atopic status.[19-28] Despite this, *TNF* gene polymorphisms are associated with asthma, asthma severity, and bronchial hyperresponsiveness.

This study was conducted on a group of pediatric patients with physician-diagnosed asthma to determine the association between the *TNF* single nucleotide variants (SNV, formerly single nucleotide polymorphisms, SNP) at position -308 and asthma, as well as its association with atopic or nonatopic asthma.

MATERIALS AND METHODS

Subjects

The Ethics Committee of Tehran University of Medical Sciences approved this study. Before peripheral blood sampling, subjects or their parents provided written informed consent. This study enrolled 86 Iranian asthma patients who were referred to the Allergy and Immunology Clinic of the Children's Medical Center hospital, with a median age of 7.99 (range 2.5–15) years. The National Asthma Education and Prevention Guidelines (Global Initiative for Asthma 2017)[29] were used to diagnose asthma in patients. All participants underwent a clinical history and physical examination, and those over the age of 5 underwent a pulmonary function test. They were assessed to determine whether they had ever experienced dyspnea or shortness of

breath. Chronic pulmonary diseases other than asthma, congenital cardiac diseases, cystic fibrosis, prematurity, neonatal intensive care unit (NICU) admission with a history of intubation, inborn errors of immunity, autoimmune diseases, any thoracic anomalies, and thoracic surgery comprised the exclusion criteria.

Since genetics is not affected by age, we selected 477 healthy individuals of the same ethnic background who had no prior medical history or family history of allergies or autoimmune illnesses from a blood transfusion center as the control group. Seventy-four patients who were at least 4 years old were evaluated for atopy status by skin prick test for common aeroallergens and food allergens. Subjects who had a negative reaction to histamine (3 mm) or a positive reaction to the negative control were disqualified. Individuals with a wheal reaction ≥3mm to at least 1 allergen were classified as having atopic asthma, and those with no reaction to allergen extract were classified as having nonatopic asthma. Asthma severity was categorized based on the daily medication required to maintain control, following the GINA 2017 guidelines: mild for step 2 medications, moderate for steps 3 or 4, and severe for steps 5.[29] Additionally, we compared polymorphisms in individuals according to hospital admission requirements due to asthma attacks (ICU vs. hospital ward vs. emergency department).

DNA Sampling and Genotyping

We collected 5 mL of whole blood in ethylenediaminetetraacetic acid (EDTA) tubes from cases and controls. In addition, 2 mL of serum was collected separately from cases for serum total immunoglobulin (IgE) assays. DNA extraction was performed using the salting out method. The purity of the DNA was determined by the Nanodrop 2000 spectrophotometer based on the ratio of optical density of 260 to 280. Cytokine typing was performed by the polymerase chain reaction with sequence-specific primer (PCR-SSP) assay. Afterwards, the *TNF* allele and genotype frequencies were obtained. The Supplementary Table displays the primer characteristics.

Statistical Analysis

The association of the allele frequencies with variables was compared using the chi-square test. The chi-square test was additionally employed for comparing the genotype and allele frequencies between the 2 categories of asthmatic patients, as well as between the asthma patients and the control group. Furthermore, we examined the genotype frequencies of patients based on the severity of their asthma and whether an asthma attack required hospitalization (ICU or extra-ICU). The odds ratio (OR), *p* value, and 95% confidence intervals (CI) for each allele in the patient and control groups and for patients with atopic and nonatopic asthma were calculated. A *p* value of <0.05 was considered statistically significant.

RESULTS

Eighty-six children with clinical or spirometry diagnoses of asthma participated in this prospective case-control study from November 2017 to April 2019. Sixty-one boys and 25 girls with asthma were recruited for the study. The control group consisted of 470 healthy individuals with no family history of allergic or autoimmune diseases. The atopic status of 74 asthma patients >4 years old was determined using a skin prick test with positive and negative controls, assessing 21 food and inhalant allergens. Clinical data and *TNF* (-380 G>A) polymorphism were examined in patients and controls.

Allele Frequencies

Allele frequencies of *TNF* (-308 G>A) were compared between case and control populations as well as between atopic and nonatopic asthma. We present the allele frequency (number and percentage), OR, *p* value, and 95% CI for each group in Tables 1 and 2. Table 1 compares asthmatic and healthy groups, while Table 2 compares atopic and nonatopic asthma. *TNF*-308A was the most significant positive allelic relationship that increased the patients' risk of developing asthma ($p<0.001$; OR, 29.55; 95%CI, 18.70– 46.40). There were no statistically significant differences in the allelic polymorphism of *TNF* (-308 G>A) between atopic and nonatopic asthma patients, nor between asthma patients based on the severity of their asthma or the need for hospitalization.

Cytokine Genotype Polymorphism

The most prevalent genotype in asthmatic patients was AA, while the most prevalent genotype in the control group was GGT. Asthma patients had a significantly greater proportion of the AA genotype at *TNF* (-308) than controls ($p<0.001$). The *TNF* gene

polymorphism (-308 G>A) did not differ significantly between atopic and nonatopic asthma. There was no correlation between the genetic polymorphism of *TNF* (-308) and asthma severity or hospitalization requirement of the patients. There was no significant difference in serum IgE levels between the genotypic groups of rs1800629 in *TNF* gene polymorphism nor between the two asthma groups. (Tables 3 and 4).

Table 1. Alleles and genotype frequencies of the tumor necrosis factor (*TNF)* gene in asthma patients

Cytokine gene	Alleles or genotypes	Controls (N=470), n (%)	Patients (N=86), n (%)	*p*	OR (95% CI)
TNF (-308)	A	156 (16.6%)	147 (85.5%)	<0.001	29.55(18.70–46.70)
	G	784 (83.4%)	25 (14.5%)		
	AA	14 (3.0%)	63 (24.4%)	<0.001	
	AG	128 (27.2%)	21 (24.4%)		
	GG	328 (69.8%)	2 (2.3%)		

OR: odds ratio; CI: confidence interval.

Table 2. Alleles and genotype frequencies of the tumor necrosis factor (*TNF*) gene in atopic vs. nonatopic asthma patients

Cytokine gene	Alleles or genotypes	Atopic asthma (N=34), n (%)	Non-atopic asthma (N=34), n (%)	*p*	OR (95% CI)
TNF (-308)	A	63 (90%)	59 (86.8%)	0.553	1.37(0.481–3.922)
	G	7 (10%)	9 (13.2%)		
	AA	27(79.4%)	25 (73.5%)	0.567	0.720(0.233–2.224)
	AG	7 (20.6%)	9 (26.5%)	0.567	
	GG	0	0		

OR: odds ratio; CI: confidence interval.

Table 3. Genotype frequencies of the tumor necrosis factor (*TNF*) gene in asthma patients and the severity of asthma

TNF (-308) gene polymorphism	Mild asthma n (%)	Moderate asthma n (%)	Severe asthma n (%)	*p*
AA	18 (27.7%)	22 (33.8%)	25 (38.5%)	0.466
AG	9 (42.5%)	6 (28.6%)	6 (28.6%)	
GG	0	0	0	

Table 4. Genotype frequencies of the tumor necrosis factor (*TNF*) gene polymorphisms in asthma patients by admission status

TNF (-308) gene polymorphism	No admission n (%)	Admission except in ICU n (%)	Admission in ICU n (%)	*p*
AA	29 (44.6%)	29 (44.6%)	7 (10.8%)	0.659
AG	11 (52.4%)	9 (42.9%)	1 (4.8%)	
GG	0	0	0	

ICU: intensive care unit.

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DISCUSSION

This study attempted to investigate the effect of the TNF-α cytokine gene SNV on asthma susceptibility. Also, its relationship with atopy, asthma severity, and total IgE levels in 2 distinct groups of children with asthma. Asthma is a complex polygenic disease,[30] and gene-gene and gene-environment interactions have a substantial effect on the disease.[4]

Numerous studies have demonstrated that, regardless of the atopy status, asthma patients have elevated total IgE levels. Our research revealed that 56.25% of patients were atopic, and 43.75% were nonatopic. The mean total IgE was 217.33 in asthma patients, 205.31 in nonatopic asthma, and 231.23 in atopic asthma patients. The total IgE level differed between atopic and nonatopic asthma (p=0.83). In line with Daneshmandi's findings and in contrast to Zhang and Amal's research, the total IgE level in our study was not associated with cytokine variations.[22,23,31]

Asthma is recognized to be primarily caused by allergen-induced Th2 inflammation. However, the Th1 inflammatory pathway has also been linked to the pathogenesis of asthma.[25,30] The elevated level of TNF-α in the airways of asthma patients induces the expression of several airway epithelial cell genes, including cytokines, chemokines, and adhesion molecules. It enhances activated eosinophil adhesion to respiratory epithelial cell cultures and fosters neutrophil chemotaxis, adherence, and transepithelial migration. IgE receptor stimulates eosinophil TNF mRNA expression and induces TNF-α production in human lung tissue. TNF-α causes transient bronchial hyperreactivity, asthma severity, and complications.

In conclusion, TNF-α is a potentially significant cytokine in refractory asthma, and preliminary investigations on a few patients have shown improvements in exacerbation rate, lung function, airway hyperresponsiveness, and asthma quality of life after anti-TNF-α medication.[11,30,32,33] Multiple independent studies have associated *TNF* polymorphism with an increased risk of developing asthma. Specifically, the *TNF* (-308G>A) gene polymorphism, which affects transcriptional activators, has been found to increase plasma levels of TNF-α in response to stimulation, both in vivo and in vitro. The polymorphic location contains the *TNF*-308G and *TNF*-308A alleles. The homozygote *TNF*-308G is the most prevalent genotype in the general population.[25]

According to some studies, *TNF -308A* allele is associated with an increased risk of developing asthma,[4,11-13,17-19, 26-28, 31,34] whereas others have found the opposite to be true.[12,35-40] Despite numerous categories of research, the effects of *TNF*-308G>A on the likelihood of developing asthma and its associated phenotypes remain debatable. Compared to the AG heterozygote variant and the GG homozygote variant, the AA homozygote variant demonstrated a greater correlation with asthma risk, according to our research. However, there is no difference in allele frequencies or genetic polymorphism between the 2 asthma groups (p>0.05). Our findings suggest that the G allele may play a more beneficial role than the A allele in asthma. and are consistent with the in vivo and in vitro observations of TNF's role in asthma inflammation.[4,11-13,17-19,26-28,31,34] Jong Hong et al. conducted research demonstrating that the TNF promoter polymorphism (-308G>A) was associated with severe bronchial hyperresponsiveness rather than asthma and atopy in Korean children with asthma.[35] On the other hand, Zhang et al. investigated individuals who carried the A allele and had an increased risk of atopy.[22] Further investigations are recommended to reach a better understanding of the relationship between these genetic polymorphisms and atopic asthma susceptibility.

Three grades of asthma severity (mild, moderate, severe) were investigated based on the amount of daily medicine needed to achieve asthma control. There was no significant difference in genotype frequencies or allele frequencies between the 2 groups. Our findings were consistent with those of Bilolikar et al., who found no association between the *TNF* gene polymorphism and the severity of asthma.[25] In contrast, Darweeshi et al. and Zaden et al. showed a correlation between the *TNF* gene polymorphism and the severity of asthma.[19,41] In addition, we examined the genotype and allele polymorphism of patients in relation to their need for hospitalization for asthma attacks (ICU and extra-ICU). Our study did not investigate allele or genotype differences in the 3 groups (no need for admission, ICU admission requirement, and extra ICU admission). The study by Daneshmandi et al. on the *TNF* (-308 G>A) polymorphism in asthma found that the AG heterozygote genotype was associated with a higher risk of developing asthma despite the fact that genotype and allelic frequencies did not differ between well-managed and poorly managed asthma.[23] Through this controversy in various studies, we should consider gene-gene and

gene-environment interaction as well as the various examined geographic and racial populations.

In conclusion, the *TNF*-308G>A polymorphism is associated with an increased risk of asthma in Iranian children but not with asthma phenotypes (atopy) or asthma severity. Recognizing distinct cytokines and cytokine receptor gene polymorphisms and their association with asthma, asthma severity, and asthma complications is of great importance nowadays, as targeted therapy is the primary treatment for various asthma endotypes and allergic diseases. Due to the limited sample size, additional research is required to detect the association between atopy and asthma complications. In addition, there was a shortage of research on different races. More accurate results will be attained if environmental factors are taken into account and reinforced in more races and more samples; thus, additional research is required.

STATEMENT OF ETHICS

This study was registered and approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.CHMC.REC.1398.033).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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