

## Association between the Polymorphism of TGF- $\beta$ 1 Gene Promoter (-509C>T) and Idiopathic Chronic Urticaria

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### ABSTRACT

Idiopathic Chronic Urticaria (ICU), the most common form (70-80%) of chronic urticaria is supposed to have immune basis causes. It is speculated that the promoter polymorphism of TGF- $\beta$ 1 gene may be involved in ICU. This condition is thought to affect at least 0.1% of the population and often can be severe and difficult to treat.

A total of 40 patients with ICU and 41 normal subjects were studied. DNA was extracted from whole blood and TGF- $\beta$ 1 promoter -509C>T polymorphism was determined by PCR-RFLP method.

Out of the 40 patients with ICU, 11 (27.5%) had CC, 26 (65%) had CT and 3 (7.5%) had TT genotypes. A higher proportion of case subjects with the C allele (CT type or CC type) was found compared with the T allele.

These results do suggest an influence of genetic variability at the promoter of TGF- $\beta$ 1 gene (-509C>T) on the occurrence of ICU. This polymorphism has been shown as a useful genetic change in our study. Further work is required to confirm this result.

**Key words:** Polymorphism; TGF- $\beta$ 1 gene; Urticaria

### INTRODUCTION

Urticaria is a blotchy rash consisting of a number of pale raised bumps or wheals surrounded by reddened skin which is thought to affect at least 0.1% of the population and often can be severe and difficult to treat.<sup>1</sup> The rash is extremely itchy and debilitating.<sup>2</sup> It affects 15-25% of people at some point in their lifetime.<sup>3</sup>

Acute urticaria is a self-limited disease and often due to allergic reactions to food or medication.<sup>4</sup> Chronic urticaria is a common skin disorder characterized by recurrent, transient, itchy wheals with individual lesions lasting less than 24 hours and affecting patients for 6 weeks or more.<sup>5-7</sup> It is most common in middle-aged women. In spite of a careful search, a causative agent is discovered only in less than 25% of cases; the remainder is designated as idiopathic chronic urticaria<sup>3</sup> There is now emerging evidence supporting an autoimmune phenomenon related to the presence of circulating functional histamine-releasing auto-antibodies reacting against the  $\alpha$ -subunit of the high-affinity IgE receptor. Studies in adults have clearly

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demonstrated that antibodies against the FcεR1α are detectable in 25-45% of cases of Idiopathic Chronic Urticaria (ICU). ICU is a cause of serious personal, social, economic, and occupational disability that has been found to be comparable to disability associated with severe coronary heart disease. It remains a major challenge in its etiology, investigation, and management.<sup>8</sup> This condition, which can persist for many years is due to the production of autoantibodies which subsequently sensitize specific mast cells in skin and tissues causing an enormous release of histamine.<sup>2</sup>

Recently, an association between chronic urticaria and the major histocompatibility complex allele, HLA-DRB1\*04, was identified. This association suggests that the disease may involve an interaction between genes, the environment, and the immune system similar to that observed in asthma and coronary artery disease.<sup>3</sup> There are vasoactive mediators such as a few cytokines (IL-3, 4, 5, 6, 8, 13 and TNF-alpha) supposed to have a pathogenic role in urticaria.<sup>9</sup>

TGF-β1 is a multifunctional polypeptide implicated in the regulation of a variety of cellular processes including growth, differentiation, apoptosis, adhesion, motility and has a key role in immune system.<sup>10</sup>

The human gene encoding TGF-β1 is on chromosome 19q13 and is highly polymorphic. The expression of TGF-β1 is influenced by polymorphisms in the TGF-β1 gene, and some of these polymorphisms may be associated with asthma and other diseases.<sup>11,12</sup> Awad et al.<sup>13</sup> identified five polymorphisms in the TGF-β1 gene; two in the promoter region at positions -800 and -509, one at position +72 in a non translated region, and two in the signal sequence at positions +869 and +915. TGF-β1 production is more variable due to cross-modulating interfaces between signaling networks within cells. Thus, the impact of polymorphism on phenotype may depend on the specific disease under study because of stimulus-specific contributions to signaling pathways involving translational (protein folding) and post-translation (secretion/activation) events (14). Recently, polymorphism has been described for -509C>T which was evaluated to be associated with several diseases.<sup>15-17</sup> In this study, we have evaluated the relationship between the polymorphism of TGF-β1 gene promoter (-509C>T) and idiopathic chronic urticaria as a new study model.

## PATIENTS AND METHODS

### Study Population

We had 40 patients with ICU from Northeastern Iran diagnosed by the immunologists in the allergy and skin clinics of a university teaching hospital. Patients were recruited between January 2004 and May 2005.

Consents were obtained from all patients. A complete history taking and physical examination were carried out. Known reasons of urticaria were excluded and skin prick test with the common allergens of the geographic region was negative in all of them. After normal results of laboratory tests (CBC-diff, ESR, BUN, Cr, ALT, AST, IgE levels), idiopathic urticaria was confirmed in 40 patients. Forty-one normal subjects age/sex matched with the case group living in the same area of Iran were selected as controls. They had no history of urticaria or any positive findings on physical examination, skin prick test or laboratory tests.

This study was confirmed by the ethic committee of Mashhad University of Medical Sciences.

### Laboratory Procedures

Blood samples were sent to Mashhad Bu-Ali Research Institute for laboratory tests. Genomic DNA was extracted by commercially DNA extraction kit (Biogene, Mashhad, Iran) using salting-out method from 10ml of whole blood, which was used for genotyping assays with the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) technique. We used primer sequences, as previously reported (18) to amplify the 741bp product containing the polymorphism site. PCR was performed in a T3 Thermocycler (Biometra, Germany). Each 20μl of PCR mixture contained 100ng of genomic DNA, 0.5 unit of Taq DNA polymerase, 1x PCR reaction buffer (10mM/L Tris-HCl, 50 mM/L KCl, 1.5 mM/L MgCl<sub>2</sub>), 0.2 mM each dNTP, and 0.5μM each primer. The reaction mixture was initially denatured at 95°C for 3 min, followed by 38 cycles of 95°C for 1 min, 62°C for 1 min, 72°C for 1 min and a final extension at 72°C for 7 minutes.

Aliquots of the PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide before digestion to control for correct amplification of the 741bp fragments. The PCR products were digested with *Eco8II* (20U/ml) (Fermentas, Germany) restriction endonuclease at 37°C for 60 minutes. The PCR products (741bp) with T allele were digested to two fragments (184 and 557bp), whereas the PCR products with C allele could not be cut by *Eco8II*. The samples were then analyzed by electrophoresis on a 3% agarose gel by IMAGO gel documentation system (B&L system, Germany) and the genotypes were determined as homozygous for TT, CC and heterozygous for CT (Figure 1).

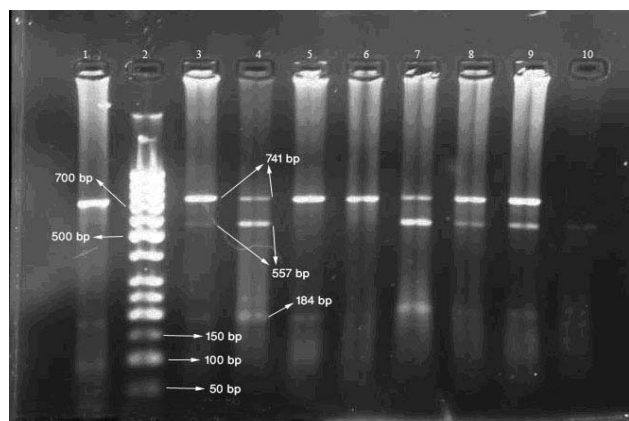
### Statistical Analysis

Statistical analysis was performed using Fisher's exact and chi-square tests. The results were considered to be significant when the P-value was less than 0.05.

**RESULTS**

**Subject Characteristics**

Out of the 40 patients with ICU, we had 32 (80%) female and 8 (20%) male showing a higher prevalence of ICU among women (4 times). The mean age of our patients was 32 years old and the most age-group commonly involved in our study was the third decade of life.



**Figure 1.** Analysis of the -509 (C>T) promoter polymorphism. Lane 1: Uncut (CC) product; Lane 2: DNA size marker (100bp); Lane 3-10: subjects with different genotypes in -509 promoter; Lane 5-6: Allele1 (CC) homozygous; Lane 3-4, 7-9: (CT) heterozygous; Lane 10: Allele 2 (TT) homozygous.

**Table 1.** Allele numbers for the -509 C>T promoter polymorphism of the TGF-β1 gene in patients with ICU and control subjects

Allele	ICU	Controls
	n=80	n=82
C	48	33
T	32	49

**Table 2.** Allele frequencies for the -509 C>T promoter polymorphism of the TGF-β1 gene in patients with ICU and control subjects

Allele	ICU	Controls
	n=80(%)	n=82(%)
C	0.6(60)	0.402(40.2)
T	0.4(40)	0.597(59.7)

**Polymorphism Detection**

Out of the 40 patients with ICU, 11 (27.5%) had CC, 26 (65%) had CT and 3 (7.5%) had TT genotypes. Of the 41 normal subjects, 3 (7.3%) had CC, 27 (65.8%) had CT and 11 (26.8%) had TT genotypes. A relatively higher proportion of control subjects carrying the T allele was found compared with those carrying the C allele (P=0.012) (Tables 1 and 2) and as

mentioned above, the difference in genotypic distribution between the controls and patients was also significant (P=0.010).

**DISCUSSION**

Gene polymorphisms are mechanisms through which individuals may exhibit variations within the range of what is considered biologically normal.<sup>18</sup> More than 100 Single Nucleotide Polymorphisms (SNPs) and other genetic variants have been identified in genes of the TGF-β1 signaling pathway, and a few of these have been associated with disease.<sup>19</sup>

-509C>T was chosen as the sole candidate SNP because previous studies suggest that it is associated with altered serum levels of TGF-β1, asthma diagnosis, asthma severity, and serum IgE levels.<sup>20</sup> -509C>T was found to be the most informative marker for association studies with asthma in one study. In fact, -509C>T is the only SNP in TGF-β1 found to be associated with atopic phenotypes, i.e. elevated IgE levels and asthma.<sup>21</sup> We were interested in the role of this genetic variance in idiopathic chronic urticaria and studied the C>T polymorphism at the -509 position of the human TGF-β1 promoter. Urticaria is a multifactorial disease which its development is dependent on several genetic and environmental factors and also evidences show that the concentration of active TGF-β1 may be predominantly below genetic control.<sup>11,13</sup> Thus, the -509C>T polymorphism of TGF-β1 as a possible cause of ICU has been assessed in the present study.

Grainger, et al.<sup>11</sup> observed that the -509C>T polymorphism is significantly associated with the TGF-β1 plasma concentration. A significant association between the -509C>T polymorphism and bone mineral density was detected in a study containing 625 postmenopausal Japanese women. The genotype T/T was found in higher frequency in 286 individuals with osteoporosis than in 170 normal controls. The frequencies of the CC, CT and TT genotypes of the -509C>T polymorphism in the studied population were 24%, 49% and 27%, respectively.<sup>22</sup> However, other authors have observed that this polymorphism is not associated with coronary artery disease, and its presence alone would not be a genetic risk factor for predisposition to heart diseases.<sup>23</sup>

In one study, Kim SY, et al.<sup>24</sup> found a trend that -509C>T and the 869T>C polymorphisms of TGF-β1 gene were associated with rheumatoid arthritis in the male population. Eric Silverman et al.<sup>25</sup> studied 527 subjects with asthma in association with TGF-β1 promoter polymorphism and concluded that the T allele of C-509T was associated with the diagnosis of asthma

and might enhance TGF- $\beta$ 1 gene transcription.<sup>19</sup> In the present study, T/T genotype was found in a higher frequency in the control than in the case group. Additionally, the T allele was also found in a higher frequency in the control group. Several diseases, including coronary vasculopathy, inflammatory bowel disease and vertebral fractures have been reported to be predisposed to TGF- $\beta$ 1 polymorphism. The significance of the association with hypertension and other cardiovascular diseases has been reported. Ohtsuka et al.<sup>26</sup> have shown a positive relation between -509C>T polymorphism and systemic sclerosis in Japanese patients.

Our results suggest that in the TGF- $\beta$ 1 gene polymorphism the T allele might be one of the genetic safe factors for ICU because subjects with the T/T TGF- $\beta$ 1 genotype had a reduced risk of developing ICU as compared with patients with the C/T or C/C genotypes. This study was carried out for the first time and we could not compare our results with other studies. It should be mentioned that we studied only a limited number of ICU patients (40 cases), and these results should be confirmed in a larger number of cases. In addition, other polymorphisms present in the coding sequence of the TGF- $\beta$ 1 gene may have a greater effect on the development of ICU.

We conclude from our data that TGF- $\beta$ 1 gene promoter polymorphism does not play a role in the development of ICU in our Iranian population. Our results may be related to the multifunctional characteristics of TGF- $\beta$ 1.

This case-control association study suggests that the -509C>T SNP of the TGF- $\beta$ 1 gene can protect individuals against ICU.

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