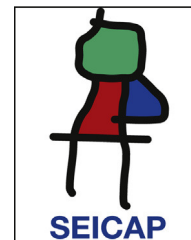




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### ORIGINAL ARTICLE

# IgE Fc $\epsilon$ R1 $\beta$ polymorphism and risk of developing chronic spontaneous urticaria: A study in an ethnic Kashmiri population



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#### KEYWORDS

Chronic idiopathic urticaria (CIU);  
Chronic autoimmune urticaria (CAU);  
Fc $\epsilon$ R1 $\beta$  (Fc epsilon receptor 1 beta);  
Autologous serum skin test (ASST)

#### Abstract

**Background:** The pathogenesis of chronic spontaneous urticaria involves interplay between the genetic and environmental factors, most of which is still poorly understood. It is well-recognized that 30–40% of chronic spontaneous urticaria is autoimmune in nature. Chronic autoimmune urticaria is caused by anti-Fc $\epsilon$ R1 $\beta$  and less frequently, by anti-IgE auto antibodies that lead to mast cell and basophil activation, thereby giving rise to the release of histamine and other proinflammatory mediators. We investigated the association between SNP loci in Fc $\epsilon$ R1 $\beta$  and chronic spontaneous urticaria and to see its relation with serum IgE levels in Kashmiri population.

**Methods:** The autologous serum skin test was used as a screening test for chronic autoimmune urticaria. PCR-RFLP was used to detect the genotype of the SNP loci. Serum IgE levels were assessed by ELISA kit.

**Results:** No significant difference was found between the study population and control group in genotype distribution (wild and variant) among Fc $\epsilon$ R1 $\beta$  loci ( $P$  value = 0.06, odds ratio = 0.29). The frequency of Fc $\epsilon$ R1 $\beta$  (C109T) in autologous serum skin test positive chronic autoimmune urticaria patients with the CT genotype was found to be statistically non-significant when compared with the wild genotype ( $P$  = 0.35). Carriers of Fc $\epsilon$ R1 $\beta$  (T allele) had a more significant risk of developing CAU than those with C allele ( $P$  = 0.01). In our population serum total IgE levels did not find any statistical significance with regard to ASST positive & ASST negative patients ( $P$  = 0.26).

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**Conclusions:** There is statistically no significant association between FcεR1β gene polymorphism and CSU in Kashmiri population; however, there is a probability of developing CSU in patients carrying FcεR1β T allele. Furthermore, serum total IgE levels had no significant association with the development of CAU.

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## Introduction

Urticaria involves the superficial portion of the dermis, presenting as well-circumscribed wheal with erythematous raised serpeginous borders and blanched centers that may coalesce to become giant wheals. Chronic urticaria is one of most common chronic inflammatory disorder prevalent in our society, defined as urticaria persisting daily or almost daily for more than six weeks. Chronic urticaria causes deterioration of quality of life.<sup>1</sup> It includes physical urticaria, cholinergic urticaria, chronic idiopathic urticaria and urticarial vasculitis. Up to 55% of the patients with chronic urticaria have an idiopathic cause. In recent years a significant number of patients (30–40%) with CSU have been reported to have an autoimmune basis for their disease including autoantibodies to IgE (5–10%) or, more commonly, to the (α-chain) of FcεR1 (35–45%).<sup>2</sup> These autoantibodies have been shown to activate blood basophils and cutaneous mast cells in vitro, with enhancement of basophil activation by complement and release of C5a. Unfortunately antibody binding tests (viz. ELISA) can yield positive tests in many autoimmune diseases, normal subjects, or patients with other forms of urticaria, but most such sera are non-functional and cannot be used to diagnose CAU. At this point of time, gold standard for detecting clinically relevant autoantibodies to FcεR1 is functional in vitro donor basophil histamine release assay (BHRA) and their specificity is confirmed by immunoassay (Western blot or ELISA), where these tests are available.<sup>3</sup>

In autologous serum skin test (ASST), autologous serum injected into patients own skin can induce a wheal and flare reaction involving mast cell activation, and removal of the antibody leads to remission.<sup>4</sup> CAU has been reported to occur in children as well.<sup>5</sup> It is often not possible to distinguish CAU from those without autoantibodies, clinically or histologically. The ASST is used as a screening test. Grattan et al., reported that the histological features of a positive ASST resemble an IgE-mediated late phase reaction.<sup>6</sup> The ASST is a useful tool for picking up patients with circulating wheal producing factors in CSU. However, its specificity as a screening test for presence of functional anti-FcεR1 is moderate while the sensitivity is high enough to safely exclude autoimmune urticaria and confirmation by demonstration of histamine-releasing activity in the patient's serum may be needed for establishing this diagnosis.

To release histamine from mast cells, the key step is a cross-linking of allergens with IgE, which is bound with the FcεR1β located on their surface.<sup>7</sup> The receptor is a tetrameric complex composed of an alpha, a beta and two

disulphide-linked gamma chains.<sup>8</sup> While the genes for the alpha and gamma subunits are both located on human chromosome 1,<sup>9</sup> the beta gene is located on 11q13 and spans about 10 kb and contains 7 exons.<sup>10</sup> Histamine release via autoantibodies against FcεR1β was noted in the pathogenic mechanism of CSU.<sup>11,12</sup> Several investigators demonstrated a positive association of gene polymorphism of the β chain of FcεR1β with high serum total IgE level, atopy or the asthma phenotype.<sup>12–15</sup> In this study, we analyzed known single nucleotide polymorphism (SNP) of FcεR1β in CSU patients, compared with other ASST positive CAU and in normal healthy controls in Kashmiri population. Furthermore we observed association of ASST positive CAU with serum total IgE levels.

## Methods and materials

The study included 120 patients of CSU and equal number of healthy controls. These patients were enrolled by the allergy clinic of department of Immunology and Molecular Medicine, Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, India. All the subjects were native, with normal controls having no history of allergy, atopy, drug hypersensitivity or family history of urticaria. All the subjects were recruited from the general population and informed consent was taken, which was then approved by SKIMS ethical committee. CSU was defined as the daily or near daily presence of pruritic wheal for more than 6 weeks without underlying etiology. Autologous serum skin test (ASST) was performed by injecting autologous serum intradermal to volar aspect of same patient's forearm, and the injected skin was examined for wheal formation 30 min later. Positive and negative controls with intradermal histamine and saline injections were carried out on the adjacent skin. The test was considered positive if the serum induced wheal was at least 1.5 mm greater than the saline wheal, (Fig. 1). The form of urticaria with positive ASST is characterized as autoreactive. In all the subjects, serum total IgE levels were estimated by performing Enzyme linked immunoassay (ELISA) kit (Fortress diagnostics, UK) following manufacturer's protocol.

## SNP genotyping for allele frequencies of the candidate gene

Genomic DNA was isolated from peripheral blood using phenol–chloroform method. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was applied to detect the genotype of SNP loci, The reference

**Table 1** Overview of the SNP genotyped in our study using PCR-RFLP.

SNP NAME	rs number	Primers	Tm (°C)	PCR product	Restriction enzyme	Digested product length
FcεR1β C-109T	1441586	Forward 5' > GTGGGGACAATTCCAGAAGA < 3' Reverse 5' > CCGAGCTGTCCAGGAATAAA < 3'	60	382	Tru91	CC:221bp, 161bp TT:182bp, 161bp, 39bp CT:221bp, 182bp, 161b 39bp

SNP(rs) number, primers, melting temperature, PCR product length, restriction enzyme, and digested products length of FcεR1β SNP loci(C109T) is shown in Table 1. PCR thermal cycler (BioRad) was used for performing reaction with proof reading polymerase by using net volume of 10 μL, including 2.05 μL of a commercial PCR master mix (Biotools B & M Labs, S.A.), 5 pmol of each primer (Sigma–Aldrich Chemicals Pvt. Ltd., India), and 10 ng of genomic DNA. The negative control was used to monitor contamination. Cycling conditions included 1 cycle at 95 °C for 5 min, 40 cycles at 95 °C for 30 s, annealing temperature for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR-amplified products were detected by 2% agarose gel electrophoresis (Fig. 2a). One sample was not included in the study due to the non-amplification of the candidate gene. A restriction enzyme (Tru91) with restriction site TGAA > TTAA was used to digest the PCR products of the loci. A 10-μL reaction mixture containing 5 units of restriction enzyme and 5-μL of PCR products was incubated for 16 h. Digested products were run on 2.5% agarose (High EEO, Highmedia Labs, India) in 1× TAE buffer. The electrophoresis was carried out at 80 V and 200 mA and photographed using the automatic digital

gel imaging system (Fig. 2b). CC genotype was considered as homozygous wild (normal), TT as homozygous variant & CT as heterozygous variant.

### Statistical analysis

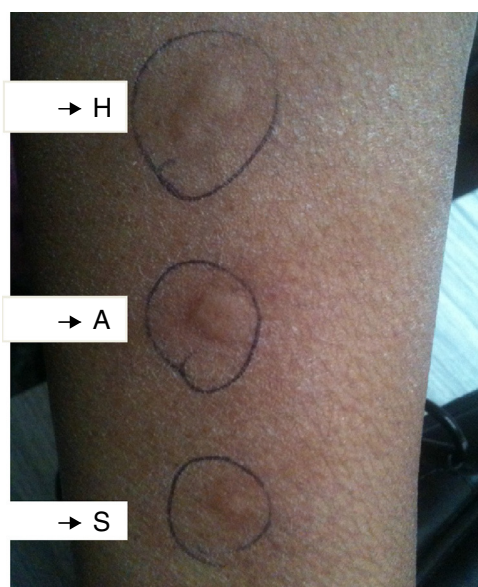
Differences in genotype distribution between the experimental and control group were analyzed using  $\chi^2$  Test. A *P* value of 0.05 or less was considered statistically significant. All statistical analyses were performed using SPSS version 13.0 (Chicago, USA).

### Results

In this study 120 CSU patients and equal number of healthy controls were included. The clinical characteristics of study subjects are summarized in Table 2. The age of patients ranged from 7 to 65 years, the mean age was 28 years with females out-numbering males, 11:1 ratio. Atopy was found in 12% of patients, 32% patients revealed the history of allergic disease, 94% patients were having pruritic rashes. As far as the total serum IgE in patients is concerned, the patients were divided into two groups. Those having the total IgE concentration of ≤100 IU/mL (group I) and group II having the total IgE concentration of >100 IU/mL. In the group I, 48% (*n*=7) patients were ASST positive and 52% (*n*=9) patients were ASST negative. In the group II, 58% (*n*=61) patients were ASST positive and 42% (*n*=43) patients were ASST negative. Thus, total IgE levels in ASST positive and negative patients varied; there existed no statistical significance in total IgE levels between ASST positive and ASST negative patients among the two groups (*P*=0.26, OR=0.54, 95% CI=0.16–1.77) as shown in Table 3.

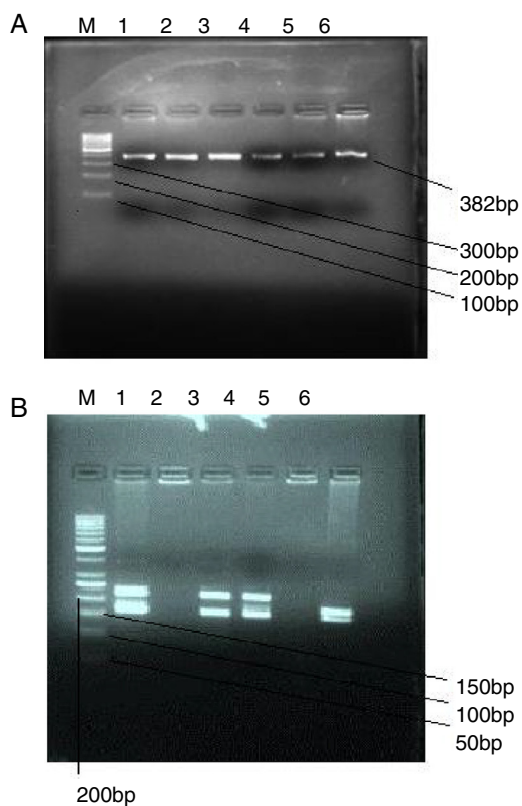
### Effect of the SNP loci

Statistically non-significant differences (*P*>0.05) were found between the cases and the control group (C/C genotype) in the genotype distribution of FcεR1β C109T in loci (Table 4). In our study, we found the frequency of C/C genotype was 69.74% (*n*=83), T/T genotype was 26.89% (*n*=32) and that of CT was 3.36% (*n*=04) in cases, where as it was 77.5% (*n*=93), 15.83% (*n*=19) and 6.66% (*n*=08) in healthy controls, respectively. The frequency of FcεR1β C109T, T/T genotype in the ASST +ve CIU group was found statistically non-significant with that of the control group



H-histamine, A-autologous serum, S-saline

**Figure 1** Shows wheal formation in ASST (autologous serum skin test) positive patient.



PCR-restriction fragment length polymorphism analysis of the polymorphism of FcεR1β C109T  
 M : 50 bp ladder  
 Lane 3 represents wild CC genotype; lane 1 & 4 represents heterozygous CT genotype  
 Lane 6 shows variant TT genotype; lanes 2 & 5 are empty.

**Figure 2** (a) Representative gel picture of the amplified product of FcεR1β gene (382 bp) product. Lane M: Molecular markers (100 bp ladder). (b) 50 bp; Lanes 1–6, amplicons from CIU blood samples.

**Table 2** Clinical characteristics of the study subjects.

Variables	CIU	NC	P value
Age (year)	28.6 ± 14.4	29.8 ± 12.4	< 0.05
Sex			
Female	92% (n = 110)	86% (n = 103)	NS
Male	8% (n = 10)	14% (n = 17)	NS
Atopy	12% (n = 14)	NA	
History of allergic disease	32% (n = 38)	NA	
Pruritus	94% (n = 110)	NA	
MPE	42% (n = 50)	NA	

CIU: chronic idiopathic urticaria, NC: normal control, NA: not applicable, NS: non significant, MPE: maculopapular exanthematous rash.

**Table 3** Total serum IgE in study subjects.

Total serum IgE	ASST +ve CAU	ASST –ve CIU	P value, OR, 95% CI
≤100 IU/mL	48% (n = 7)	52% (n = 9)	0.26, 0.54 (0.16–1.77)
>100 IU/mL	58% (n = 61)	42% (n = 43)	

ASST: autologous serum skin test, CIU: chronic idiopathic urticaria, OR: odds ratio.

**Table 4** Analysis of association between FcεR 1β C-109T and CIU.

Genotype	Cases <i>n</i> = 119 (%)	Controls <i>n</i> = 120 (%)	<i>P</i> value, OR (95% CI)
<i>CT (heterozygous variant)</i>	04 (3.36)	08 (6.6)	(CT vs. CC) 0.35 0.56, (0.13–2.15)
<i>CC (wild)</i>	83 (69.74)	93 (77.5)	(CC vs. TT) 0.06 0.29 (0.06–1.30)
<i>TT (homozygous variant)</i>	32 (26.89)	19 (15.83)	
<b>5 Alleles</b>			
C	170	194	0.01
T	68	46	0.6 (0.37–0.92)

( $P=0.06$ , OR = 0.29, 95% CI = 0.06–1.30). Similarly, the frequency of FcεR1β (C109T) in ASST +ve CAU patients with the CT genotype was found to be statistically non-significant when compared with the wild genotype ( $P=0.35$ , OR = 0.56, 95% CI = 0.13–2.15).

Also, it was seen that Carriers of FcεR1β (T allele) had a more significant risk of developing CAU than those with C allele ( $P=0.01$ , OR = 0.6, 95% CI = 0.37–0.92) as shown in Table 4.

## Discussion

We investigated single SNP of FcεR1β gene that might be associated with CSU pathogenesis, further we analyzed the association of total serum IgE levels with the ASST positive CSU patients. In the present study, no significant differences were found in the allele and genotype frequencies of SNP, thereby suggesting that the high affinity receptor gene polymorphisms may not be related to the development of CSU phenotype in our population. The high affinity IgE receptor is responsible for initiating allergic response. The binding of an allergen to the receptor bound IgE leads to mast cell activation and the release of histamine, which are responsible for clinical manifestations of urticaria.<sup>17</sup>

Polymorphisms of the FcεR1β gene have been reported to be associated with the atopy, total serum IgE level, bronchial hyper responsiveness, asthma and the basophilic histamine-releasing activity of asthmatic patients.<sup>13–16</sup> Since no previous study has been made on the association of FcεR1β gene polymorphisms and urticaria, this study, therefore, is the first of its type to investigate whether there is any significant association between the FcεR1β gene polymorphism and the CSU phenotype in a Kashmiri population or not. Also, autoimmunity against the high affinity IgE receptor has been reported in chronic urticaria and CAU.<sup>10,11</sup> In the present study, we found no significant associations between the SNPs of FcεR1β gene and CSU, however, in this study, serum total IgE levels were found varied to different ranges in CAU patients and most of ASST positive CSU patients were found to have high levels of serum total IgE.

Thus we conclude that there is no statistically significant association between FcεR1β gene polymorphism and CSU in Kashmiri population; however, there is a probability of developing CSU in patients carrying FcεR1β T allele.

Furthermore, serum total IgE levels had no significant association with the development of CAU.

## Financial support

The study was all self funded.

## Ethical disclosures

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

## Conflict of interest

The authors declared no conflict of interest.

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