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ORIGINAL ARTICLE

Single nucleotide polymorphisms of *IL-2*, but not *IL-12* and *IFN-γ*, are associated with increased susceptibility to chronic spontaneous urticaria

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Abstract
Background: A clear picture of interaction of Th1/Th2 cytokines in pathogenesis of chronic spontaneous urticaria (CSU), remains elusive. Impaired IFN-γ production and decreased levels of IL-2 have been reported. The aim of this study was to evaluate the association of Th1 cytokines; IL-2, IL-12 and IFN-γ polymorphisms with CSU.
Methods: 90 patients with CSU and 140 age-sex matched subjects were included in this study. DNA samples were evaluated through PCR-SSP assay in order to detect single nucleotide polymorphisms of IL-12 (A/C −1188) or (rs3212227), IFN-γ (A/T UTR5644) or (rs2069717) and IL-2 (G/T −330 and G/T +166) or (rs2069762 and rs2069763).
Results: G allele at −330 at promoter region of IL-2 gene was overrepresented in CSU. Heterozygotes (GT) at this locus and heterozygotes at +166 of IL-2 gene (GT) were more prevalent in CSU group. Additionally, the haplotype GT for loci −330 and +166 of IL-2 gene was powerfully associated with CSU (OR (95%CI) = 57.29 (8.43–112.7)).

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Conclusions: SNP at position −330 and +166 of IL-2 gene are differently expressed in CSU. The haplotype GT of IL-2 at −330 and +166 might confer vulnerability to a number of immunological disorders in Iranian region.

Introduction

Recurrence or persistence of pruritic erythematous wheals or angio-edema for at least six weeks is designated as chronic urticaria.[1] Chronic spontaneous urticaria (CSU) is a term that has now replaced the two terms of chronic idiopathic urticaria (CIU) and chronic autoimmune urticaria (CAU), the later characterized by positive serum anti-FcεR1β and/or anti-IgE auto antibodies.[2] Patients with CSU have a lower quality of life; experience more of symptoms of depression, anxiety and sleep difficulties and also have reduced working capacity.[3] A strong correlation exists between the activity of CSU, determined by urticaria activity score 7, and quality of life scores, determined by chronic urticaria quality of life questionnaire (CU-Q2oL).[4] Spontaneous urticaria was first devised to describe occurrence of spontaneous wheals or angio-edema for no known external cause apparent at clinical examination.[5] While all types of urticaria can be chronic in nature, physical urticarial to name some, the term chronic spontaneous urticaria has now replaced the former term chronic idiopathic urticaria, to rightfully represent the chronic nature and unknown eliciting factor of this subgroup of urticaria.[5] A wide array on underlying predisposing factors have been sub-classified or identified as causative factors in chronic urticaria, among which the role of functional auto-antibodies and chronic infections have been emphasized.[5] Autoimmune urticaria, for instance was formerly classified as a unique entity, by the presence of high affinity IgE receptor antibodies. Meanwhile, in as much as 50% of sera of patients with CSU, high affinity auto-anti FcεR1, anti-IgE antibody or mast-cell activating non-immunoglobulin mediators can be detected,[6] and a positive autologous serum skin test (ASST) proves an immunological substrate in 25–45% of CSU (former CIU) patients.[7]

A powerful association has been found between FCER gene (FC epsilon receptor gene 1) α and β chains, polymorphisms, with CSU.[8,9] The high-affinity IgE receptor, FCε receptor 1, on basophils and mast cells is the primary site of action for activating auto-antibodies to cross-link the receptors and initiate a response. This mechanism is however confined to patients with high total IgE (RIST assay) or patients with positive ASST test. There is a mixed and heterogeneous cytokine network of both Th1 and Th2 response in most patients with CSU, and the spontaneous nature of CSU justifies this heterogeneity. In fact an individual immune profile might underlie each case of CSU.[10] sIL-2R and TNF-α concentrations are higher in serum of atopic patients with CSU, while the Th2 profile cytokines are the prevailing cytokines in patients with chronic spontaneous angio-edema.[10]

We have recently shown that single nucleotide polymorphisms (SNPs) of pro-inflammatory cytokines such as IL-6 and TNF-α[1] as well as IL-10 and TGF-β are associated with CSU.[11] Although the association of Th1 cytokines with several diseases has been investigated,[12–17] it has not been studied in CSU. Herein, the possible association of IL-2, IL-12 and IFN-γ SNPs with CSU was studied in order to elucidate the underlying immune dysregulation in patients suffering from CSU.

Materials and methods

Study design

This case–control study comprised a number of 90 patients (75 females) with CSU, who were selected by simple randomization from referrals to the Children's Medical Center, the Pediatrics Center of Excellence in Tehran, Iran. The diagnosis of Chronic Urticaria was primarily made with a history of at least two recurrences of wheals and/or angio-edema in a weak that lasted for six weeks or more, according to standard international guidelines.[18] To ascertain the spontaneous nature of urticaria, detailed history including the time of onset, duration, characteristics and distribution of lesions and also history of associated illnesses, food or drug allergy was taken. Subjects with any sign of physical urticaria, cold urticaria, urticarial vasculitis and food or drug induced urticaria were excluded from the patients group. Finally, specific laboratory tests were performed; including C3, C4, CH50, and C1-inhibtor (C1-INH) in order to exclude patients with complement deficiencies. Additionally, a number of 140 age-sex matched subjects with no evidence of allergic or autoimmune disorders were randomly selected as control group from the control bank of our research center.[19] This study was approved by the local ethics committee and institutional review board of Tehran University of Medical Sciences. The participants were provided with detailed information about the aim and protocol of the study and signed informed consent forms.

Sampling and genotyping

The amount of 5 ml of whole blood was taken from all participants and preserved with ethylene-diamine-tetra-acetic acid (EDTA) until investigation. Genomic DNA was extracted from peripheral blood mono-nucleated cells using phenol–chloroform method.[20] The extracted DNA was amplified using a PCR Techne Flexigene apparatus (Roche, Cambridge, UK) as explained before.[12] Polymerase chain reaction with the Sequence Specific Primers (PCR-SSP) assay was employed (PCR-SSP kit, Heidelberg University,

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