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IL-18 gene polymorphisms in aphthous stomatitis vs. Behçet's disease in a cohort of Egyptian patients

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OBJECTIVE: A clinical investigation of the potential correlation of two single-nucleotide polymorphisms at −137 (G/C) and −607 (C/A) in the promoter region of the IL-18 gene, with the susceptibility to aphthous stomatitis and Behçet's disease.

PATIENT AND METHODS: This study included 80 aphthous stomatitis patients and 80 patients with Behçet's disease. Eighty healthy subjects were enrolled as a control group. IL-18 single-nucleotide polymorphisms at −607 and −137 regions were analyzed using polymerase chain reaction–restriction fragment length polymorphism analysis.

RESULTS: The genotype and allele distributions of the two regions did not differ significantly between patients with aphthous stomatitis and controls. The genotype and allele distributions at −607 were significantly different between patients with Behçet's disease [CC (P = 0.044), C allele (P = 0.043), A allele (P = 0.043)], and controls. The frequency of the GG genotype at position −137 in patients with Behçet's disease was associated only with a higher rate of ocular manifestations (OR= 1.4, CI= 0.76–2.7, P = 0.031).

CONCLUSION: IL-18 gene polymorphisms were not associated with any susceptibility to aphthous stomatitis, while a positive association was found with patients with Behçet's disease regarding −607 promoter site. Moreover, patients with Behçet's disease carrying the GG genotype at position −137 had a higher risk of developing ocular manifestations.

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Introduction

Recurrent aphthous stomatitis (RAS) is a common condition characterized by recurrent episodes of oral ulceration that was reported as the most common inflammatory ulcerative condition of the oral mucosa (1, 2), and it is an important condition as it can be distressing and cause suffering and pain. Three clinical forms are described: minor (MiRAS) that accounts for 80% of RAS patients, major (MaRAS), and herpetiform ulceration (HU), according to the classification of Stanley (3). The ulcers usually occur on the non-keratinized mucosa and heal spontaneously, without scarring, except for MaRAS that are similar to MiRAS, but are more than 10 mm in diameter, and the ulceration is deeper. Because the lesions are larger, healing usually takes longer (about 20 to 30 days) and may leave scars (4).

It has been suggested that RAS is precipitated by local trauma in an individual genetically predisposed to an exaggerated or abnormal cytokine cascade. In turn, this leads to cell-mediated damage to focal areas of the oral mucosa (5). Genetic factors are thought to play an important role in the development of RAS; about 24–46% of patients report a positive family history (6).

Furthermore, there is significantly higher disease concordance in monozygotic than dizygotic twins (7). Nevertheless, individual variability in disease susceptibility suggests a polygenic mode of inheritance with extrinsic factors such as trauma modulating the disease expression (8). Indeed, elevated levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin-(IL)-2, IL-4, and IL-5 have been detected in ulcer tissue and raised levels of IL-6 in the circulation (9).

Another inflammatory disease with oral ulcers, periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome showed *IL-18* gene expression during periods of activity. PFAPA is a non-hereditary auto-inflammatory disease, characterized by relatively regular recurrence of febrile episodes of 3–6 days duration, accompanied by aphthous stomatitis, pharyngitis/tonsillitis, and/or cervical adenitis. Although its etiology is still to be elucidated, a recent study suggested an environmentally triggered activation of complement and IL-1β/IL-18 during PFAPA syndrome flares, with induction of Th1 chemokines and subsequent retention of activated T cells in peripheral tissues (9–11).

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Behçet's disease (BD) is a multisystemic inflammatory disease classified as a vasculitis, in which the presence of recurring oral ulcerations, plus any two signs of genital ulceration, skin lesions (e.g., pustules or nodules), or eye lesions (e.g., uveitis or retinal vasculitis) is diagnostic for the disease. Oral ulcers are categorized as the first clinical presentation in the majority of patients and are deemed to be present in BD when observed by the physician or patient at least three times in the course of a year (12). Although the pathogenesis is unclear, environmental, genetic, and autoimmune factors have been considered; in addition, polymorphisms of many cytokines have been identified, including IL-1, IL-12, IL-4 (13), and IL-18 (14).

IL-18 is a proinflammatory cytokine that mediates T-helper (Th)-1-polarized immune responses, and elevated levels of IL-18 have been observed in the sera and bronchoalveolar lavage fluid of patients with active BD (15). IL-18 can enhance the production of TNF-α, granulocyte–monocyte-colony-stimulating factor (GM-CSF), and IFN-γ, which were found to be elevated in BD (16). It has been reported that BD patients have higher serum levels of IL-18 than controls, and its concentration is related to disease activity (17).

IL-18 gene is located on chromosome 11q22.2–22.3 (18), and several polymorphisms within IL-18 promoter gene have been associated with different inflammatory and autoimmune diseases (19–23). In addition, many investigators identified a significant higher frequency of −137 GG and the CC −607 genotypes in BD patients in comparison with controls (24, 25).

The contribution of single-nucleotide polymorphisms (SNPs) in *IL-18* to variations in expression and activity of this inflammatory cytokine has become important for the understanding of diseases related to immune function. In this regard, the aim of this work was twofold: to investigate the potential role of two polymorphisms (−137 and −607) within the promoter of the *IL-18 gene* in the susceptibility to RAS and BD, compared to healthy controls in Egyptian patients and additionally to find out its association, if any, with the clinical severity of either of the two conditions.

Subjects and methods

In this case–control study, 80 patients with RAS, 80 patients with BD, and 80 age- and gender-matched healthy individuals were selected and enrolled after signing an informed consent. Both the protocol and consent forms were reviewed and approved by the Medical Ethical Committees of Al Azhar University and Ain Shams University. This study was conducted according to the Declaration of Helsinki Principles and according to the principles good clinical practice.

For RAS patients, they were selected from the outpatients' clinic of the Oral Medicine Department of Al Azhar University, Faculty of Dental Medicine for girls, during the period of July 2012 to November 2013.

Patients with Behçet's disease were selected from the outpatients' clinics of the Dermatology, Rheumatology, and Oral Medicine Departments of Ain Shams University, Faculty of Medicine, during the period from August 2012 to September 2013. The control participants were selected from visitors and hospital staff of the Faculty of Dental Medicine for girls, Al Azhar University.

Patients and controls were subjected to an assessment protocol that included careful history review, general assessment of health, and full dermatological and oral examination. In addition, blood samples were obtained for assessment of complete blood count (CBC), erythrocyte sedimentation rate (ESR), serum B12, serum folate, and C-reactive protein (CRP).

1) RAS patients: Eighty patients with RAS, unrelated to each other, were included in this group (Group 1) [age range: 22–42 years; mean age ± SD = 37.33 ± 24.64 years, comprising 29 men and 51 women). All subjects were clinically assessed, and the presence of aphthous ulcers was confirmed. Inclusion criteria included: (i) a 6-month or longer history of regularly recurrent episodes of oral aphthous ulceration, and the ulcers were strictly limited to the oral cavity (Fig. 1), (ii) at least two ulcers per month for the previous 6 months, (iii) normal full blood count, serum B12 (200–900 pg/ml), red cell folate (110–700 μg/l).
2) BD patients: For 80 BD patients, the International Study Group (ISG) criteria for diagnosis of BD were used (Table 1). Patients were thoroughly examined for oral lesions, genital lesions, skin lesions (pseudofolliculitis, acne-like lesions, erythema nodosum, thrombophlebitis, etc.). They were referred to ophthalmology, internal medicine, and rheumatology departments for eye, chest, heart, renal, gastrointestinal system, nervous system, and joints examination. The pathergy test, a 2-mm papule induced 24 to 48 h after intradermal puncture, is one of the 4 additional criteria of diagnosis. It was carried out in case of the presence of only single additional criterion. All patients were unrelated to each other (Group 2; age range: 28–40 years; mean age ± SD = 34.24 ± 21.53 years, comprising 62 men and 18 women).

Exclusion criteria for both disease groups included: (i) pregnancy, (ii) history of systemic disease in which oral

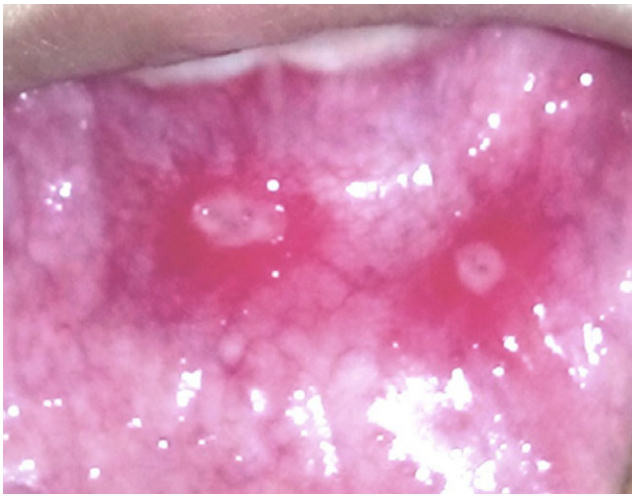

Figure 1 A clinical photograph of a 24 male patient having two minor aphtha in the mucosal side of the lower lip.