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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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Keywords: aphthous stomatitis; Behçet's disease; gene polymorphism; IL-18

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

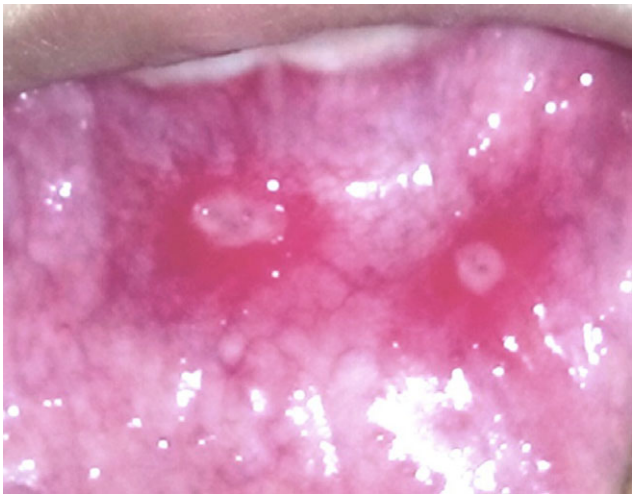

Figure 1 A clinical photograph of a 24 male patient having two minor aphtha in the mucosal side of the lower lip.

Table 1 International classification criteria of Behcet's disease (26)

In the absence of other clinical explanations, patients must have:
1. Recurrent oral ulceration (aphthous or herpetiform) observed by the physician or patient recurring at least three times in a 12-month period;
And two of the following:
2. Recurrent genital ulceration.
3. Eye lesions: anterior uveitis, posterior uveitis, cells in the vitreous by slit-lamp examination, or retinal vasculitis observed by an ophthalmologist.
4. Skin lesions: erythema nodosum, pseudofolliculitis, papulopustular lesions, or acneiform nodules in post-adolescent patients not on corticosteroids.
5. Pathergy, read by a physician at 24–48 h

ulceration may be a feature, for example, celiac disease, Crohn's disease, ulcerative colitis, anemia, neutropenia, or AIDS, (iii) concurrent medication with systemic steroids, immune modulators, or cytotoxics.

3) The control group (CL): To set-up a control group (Group 3), 80 healthy participants, unrelated to any of the patients, were randomly selected to share in this current study (age range: 16–46 years; mean age ± SD = 33 ± 16 years; 37 male and 43 female). They were clinically as well as laboratory free of any abnormality.

Technical steps

IL-18 SNPs analysis was carried out in all patients and control subjects. SNPs were analyzed by PCR amplification and restriction fragment length polymorphism (RFLP) analysis according to Refs. (27). One cubic centimeter (cc) of venous blood was collected from each patient and control subject *n* EDTA tubes. All blood samples were processed on the same day of collection.

Genomic DNA was extracted from whole blood using Gene JETTM genomic DNA purification kit (Fermentas, Waltham, MA, USA) according to the manufacturer instructions. Purified DNA samples were stored at −20°C till used in the amplification step. The extracted DNA concentration was detected through measurement by ultra-violet (UV) spectrophotometer. Readings were taken at wavelengths of 260 and 280 nanometer (nm). The extracted DNA concentration ranged from 20 to 30 ng DNA/μl.

Primers sequences, cycling conditions, and the restriction enzymes used are summarized in Table 2. The reaction mixture contains the following: 1 μg of genomic DNA, 25 μl of Dream Tag PCR Mastermix (2X; Fermentas), 0.5 mM of each primer (Fermentas), water (nuclease free) to a final volume of 50 μl. All reagents were prior vortexed, and 25 μl of mineral oil was added to the reaction mixture and carried out in thermal cycler (Biometra GmbH, Göttingen, Germany).

For *IL-18*-607C/A polymorphism, the −607A allele was cut into two fragments of 101 and 70 bp, while the −607 C allele remains uncut (171 bp).

For *IL-18*-137G/C polymorphism, the −137 G allele was cut into two fragments of 107 and 24 bp, while the −137C allele remained uncut (131 bp).

PCR-RFLP products were visualized using 1% agarose gel electrophoresis stained by ethidium bromide and visualized by UV light.

Statistical analysis

The collected data were analyzed using SPSS version 16 software (SPSS Inc., Chicago, IL, USA). Data were presented as number and percentages, and odds ratio (OR) and the corresponding 95% confidence interval (CI) were also calculated. $P < 0.05$ was considered significant. Chi-squared (X^2) test was used to compare between two independent groups with regard to the categorical data. Pearson correlation coefficient (r) test was performed to study the possible association between each two variables.

Results

Table 3 represents the clinical features of the study patients and control subjects. Patients with RAS presented with minor type in 69 (86.25%), major type in 6 (7.5%), and herpetiform type in 5 (6.25%). They showed normal laboratory investigations. All BD patients had recurrent oral ulcers, while 75 patients (93.75%) had cutaneous lesions, 68 patients (85%) had recurrent genital ulcers, 56 patients (70%) had ocular affection, 38 patients (47.5%) had arthritis, 23 patients (28.75%) had thrombophlebitis, 13 patients (16.25%) had cardiopulmonary affection, 10 patients (12.5%) had gastrointestinal manifestations, and none of the patients had neurobehcet's disease. They showed moderate leukocytosis, high ESR, and positive CRP (in 60 patients, 75%).

IL-18 genotypes in RAS patients vs. controls

The genotype distribution and allele frequencies for *IL-18* -607C/A and *IL-18* -137G/C SNPs in RAS patients and the control group are shown in Table 4 and Fig. 2.

Table 2 Primer sequences for genes, cycling conditions, and restriction enzymes used in PCR

	Primer sequence	*Cycling conditions*		*Restriction enzyme*
IL-18 -607C/A	F:5′-GCCCTCTTACCTGAATTTTGGTAGCCCTC -3′ R:5′-AGATTTACTTTTCAGTGGAACAGGAGTCC -3′	95°C 3 min 95°C 30 s 56°C 30 s 72°C 1 min 72°C 15 min	30 cycles	*Tru1*I (Fermentas)
IL-18 -137G/C	F:5′- ATGCTTCTAATGGACTAAGGA -3′ R:5′- GTAATATCACTATTTTCATGAATT -3′	95°C 3 min 95°C 30 s 43°C 30 s 72°C 1 min 72°C 15 min	30 cycles	*Eco*R1 (Fermentas)

Table 3 Clinical features of the study patients and control subjects

Patient clinical criteria	*RAS*	*BD*	*CL*
Number	80	80	80
Sex (female/male)	51:29	18:62	43:37
Age (years):			
Range	22–42	28–40	16–46
Mean ± SD	37.33 ± 24.64	34.24 ± 21.53	33.23 ± 16.11
Presence of RAS	100%	100%	None
Rate of RAS recurrence:			
2 ulcers/month	52 (65%)	43 (53.75%)	–
3 ulcers/month	28 (35%)	37 (46.25%)	–
Ulcer size, No. (%)			
Minor	69 (86.25%)	74 (92.5%)	–
Major	6 (7.5%)	4 (5%)	–
Herpetiform	5 (6.25%)	2 (2.5%)	–
Genital ulcer:	–	Present in 68 of patients (85%)	–
Ocular lesions	–	Present in 56 of patients (70%)	–
Blood Lab. findings:			
RBCs	Normal	Normal	Normal
WBCs	Normal	Moderate leukocytosis	Normal
Platelets	Normal	Normal	Normal
Serum B12 (pg/ml):			
Range	200–900	200–900	200–900
Mean ± SD	501.88 ± 35.02	520.44 ± 168.98	278.49 ± 190.06
Red cell folate (μg/L):			
Range	110–700	110–700	110–700
Mean ± SD	435.03 ± 21.11	557 ± 6.55	547 ± 3.78
ESR (mm/h):			
Range	10–30	20–150	10–30
Mean ± SD	20 ± 5	70.88 ± 37.003	17 ± 6
CRP			
Positive (+ ve)	0	60 (75%)	0
	80 (100%)	20 (25%)	80 (100%)
Negative (− ve)			
Positive Pathergy test	–	(+ ve) in 71 (88.75%)	–
Arthritis	–	Present in 38 of the patients (47.5%)	–
Skin lesions:			
Erythema nodosum	–	(+ ve) in 32 (40%)	–
Subcutaneous thrombophlebitis	–	(+ ve) in 27 (33. 75%)	–
	–	(+ ve) in 21 (26.25%)	–
Acne-like lesions			
Thrombophlebitis	–	23 patients (28.75%)	–
Cardiopulmonary affection	–	13 patients (16.25%)	–
Gastrointestinal manifestations	–	10 patients (12.5%)	–

BD, Behcet's disease; CL, controls; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RBCs, red blood cells; RAS, recurrent aphthous stomatitis; WBCs, white blood cells.

As for the *IL-18* -607C/A promoter SNP analysis, the homozygous CC genotype was detected in 22.5% of RAS patients and heterozygous CA in 48.75%, whereas homozygous AA genotype was detected in 28.75% of patients. The frequency of C allele was 53.75%, and the frequency of A allele was 46.25%. These values did not differ signifi-

Table 4 Distribution of IL-18 genotypes and alleles frequencies in RAS patients and the control group

Genotypes		*Controls* (N = *80)* N *(%)*	*RAS patients* (N = *80)* N *(%)*	*OR*	*95% Confidence interval of OR*	P
IL-18 -607C/A	CC	20 (25)	18 (22.5)	0.87	0.42–1.8	0.71
	AC	42 (52.5)	39 (48.75)	0.86	0.46–1.6	0.63
	AA	18 (22.5)	23 (28.75)	1.4	0.68–2.8	0.37
	C allele	82 (51.25)	86 (53.75)	1.1	0.7–1.7	0.65
	A allele	78 (48.75) (47.4)	74 (46.25)	0.9	0.58–1.4	0.65
IL-18 -137G/C	GG	33 (41.25)	37 (46.25)	1.2	0.65–2.3	0.52
	GC	35 (43.75)	35 (43.75)	1.0	0.53–1.8	1.0
	CC	12 (15) 4 (15.3)	8 (10)	0.63	0.24–1.6	0.34
	G allele	101 (63.13)	109 (68.125)	1.24	0.79–1.98	0.35
	C allele	59 (36.87)	51 (31.875)	0.8	0.5–1.3	0.35

RAS, recurrent aphthous stomatitis.

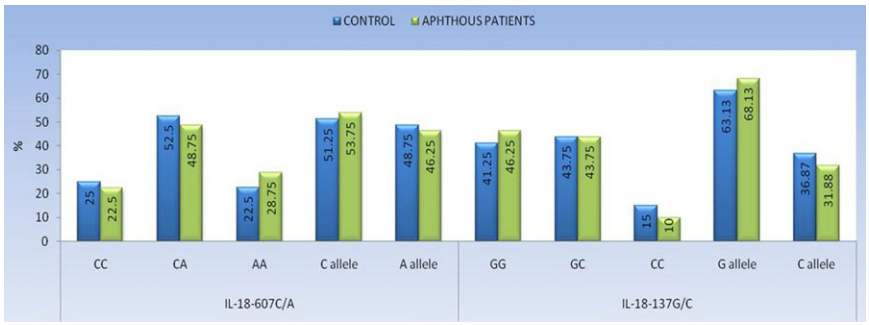

Figure 2 The genotype distribution and allele frequencies for IL-18 -607C/A and IL-18 -137G/C SNPs in RAS patients and the control group.

cantly from the control group (CC 25%, CA 52.5%, AA 22.5%, C allele 51.25%, and A allele 48.75%).

Regarding the −137G/C promoter SNPs, the homozygous GG genotype was detected in 46.25% of RAS patients, heterozygous GC genotype was detected in 43.75%, and homozygous CC genotype was detected in 10%. The frequency of G allele was 68.125%, and the frequency of C allele was 31.875%. In the control group, the GG genotype was detected in 41.25% of subjects, GC in 43.75%, and CC in 15%. The frequency of G allele was 63.13%, and the frequency of C allele was 36.87%. These results show insignificant difference between RAS patients and the control group ($P > 0.05$).

IL-18 genotypes in BD patients vs. controls

The genotype distribution and allele frequencies for IL-18 -607C/A and IL-18 -137G/C SNPs in BD patients and the control group are shown in Table 5 and Fig. 3.

About the *IL-18* -607C/A promoter SNP analysis, the homozygous CC genotype was detected in 40% of BD patients and heterozygous CA in 45%, whereas homozygous AA genotype was detected in 15% of patients. The frequency of C allele was 62.5%, and the frequency of A allele was 37.5%. In the control group, the CC genotype was detected in 25%% of subjects, CA in 52.5%, and AA in 22.5%. The frequency of C allele was 51.25%, and the frequency of A allele was 48.75%.

The frequency of CC genotype was significantly higher (OR = 2.0, 95% CI = 1.01–3.9, $P = 0.044$) in BD patients compared with the control group. CA and AA genotypes show insignificant variations between patients and the control group ($P = 0.34$ and 0.23, respectively). The frequency of C allele was significantly higher (OR = 1.59, 95%, CI = 1.02–2.48. $P = 0.043$). However, the frequency of A allele was significantly lower (OR = 0.63, 95% CI = 0.4–0.98, $P = 0.043$) in BD patients compared with the control group.

As regards −137G/C promoter SNPs, the homozygous GG genotype was detected in 50% of BD patients, heterozygous GC genotype was detected in 42.5%, and homozygous CC genotype was detected in 7.5%. The frequency of G allele was 71.25%, and the frequency of C allele was 28.75%. These values did not differ significantly from the control group (GG 41.25%, GC 43.75%, CC 15%, G allele 63.13%, and C allele 36.87%).

Association between study groups and the genotype frequencies at position −137

The genotype distribution and allele frequencies for *IL-18* -607C/A and *IL-18* -137G/C SNPs did not show significant differences based on the clinical parameters of RAS patients, that is, gender, disease duration, mean age at onset, and clinical disease manifestations ($P > 0.05$), including size, number, and rate of recurrence.

Although the frequency of the GG genotype at position −137 was high in RAS (OR = 1.2, CI = 0.65–2.3, $P = 0.5$), the association between the genotypes at position −137 and the severity of oral ulcerations (e.g., rate of recurrence and number) remained statistically insignificant when multiple logistic regression analysis was performed using the Enter method.

Table 5 Distribution of IL-18 genotypes and alleles frequencies in patients with BD and the control group

Genotypes		*Controls (*N = *80)* N *(%)*	*BD patients (*N = *80)* N *(%)*	*OR*	*95% Confidence interval of OR*	P
IL-18 -607C/A	CC	20 (25)	32 (40)	2.0	1.01–3.9	0.044*
	CA	42 (52.5)	36 (45)	0.74	0.4–1.4	0.34
	AA	18 (22.5)	12 (15)	0.61	0.27–1.36	0.23
	C allele	82 (51.25)	100 (62.5)	1.59	1.02–2.48	0.043*
	A allele	78 (48.75)	60 (37.5)	0.63	0.4–0.98	0.043*
IL-18 -137G/C	GG	33 (41.25)	40 (50)	1.4	0.76–2.7	0.27
	GC	35 (43.75)	34 (42.5)	0.95	0.5–1.77	0.87
	CC	12 (15) 4 (15.3)	6 (7.5)	0.45	0.16–1.3	0.14
	G allele	101 (63.13)	114 (71.25)	1.45	0.9–2.3	0.122
	C allele	59 (36.87)	46 (28.75)	0.69	0.34–1.1	0.122

RAS, recurrent aphthous stomatitis; BD, Behçet's disease.
*Significance was considered when *P*-value ≤ 0.05.

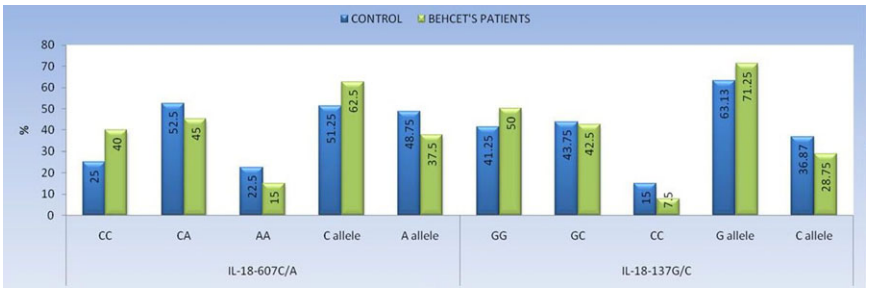

Figure 3 The genotype distribution and allele frequencies for IL-18 -607C/A and IL-18 -137G/C SNPs in BD patients and the control group.

Regarding BD, the genotype distribution and allele frequencies for *IL-18* -607C/A and *IL-18* -137G/C SNPs did not show significant differences based on the clinical parameters of BD patients, that is, gender, disease duration, mean age at onset, and clinical disease manifestations ($P > 0.05$), apart from ocular manifestations.

Concerning the frequency of the GG genotype at position −137, it was significantly higher in BD patients with ocular manifestations than in patients without (25/30 patients; 83.3% vs. 30/50 patients; 60%; $P = 0.032$). The OR value for the development of the ocular manifestations in BD patients with the GG genotype was 4.3 (95% CI, 1.1 to 15.1). When multiple logistic regression analysis was performed using the Enter method, the association between the genotypes at position −137 and the ocular ulcerations in BD remained statistically significant (B coefficient = 2.142, $P = 0.031$).

Hardy–Weinberg equilibrium calculations

As all Hardy–Weinberg equilibrium calculations are non-statistically significant as shown in Table 6, then all the observed genotypes are consistent with Hardy–Weinberg equilibrium. Calculation of Hardy–Weinberg equilibrium was performed using Court-Lab–HW calculator.

Discussion

Single-nucleotide polymorphisms (SNPs) are commonly found in humans, with an incidence of 1% in the whole population (28). These genetic variations are influenced by many factors, including race and environment. Identifying relationships between SNPs and disease pathology is critical for the development of novel treatment and preventive measures for a variety of human diseases (29).

IL-18 is a pleiotropic cytokine involved in the amplification of inflammatory responses which plays a role in many illnesses with a relevant chronic inflammatory component, playing an important role as a modulator of immune responses (23). IL-18 was first discovered as a potent IFN- γ inducing factor, produced mainly by macrophages, dendritic cells, Kupffer cells, keratinocytes, synovial, fibroblasts, epithelial cells, and osteoblasts cells and plays a crucial role in regulation of both innate and acquired immunity. It has multiple functions including inducting TNF-α, enhancing the cytotoxicity of NK cells through upregulation of FAS ligand, and inducing proinflammatory cytokines and chemokines (30, 31). It was also found that IL-18, in synergy with IL-12 or IL-21, enhances IFN-γ production in human NK and T cells (32, 33).

Table 6 Hardy–Weinberg equilibrium calculations

Genotypes	*Controls* (N = *80*)	*RAS patients* (N = *80*)	*BD patients* (N = *80*)
IL-18 -607C/A	$X^2 = 0.21$	$X^2 = 0.53$	$X^2 = 0.13$
	$P = 0.650$	$P = 0.849$	$P = 0.721$
IL-18 -137G/C	$X^2 = 0.29$	$X^2 = 0.004$	$X^2 = 0.11$
	$P = 0.590$	$P = 0.947$	$P = 0.738$

RAS, recurrent aphthous stomatitis; BD, Behçet's disease.

Several variations within the *IL-18 gene* promoter region are responsible for changes in the transcription rate (33). Many studies have cloned and analyzed the promoter region of *IL-18* to characterize gene expression and regulation (29).

By means of various analyses, several SNPs have been identified in the promoter region of *IL-18* at −137, −607, and −656 loci (34). The −607C/A (rs1946518) variation is located in the binding region of nuclear factor cAMP-response element-binding protein and histone H4 transcription actor. The SNP affects biologic functions of *IL-18*, and, thus, this locus is currently the most widely studied *IL-18* polymorphism (35).

In the present study, we selected two functional *IL-18* promoter polymorphisms (−137 and −607), which were suggested to alter the IL-18 promoter expression at transcription level and confirmed to have an impact on *IL-18 gene* activity in its promoter (22, 36).

Various studies of the *IL-18* polymorphism have focused on allergic diseases, viral infections, autoimmune diseases, and cancers. Results indicate that the −607C/A (rs1946518) locus is correlated with allergic asthma, allergic rhinitis, nasopharyngeal cancer, chronic hepatitis B virus, and human immunodeficiency virus infection, among other diseases (37–40).

Up to our knowledge, this is the first report of the role of *IL-18 gene* polymorphisms in RAS and BD among Egyptians. Our results showed that the genotype and allele distributions of the two SNPs at positions −137 (G/C) and −607C/A did not differ significantly between patients with RAS and controls. Although the results did not reach a statistical significance, patients with RAS had somewhat higher frequencies of the G allele at position −137. On the other hand, the genotype CC was present at a non-significantly higher frequency in the controls compared with those in the RAS patients further suggesting that the

genotype SNP-607-AC/CC can protect the individuals suffering from recurring oral ulcerations.

Different authors are still in controversy about the complex pathogenesis of the disease. As this is the first report concerning the genotype and allele distributions of the two SNPs at positions −137 (G/C) and −607 (C/A) of *IL-18* and RAS, additional studies with larger sample sizes will be required to confirm these preliminary findings.

In contrast, the frequency of CC genotype in our study was significantly higher in BD patients compared with the control group, contrary to Jang et al., results. The frequency of C allele was significantly higher, while the frequency of A allele was significantly lower in BD patients compared with the control group. Furthermore, our BD patients had somewhat higher frequencies of the G allele at position −137, although insignificantly different from controls, in agreement with Jang et al. (41).

It was also noticed that BD patients carrying the GG genotype at position −137 (OR = 1.4) had a higher risk of developing the ocular lesions, possibly due to higher IL-18 levels with further immune dysregulation. This is consistent with the previous finding showing that subjects homozygous for G at position −137 had higher levels of *IL-18* mRNA when compared with other genotypes (14, 33).

The association of two functional polymorphisms in the *IL-18* promoter, −607C/A (rs1946518) and −137G/C (rs187238), with the risk of BD was investigated previously in different populations. Our results showed similar association concerning the −607 (C/A) SNP in the Korean population where BD patients had a significantly higher frequency of the −607 C allele (60.7% vs. 48.1%, OR 1.668, 95% CI 1.129–2.464, $P = 0.0101$) (23). On contrary, opposite results were observed in the Turkish population, where a genetic association between allele 'A' in rs1946518 (−607 C/A) and BD (39% vs. 19%, OR = 1.48, 95% CI = 1.10–1.97, $P = 0.0088$) was detected (24). However, considerable interethnic variability has been reported for the distributions of genotypes of the *IL-18* promoter polymorphisms (42, 43).

Ultimately, in this current study, patients with RAS and BD had somewhat higher frequencies of the G allele at position −137. Therefore, we could speculate that overexpression of IL-18 associated with the GG genotype at position −137 may cause IFN-γ production in T cells and NK cells in local cellular environment, resulting in the development of certain inflammatory lesions in both RAS and BD patients, for further studies.

However, some limitations in our study need to be addressed. First, this study was hospital-based case–control study, so patients were unrepresentative of the general population. Second, the sample size of the RAS and BD cases was not large enough to detect SNPs. Finally, although every effort was made in this study to achieve a gender concordance between patients and controls, that was almost reached only with RAS patients. Actually, this could be attributed to the fact that, in BD, it is customary to believe that men are more affected than women; the male-to-female ratio is reported from 5.37 to 1 in Egypt. However, no strong association was found between the male gender and major organ involvement (44), including the ocular manifestations, either with clinical relevance or therapeutic outcome (45).

Future studies on the other IL-18 sequence variants and their biologic function are also needed to further understand the role of the IL-18 polymorphisms in determining the risk of RAS and BD.

Moreover, as genetic polymorphisms often vary significantly between the different ethnic groups, hence, further studies are warranted to clarify the association of the IL-18 polymorphisms with the risk of RAS and BD in diverse ethnic populations.

Conclusion

According to our results, although there was no evidence for a genetic association conferred by the two SNPs at positions −137 and −607 in the promoter region of the *IL-18 gene* with respect to a susceptibility to RAS, a positive association was found in case of BD patients regarding −607 promoter region.

Concerning RAS patients, even if there is no convincing evidence to support a correlation between *IL-18 promoter gene* polymorphisms and RAS, it was found that RAS patients had somewhat higher frequencies of the G allele at position −137. This can partially explain the more likelihood of clinical severity in this category of patients. Regarding the BD patients carrying the GG genotype at position −137, they had a higher risk only of developing ocular manifestations.

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Conflict of interest

Neither of the authors is aware of any financial or personal relationships with people or organization of pharmaceutical manufacturer in this study that could have inappropriately influenced (biased) the work described in this study.