

Association of Genetic Variants of the Interleukin-17F rs763780 and its Circulating Level in Psoriasis Patients in Egypt

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Psoriasis is a chronic autoimmune disease affecting the skin and joints, IL-17 family has been shown to be the major effector cytokine in its pathogenesis. This study aimed to investigate genetic polymorphism of IL-17F (rs763780) and evaluate the impact of this polymorphism on circulating levels of IL-17F as a potential risk locus for psoriasis. 60 patients suffering from chronic plaque psoriasis and 60 healthy controls were genotyped for the IL-17F (rs763780) using an Amplification Refractory Mutation System - PCR (ARMS-PCR) method. Measurement of serum IL-17F was also done by ELISA. There was a significant difference in frequency between TT and TC genotypes (OR= 2.74, 95%CI=1.04 -7.4, P=0.04) and TT and CC genotypes (OR=10.9, 95%CI=1.3-91.3, P=0.007). Moreover, the TC and CC genotypes were associated with increased risk of psoriasis in comparison with the TT genotype. (OR= 3.8, 95% CI: 1.5–9.4, P= 0.003). The mutant allele, C, was significantly associated with an increased risk of psoriasis compared to that with the wild T allele, T (OR= 4.1, 95% CI: 1.9– 9.1, P= 0.0002). Serum level of IL-17F was higher among psoriasis patients (25.7±3.8pg/ml) than healthy controls ((15.1±2.1pg/ mL). In conclusion, IL17F polymorphism (rs763780) is associated with increased risk of psoriasis and may influence the level of production of IL-17F with subsequent effects on the pathogenesis of psoriasis.

Psoriasis is a chronic autoimmune (sometimes considered as autoinflammatory) disease affecting the skin and joints [1]. The role of immune system in pathogenesis of psoriasis is well established [2], however; what initiate the psoriatic reaction is still an enigma [3]. T cell (especially Th1, Th17, and Th22) had been widely investigated in psoriasis and several directed drug therapies has been used in psoriasis [4]. Th 17 has been investigated and targeted in psoriasis in the last years [5]

IL-17 family (IL-17A-F) has been shown to be the major effector cytokine in the pathogenesis of psoriatic disease [6]. Within this family, IL-17A, IL-17C, and IL-17F are implicated in psoriasis pathogenesis [7,8]. Several drugs targeting the IL-17 cytokine family (IL-17A, IL-17F, and IL-17 receptor A) in the treatment of psoriasis and psoriatic arthritis have been developed [8,9]

In addition to T helper 17 (Th17) cells, current data indicate other cellular sources of IL-17 such as mast cells, $\gamma\delta$ T cells, $\alpha\beta$ T cells, and innate lymphoid cells[10,11]. IL-17 targets different cells in psoriasis such as keratinocytes, endothelial cells, and innate immune cells. In keratinocytes it stimulates synthesis of antimicrobial peptides, proinflammatory cytokines, chemokines and proliferative cytokines [8]. IL-17 upregulates synthesis of IL-6, IL-8, and expression intracellular adhesion molecule-1 in endothelial cells with subsequent tissue inflammation and increase in procoagulant activity [12]. It also promotes the production of proinflammatory mediators by fibroblasts, epithelial and endothelial cells and has a proinflammatory effect on antigen presenting cells [13].

Polymorphisms affecting IL-17 family have been associated with increased risk of

inflammatory, infectious, autoimmune or neoplastic disorders [11]. Data that link certain alleles with demographic, clinical and therapeutic features of psoriasis is limited and further studies in larger populations are needed to detect the effect of these polymorphisms [11].

Aim of the study: We aimed to investigate the genetic polymorphism of IL-17F (rs763780) and evaluate the impact of this polymorphism on circulating levels of IL-17F as a potential risk locus for psoriasis.

Patients and Methods

This prospective case–control study was carried out from January 2019 to December 2019, and included 120 subjects divided into two groups; the first group included 60 patients suffering from chronic plaque psoriasis recruited from patients attending the Dermatology Outpatients Clinic in Benha University Hospitals; and the second group included 60 age and sex matched apparently healthy individuals as a control group.

The study was conducted after approval of the Ethics Committee of Research of Faculty of Medicine, Benha University, Egypt. An informed consent was obtained from each participant before enrollment in the study.

Inclusion and exclusion criteria

Patients from both sexes who were diagnosed clinically and confirmed by a grattage test as a psoriatic patient with more than 6 months of psoriasis duration and did not receive treatment for psoriasis in last month were included in this study.

Patients with unstable psoriasis (erythrodermic or pustular psoriasis), other skin diseases, autoimmune disease, pregnant or lactating females, hepatic, renal, hematological, gastrointestinal, with malignancy or received chemotherapy in the last 6 months were excluded from this study.

A complete medical history was taken from patients regarding the duration, onset, course, family history, and previous treatment of psoriasis. A complete dermatological examination was done to assess the distribution, site and severity of psoriasis. Psoriasis severity was assessed using psoriasis area severity index (PASI) [14].

Laboratory Procedures

Blood samples: 4 ml of venous blood were taken under a complete aseptic technique from all participants and divided into two parts. The first part collected in EDTA containing blood collection tube and was stored at -80 till used in DNA extraction and amplification. The second part of blood allowed to clot for 30 minutes before centrifugation for 15 minutes, then the obtained serum was collected and stored at -80°C for ELISA.

Measurement of IL-17F by ELISA

The interleukin 17F was measured in serum using a commercially available ELISA kit (Human Interleukin-17F, Catalog No: E1955h) according to the manufacturer's protocol. This immunoassay kit allows for the in vitro quantitative determination of human Interleukin-17F ("Sandwich" enzyme-linked immunosorbent assay).

Genotyping of IL-17F (rs763780): by Amplification Refractory Mutation System -PCR (ARMS-PCR) method (*Chaitanya et al., 2014* [15]).

Principle: ARMS-PCR is a method used for the analysis of single nucleotide polymorphisms (SNPs), based on allele specific amplification of a desired fragment using primers corresponding to each allelic variant that allow amplification of test DNA only when the target allele is contained within the sample.. ARMS-PCR amplified the 2 alleles in two different PCRs (each PCR tube contains four primers). The region flanking the mutation is amplified by a common (outer) primer, producing a non-allele-specific common amplicon. Two allele-specific (inner) primers are designed in opposite orientation and, in combination with the common primer, can simultaneously amplify both the wild-type and the mutant amplicons.

Extraction of genomic DNA: Genomic DNA was extracted from whole blood using a commercial kit (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions, the purified DNA was stored at -20°C until used in amplification step.

Amplification of IL-17F (rs763780) : Amplification was done using primers of human growth hormone (HGH) (control band) to ensure the success of PCR reaction and amplify a sequence of 426 bp, control primer (sense), 5'- CCT TCC AAC CAT TCCCTT A-3'; control primer (antisense), 5'-

TCACGG ATT TCT GTT GTG TTTC-3'. For IL-17F (rs763780) detection, two inner primers (allele specific primers); Allele T specific forward; 5'- GAT ATG CACCTC TTA CTG CAC TT-3'. Allele C specific forward 5'-GAT ATG CAC CTC TTA CTG CAC TC-3' and a common reverse 5' CACCAAGGCTGCTCTGTTTCTT3' was used.

Two PCR runs were performed independently for each sample where one contained T allele-specific forward primer and common reverse primer, and the other reaction tube contained C allele-specific forward primer and common reverse primer. The T-specific primer will amplify only if 'T' is present at 763780 location in IL-17F gene, and C-specific forward primer will amplify only if 'C' is present at 763780 location in IL-17F gene. As both these allele-specific forward primers have a common reverse primer so the size of the amplicon is same (106 bp).

The PCR reaction mixture contained 25 µl Maxima Hot Start PCR Master Mix (2X) (Thermo Scientific, EU Lithuania), Master Mix (2X) (Thermo Scientific, EU Lithuania), 15 µl of tested DNA, 0.5µM of each primer (Biosearch Technologies, USA) and water, nuclease free was added to a PCR mixture to give a final volume of 50 µl. All reagents were prior vortexed, and finally 25 µL of mineral oil were added to the reaction mixture. Water, nuclease-free, was used as a negative control in each PCR run

Thermal Cycler (Biometra, Germany) was used in running PCR cycles with the following conditions: 1 cycle of 94°C for 3 minutes (Initial denaturation step) followed by thirty repeated cycles of denaturing at 95 °C for 30 seconds, annealing at 60°C for 1 minute and extension at 72 °C for one minute, a final extension step was done at 72°C for 10 minutes then hold at 4 °C.

Ten µL of each amplified DNA & 50 bp ladder (molecular weight marker) (Fermentas, Germany) were separated by electrophoresis using 2% agarose

gel containing stained with ethidium bromide and visualized by UV. The IL-17F (rs763780) genotypes were assessed for the presence/absence of PCR amplicon (106 bp), corresponding to the specific allele. Quality assurance was confirmed through: Internal positive amplification control to amplify 426 bp for two outer primers (control band) in each PCR tube. Negative control reaction was confirmed by inserting a tube containing all components needed for amplification except DNA template in each amplification run.

Statistical Analysis

The data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 20. Qualitative data were expressed in numbers and percent; quantitative data were expressed in mean and standard deviation. In comparison, between the different groups, the significance of difference was tested using the student t test to compare between two groups of numerical (parametric) data, ANOVA (analysis of variance) used to compare between more than two groups of numerical (parametric) data, post-hoc test used to detect difference in-between groups. Inter-group comparison of categorical data was performed by using chi square test (X²-value), Odds Ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the risk. *P* value <0.05 was considered statistically significant.

Results

Study was conducted on 60 psoriasis patients and 60 healthy controls volunteers. Data of cases and control were summarized in Table 1

Table 1. Demographic data for psoriasis patients and controls

	Psoriasis group (n=60) N (%)	Control group (n=60) N (%)	<i>P</i> -value
Age	48.9±1.6	48.6±1.4	NS
Sex (M/F)	32/28	30/30	NS
Family history	21 (35%)	1 (1.7%)	<0.001
Disease duration (month)	8.3 ± 1.4		
PASI score	11.2		

PASI, psoriasis area severity index, *P*>0.05 is not significant (NS).

Regarding the genotype and allele frequencies (Fig 1 & Table 2), TT was 63.3% and 86.7%, TC was 23.3% and 11.6% and CC was 13.4% and 1.7% in psoriasis and control groups respectively, there were a significant difference in frequency between TT and TC genotypes (OR= 2.74, 95%CI=1.04 -7.4, $P=0.04$) and TT and CC genotypes (OR=10.9, 95%CI=1.3-91.3, $P=0.007$). Moreover, the TC and CC genotypes were associated with increased risk of psoriasis when compared with the TT genotype (OR= 3.8, 95% CI: 1.5– 9.4, $P=0.003$).

Regarding to allele frequencies, the frequency of both T and C alleles showed significant difference between psoriasis patient and control (75%, 25% and 92.5%, 7.5% respectively). The mutant C allele frequency was more common in psoriasis patients and was significantly associated with an increased risk of psoriasis compared to the major T allele (OR= 4.1, 95% CI: 1.9– 9.1, $P=0.0002$).

Serum level of L-17F was higher among psoriasis patients (25.7 ± 3.8 pg/ml) compared with healthy control subjects (15.1 ± 2.1 pg/ml) ($P<0.001$) (Table 3).

The consequence of the minor C allele of the IL-17F rs763780 was analyzed regarding to serum concentrations of IL-17F between different genotypes of psoriasis patients (table 4). The difference between the three genotypes was statistically significant ($P=0.002$). However, by post-hoc analysis there were statistically significant difference between TT and TC and between TT and CC only.

There was no significant difference between different genotype groups and clinical parameters of psoriasis patient, (age, sex, family history, Disease duration and PASI score) (Table 5).

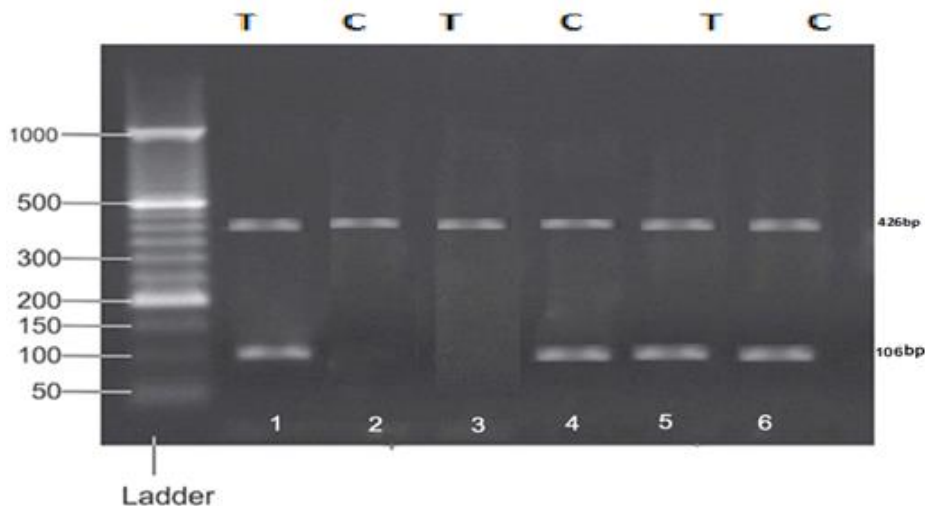


Figure 1. Gel electrophoresis of amplified ARMS-PCR products of IL-17F(rs763780); The amplification in T-specific PCR and no amplification in C-specific PCR indicate a TT homozygous genotype which is shown in lanes 1&2, amplification in only C specific PCR, and no amplification in T-specific PCR indicates a CC genotypes. The amplification in both the allele-specific PCR indicates a TC heterozygous genotype as shown in lanes 5 and 6.

Table 2. Genotypes and Alleles frequency distribution of IL17F(rs763780) in psoriasis patients and healthy controls

IL17F rs763780	Psoriasis (n =60) N (%)	Controls (n =60) N (%)	OR (95% CI)	*P-value
Genotype				
TT	38 (63.3)	52 (86.7)		
TC	14 (23.3)	7 (11.6)	2.74 (1.01–7.4)	0.04
CC	8 (13.4)	1(1.7)	10.9(1.3- 91.3)	0.007
TT	38(63.3)	52(86.7)		
TC+CC	22(36.7)	8 (23.3)	3.8 (1.5– 9.4)	0.003
Allele				
T	90 (75)	111 (92.5)	4.1 (1.9– 9.1)	0.0002
C	30 (25)	9 (7.5)		

OR: Odds ratio. 95% CI: 95% Confidence Interval. $P < 0.05$ is significant.

Table 3. Serum Concentrations of IL17F in Psoriasis patients and healthy Controls

Groups	IL17F Mean \pm SD (pg/mL)	P value
Psoriasis patients	25.7 \pm 3.8	<0.001*
Healthy Controls	15.1 \pm 2.1	

* $P < 0.05$ is significant.

Table 4. Serum concentrations of IL17F in different genotypes

IL17F (rs763780)	Serum IL17F Mean (pg/mL)	P value ⁺	Post-hoc test
TT	23.5 \pm 2.6	0.002	$P_1 < 0.05$
TC	25.9 \pm 3.8		$P_2 < 0.001$
CC	27.8 \pm 4.9		$P_3 > 0.05$

⁺ ANOVA test, $P < 0.05$ is significant; P_1 between TT and TC, P_2 between TT and CC, P_3 between TC and CC.

Table 5. Clinical parameters among different genotypes of Psoriasis patients

	TT (n=38)	TC (n=14)	CC (n=8)	P-value
Age	49.3 \pm 1.5	48.7 \pm 1.6	48.6 \pm 1.7	NS ⁺
Sex (M/F)	19(50%) / 19(50%)	8(57.1%) / 6(42.9%)	5 (62.5%) / 3(37.5%)	NS [£]
Family history	11 (56.3%)	6 (52.2%)	4 (52.9%)	NS [£]
Disease duration (month)	6.5 \pm 1.5	6.3 \pm 1.4	7.2 \pm 1.3	NS ⁺
PASI score	8.1 \pm 1.1	7.9 \pm 1.7	8.3 \pm 1.2	NS ⁺

⁺ ANOVA test, [£] Chi square test, $P > 0.05$ is not significant (NS).

Discussion

Genome-wide association studies (GWASs) identified common variants associated with different dermatologic diseases which allow understanding of the biologic pathways involved in pathogenesis and open the way for new drug targets. In psoriasis, about 50 genetic loci associated with risk of the disease have been identified [16]. IL-17 family polymorphisms have been linked with the severity and response to treatment in psoriasis, as well as with the risk of infectious, inflammatory, autoimmune and neoplastic disorders [17]. The effect of some gene polymorphisms on the increased risk of developing psoriasis and response to treatment is the focus of attention in several studies [18].

We investigated the IL-17F (rs763780) polymorphism in psoriasis patient and the serum level of IL-17F in comparison to healthy volunteers as a control. The presence of minor allele, C, in psoriasis patients was significantly higher than that in the control group suggesting a relationship between this polymorphism, with T>C substitution, and the increased susceptibility to the disease.

Limited number of studies on IL17F (rs763780) polymorphism in psoriasis have been published. Some studies reported an association between this polymorphism and psoriasis susceptibility. In agreement with our results, 2 studies conducted on Korean population confirmed the association between IL17F (rs763780) polymorphism, with presence of allele C, and psoriasis [19,20]. Significant association between this polymorphism and the response to treatment in Spanish and Italian population was reported [21,22]. An Indian study reported the association of IL-17F (rs763780) polymorphism but in contrary to our results,

the frequency of C allele decreased in psoriasis compared with the control group (36.5 vs. 45.7%) suggesting the protective role of C allele against psoriasis [23]. Other studies in Japanese, Spanish and Polish population found no significant differences in the frequency of IL17F genotypes between psoriasis patients and healthy volunteers [17,24,25].

We found that the serum level of IL-17F was not only significantly elevated in psoriasis patients but also varies significantly between different genotypes, suggesting an association between this polymorphism and the expression of IL-17F and pathogenic role that this cytokine might have in pathogenesis of psoriasis. Similar finding was reported in Asian study where IL-17F serum level was significantly higher in Psoriasis patients with mutant allele [26]. Many studies assess the level of IL-17 in psoriasis and all concluded significant elevation of IL-17 serum levels which were correlated with disease severity [27–29].

In agreement with previous studies, no significant difference between different genotype groups and psoriasis-related clinical parameters was detected in this study. This suggests insufficiency of this single nucleotide polymorphism to influence the clinical criteria and presentation of the disease [25,26].

A limitation in our study is the small number of included patients. Despite of our results and the limited published data, further comparative studies on different racial groups are required to confirm and understand the role of this IL-17F polymorphism in psoriasis.

In conclusion, IL17F polymorphism (rs763780) is associated with increased risk of psoriasis and may influence the level of production of IL-17F with subsequent

effects on the pathogenesis of psoriasis. Further studies on larger cohort are recommended to confirm the data and elucidate the molecular mechanisms associated with this polymorphism in different ethnic groups.

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