Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.05.006

# Interleukin-4 (C-589T) and Interleukin-17 (-197 G>A) Gene Polymorphisms in Psoriasis patients in Thi-Qar province south of Iraq

Salah Mahdi Muttar[1*], Hassan Risan Mubarek[2], Ahmed Abdulhussein kawin[3]
[1] General directorate of Thi-Qar education, Ministry of Education, Thi-Qar, Iraq.
[2] Department of biology, College of pure education, University of Thi-Qar, Thi-Qar, Iraq.
[3] Department of dermatology, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq.
***Corresponding author:** Salah Mahdi Muttar, General directorate of Thi-Qar education, Ministry of Education, Thi-Qar, Iraq, Email: Salahmahdy_mo21.bio@utq.edu.iq

**Submitted: 14 January 2023; Accepted: 13 February 2023; Published: 18 March 2023**

## ABSTRACT

Psoriasis is the common immune-mediated skin diseases which caused by the interaction between genetic and environmental risk factors. The present study aimed to determine the relationship between IL-17 (-197G>A) and IL-4 (C-589T) polymorphisms and susceptibility to psoriasis. This study included 100 patients and 50 healthy. 2-3 ml of Blood samples were collected in EDTA tube and preserved at -20 temperature for DNA extraction. IL-4 and IL-17 were amplified by Polymerase Chain Reaction (PCR) technique. In this study, 45% of psoriasis patients and 50% of healthy possessed GG genotype of IL-17 (-197 G>A) (wild genotype). 35% of patients and 30% of healthy have GA genotypes with non-significant differences compared to wild genotype (OR=0.77, 95% CI= 0.35-1.67). The same ratio of patients and healthy (20%) have homozygous AA genotype with non-significant differences compared to GG genotype (OR=0.90, 95% CI= 0.36-2.22). As for the IL-4, the CT and TT genotypes showed a higher frequency in the healthy group (36% and 20%, respectively) (OR=2.38, 95% CI= 1.08-5.24) compared to patients' group (22% and 14% for CT and TT, respectively (OR = 2.07, 95% CI= 0.80-5.34). The T allele frequency was 25% in psoriasis patients and 38% in the healthy group with significant differences (OR = 1.83, 95% CI= 1.09-3.07).

**Keywords:** *polymorphisms, Interleukin-4, Interleukin-17, psoriasis, PCR*

## INTRODUCTION

Psoriasis is a chronic autoimmune disease characterized by severe skin lesions caused by disturbed immune cells (Kamiya.et al., 2019). According to a National Psoriasis Foundation (NPF) report (2017), psoriasis is widespread and affects approximately 125 million people, making up 2-3% of the world's population (Springate.et al, 2017).

Psoriasis is a multifactorial disease caused by immune, genetic, and environmental factors (Carvalho and Hedrich, 2021). Common triggers for psoriasis include stress, mild trauma, chronic infections, sunburn, use of certain medications, smoking, obesity, and stress. Excessive alcohol consumption can also play a role in psoriasis (Huang, et al., 2019). Heritability is estimated at 66-90% among the highest rates among all multifactorial genetic diseases indicating the significant influence of genetic susceptibility (Oka et al., 2012; Lonnberg et al., 2013).

IL-17 acts directly on many immune and non-immune cells and promotes psoriasis skin properties (Lowes, 2014). Inflammation mediated by IL-17 cytokinesis is essential for host protection and survival against infection (Li et al., 2018). However, elevated IL-17 levels in different inflammatory conditions can lead to the development of inflammatory responses and may be involved in autoimmune pathology (Chamoun et al., 2018). IL-17-induced inflammation is usually controlled by regulatory T cells and Anti-inflammatory cytokines, for example, IL-4 is a multifunctional cytokine that can be used safely and effectively to correct imbalances in immune function without causing risks of severe immunosuppression (Mills, 2022). In addition to the ability of IL-4 to regulate immunity, it reduces inflammatory responses. It is considered an important cytokine for tissue repair as it counterbalances the effects of proinflammatory type 1 cytokines and inhibits IL-17 expression in T cells (Hahn and Ghoreschi, 2017). The present study aimed to investigate the relationship between IL-17 polymorphism (-197 G>A and IL-4 (C-589T) with psoriasis risk in Thi-Qar province.

## MATERIALS AND METHODS

### *Samples Collection*

150 samples of blood were collected from (100 patients and 50 healthy) in Al-Nahrain Laboratory for pathological analyzes in Thi-Qar province. 5ml of Blood were collected by EDTA tube at a temperature of (-20°C) for DNA extraction.

### *DNA Extraction*

DNA Extracted from each sample by Geneaid kit according to leaflet attached to Kit.

### *Polymerase Chain Reaction (PCR)*

The target region of IL-17 and IL-4 were amplified by PCR technique using specific primers (Table 1). The total reaction (20 µl) carried out by Green Master Mix according to leaflet attached to Kit (Table 2). The PCR program of each gene shown in Tables (3) and (4).

**TABLE 1:** the primer used in amplification of IL-4 and IL-17

| Gene | Primer sequences | Product size (bp) | References |
|---|---|---|---|
| IL-4 | F-5- CCAAGGGCTTCCTTATGGGT-3 R-5-GGGCCAATCAGCACCTCTCT-3 | 531 | This study |
| IL-17 | F 5- ACCTGGCCAAGGAATCTGTG-3 R 5-GCAAGAGCATCGCACGTTAG-3 | 526 | This study |

F : Forward R : Reverse bp : base pair

**TABLE 2:** green master mix components

| Chemicals | Volume |
|---|---|
| Master Mix | l µ5 |
| Primer Forward | lµ 1 |
| Primer Reverse | lµ 1 |
| DNA | µl 5 |
| D.W. | µl 8 |
| Total volume | µl 20 |

**TABLE 3:** PCR program of IL-4 gene

| No. of Stages | Steps | Temperature | Time | No. of Cycle |
|---|---|---|---|---|
| 1 | Inition denaturation | C °95 | 10 min | 1 |
| 2 | Denaturation | C °95 | sec. 35 | 30 |
| | Annealing | C °60 | sec. 35 | |
| | Extension | C °72 | sec. 35 | |
| 3 | Final extension | C °72 | 10 min | 1 |

**TABLE 4:** PCR program of IL-17 gene

| No. of Stages | Steps | Temperature | Time | No. of Cycle |
|---|---|---|---|---|
| 1 | Inition denaturation | C °94 | 10 min | 1 |
| 2 | Denaturation | C °94 | sec. 35 | 30 |
| | Annealing | C °62 | sec. 35 | |
| | Extension | C °72 | sec. 35 | |
| 3 | Final extension | C °72 | 10 min | 1 |

***Statistical Analysis***

Statistical analysis was carried out for all studied samples using SPSS program. Chi square test was used to found the significant differences between study groups at significant level P≤0.05. The odd ratio (OR) test was also used to study the frequency of of IL-17 and IL-4 genotypes.

***The Results***

The present study showed that the highest percentage of patients and healthy subjects possessed GG genotype of IL-17 (-197 G>A) (wild genotype) 45% and 50% for patients and healthy, respectively. 35% of patients and 30% of healthy have GA genotypes with non-significant differences compared to wild genotype (OR=0.77, 95% CI= 0.35-1.67). The same ratio of patients and healthy (20%) have homozygous AA genotype with non-significant differences compared to GG genotype (OR=0.90, 95% CI= 0.36-2.22).

The frequency of G allele was 62.5% and 65% in patients and healthy, respectively. However, the frequency of A allele was 37.5% and 35% in patients and healthy, respectively with non-significant differences compared to GG genotype (OR=0.89, 95% CI= 0.54-1.48). Table 5.

**TABLE 5:** IL-17(-197 G>A) genotype among patients and Healthy group

| Genotype | Patients (N=100) | Healthy (N=50) | OR | CI 95% |
|---|---|---|---|---|
| GG | 45 (45%) | 25 (50 %) | 1.00 | ——— |
| GA | 35 (35%) | 15 (30 %) | 0.77 | 1.67 - 0.35 |
| AA | 20 (20%) | 10 (20%) | 0.90 | 2.22 - 0.36 |
| Total | 100 ( 100% ) | 50 ( 100% ) | | |
| G | 125 (% 62.5) | 65 (% 65) | 1.0 | ——— |
| A | 75(% 37.5) | 35 (% 35) | 0.89 | 1.48 - 0.54 |
| Total | 200( %100 ) | 100( %100 ) | | |
| OR: Odd Rations 95% CI Confidence Interval | | | | |

J Popul Ther Clin Pharmacol Vol 30(5):e47–e53; 18 March 2023.

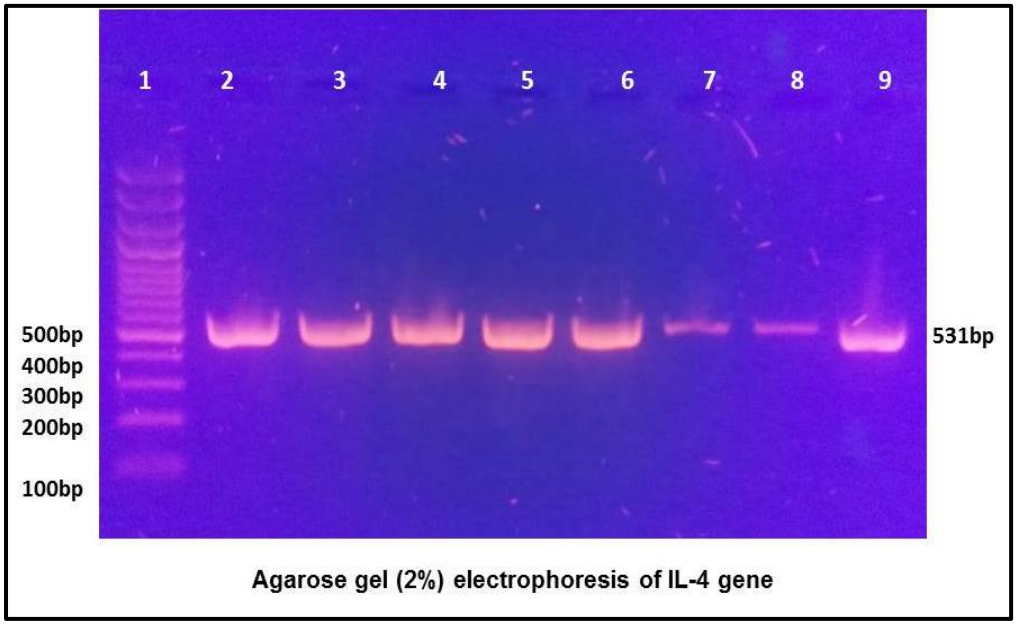
The current study showed that the percentage of patients who had the wild genotype (CC) was higher (64%) compared to healthy group who had the same genotype (44%). Out of 100 patients, 22% have CT genotype and 14% have TT genotype. As for healthy people, 36% have CT genotype and 20% have TT genotype with significant differences compared to wild genotype (OR=2.38, 95% CI= 1.08- 5.24) and (OR=2.07 , 95% CI= 0.80-5.34) for CT and TT, respectively.

The frequency distribution of the C and T alleles of the IL-4 mutant gene (C-589T) showed in table (6). The frequency of the C allele in the patient's group was 75% and in the healthy group was 62%. The T allele recorded a higher percentage in the healthy group (38%) compared to psoriasis patients (25%) (OR = 1.83, 95% CI= 1.09- 3.07).

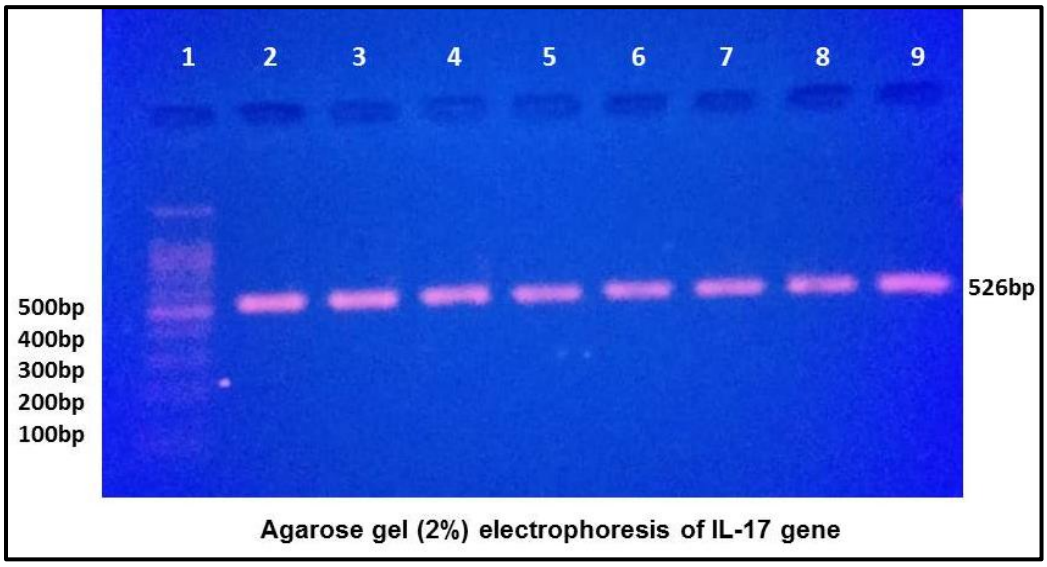
**TABLE 6:** IL-4 (C-589T) genotype among patients and control group

| Genotype | Patients (N=100) | Healthy (N=50) | OR | CI 95% |
|---|---|---|---|---|
| CC | 64(% 64) | 22(% 44) | 1.00 | ——— |
| CT | 22(% 22) | 18(% 36) | 2.38* | 5.24 - 1.08 |
| TT | 14(% 14) | 10(% 20) | 2.07* | 5.34 - 0.80 |
| Total | 100(% 100) | 50(%100) | | |
| C | 150(% 75) | 62(% 62) | 1.0 | ——— |
| T | 50(% 25) | 38(% 38) | 1.83* | 3.07 - 1.09 |
| Total | 200(%100) | 100(%100) | | |
| OR: Odd Rations 95% CI Confidence Interval | | | | |

PCR products were electrophoresed on an agarose gel with a concentration of 2%, and the results were visualized on a UV light transilluminator. The appearance of a band at the base pair 531 indicates amplification of IL-4 gene, while the appearance of a band at the base pair 526 indicates amplification of IL-17gene.




Agarose gel (2%) electrophoresis of IL-4 gene




## DISCUSSION

Psoriasis is a chronic immune-mediated disorder with cutaneous and systemic manifestations. Genetic predisposition, environmental factors, and immune imbalance contribute to the pathogenesis of psoriasis (Zhou and Yao, 2022). In last years, several genes that predispose an individual to psoriasis have been identified, many of which involve mediators of immune signaling pathways that underscore the importance of the immune system in the pathogenesis of psoriasis. These genes include those of inflammatory interleukins and immune mediators. In particular, a review of SNP analyzes from multiple studies showed an association between psoriasis and loci of the Th17 pathway, including IL-17, which is a major driver of human autoimmune diseases, particularly psoriasis, as well as subsets of IL-12, IL-23, and the Th2 pathway, including IL-4, IL-13 and adaptive immune pathways involving CD8 T cells (Kimmel and Lebwohl, 2018). In people who have a genetic predisposition, stimulation of these genetic sites leads to the development of psoriasis, which results in functional disorders in immune cells and an overproduction of their own cytokines (Coimbra et al., 2010; Kamiya et al., 2019). These cytokines activate keratinocytes, which in turn produce a variety of cytokines, chemokines, and antimicrobial peptides. This inflammatory cascade results in excessive proliferation of keratinocytes in the epidermis and in the lining of blood vessels, leading to hyperplastic epidermis and the development of psoriasis (Dopytalska, et al, 2021; Tashiro and Sawada, 2022).

In this study, the GA genotype IL-17 (-197 G>A) was a higher in the psoriasis patients compared to the healthy subjects. However, no statistically significant differences were observed (OR = 0.77). Also, there were no significant differences between GG and AA genotypes compared to wild genotype in psoriasis patients and healthy. The frequency of G allele was lower in patients compared to healthy, while the A allele was higher in patients with non-significant difference was observed. In a similar result, Białecka et al., (2016) did not find any significant differences in the genotypes of -197 G>A between patients and healthy subjects. Also, the study of Kim et al, 2017 did not show any association between the IL-17 polymorphism (-197 G>A) and psoriasis in the Korean population. In the study of Hamza et al., (2021), it was found that the G allele was 79% and 80.5% in the healthy and patients, respectively, while the A allele was 21% and 19.5% in the healthy and patients, respectively with non-significant difference between patients and healthy subjects (p. value=0.759). On the other hand, Mohamed et al., (2020) revealed an association of the IL-17(-G197A) polymorphism with several autoimmune and inflammatory diseases such as inflammatory bowel disease and psoriasis. Another study showed that the frequency of A allele was significantly higher in

psoriasis patients compared to healthy subjects (p. value = 0.001) (Sanad et al., 2022).

As for the IL-4 gene (C-589T) polymorphism, our study found that the increased frequency of the CT and TT genotypes and the T allele in the healthy group compared to psoriasis patients could indicate that the the CT and TT genotypes of IL-4 (C-589T) may play an important role in preventing psoriasis. This result is consistent with the study conducted in India, which indicated that there were high rates of the CT and TT genotypes in the healthy group compared to the patients. (P.value=0.001). The risk of developing psoriasis may be determined by the presence of the IL-4 gene and its genotypes CT and TT are associated with psoriasis. The association between IL-4 and disease reflects the effects of cytokinesis in the balance between Th1, Th17, and T-reg activity where linked Psoriasis with up-regulation of T-helper (Th)-1 and T-helper (Th)-17 cytokines and relatively down-regulation of T-helper (Th)-2 and T-Organizational (T-reg) cytokines (Indhumathi et.al., 2017). However, this study did not agree with the findings of Smolnikova et al., (2019) who found that CT and TT genotypes were higher in the patients than healthy. Also, a study of Craven et al. (2001) did not find any significant results in the frequency of the T allele in patients and healthy groups.

## CONCLUSIONS

This study showed no significant differences between psoriasis and genotypes of IL-17(-197 G>A). However, the relationship was found between psoriasis and genotypes of IL-4 (C-589T).

## REFERENCES


1. Białecka, M., Ostasz, R., Kurzawski, M., Klimowicz, A., Fabianczyk, H., Bojko, P., ... & Drozdzik, M. (2016). IL17A and IL17F gene polymorphism association with psoriasis risk and response to treatment in a polish population. Dermatology, 232(5), 592-596.
2. Carvalho, A. L., & Hedrich, C. M. (2021). The molecular pathophysiology of psoriatic arthritis the complex interplay between genetic predisposition, epigenetics factors, and the microbiome. Frontiers in molecular biosciences, 8, 662047.
3. Chamoun, M. N., Blumenthal, A., Sullivan, M. J., Schembri, M. A., & Ulett, G. C. (2018). Bacterial pathogenesis and interleukin-17: interconnecting mechanisms of immune regulation, host genetics, and microbial virulence that influence severity of infection. Critical reviews in microbiology, 44(4), 465-486.
4. Coimbra, S., Oliveira, H., Reis, F., Belo, L., Rocha, S., Quintanilha, A., & Santos-Silva, A. (2010). Interleukin (IL)-22, IL-17, IL-23, IL-8, vascular endothelial growth factor and tumour necrosis factor-α levels in patients with psoriasis before, during and after psoralen–ultraviolet A and narrowband ultraviolet B therapy. British Journal of Dermatology, 163(6), 1282-1290.
5. Craven, N. M., Jackson, C. W., Kirby, B., Perrey, C., Pravica, V., Hutchinson, I. V., & Griffiths, C. E. M. (2001). Cytokine gene polymorphisms in psoriasis. British Journal of Dermatology, 144(4), 849-853.
6. Dopytalska, K., Ciechanowicz, P., Wiszniewski, K., Szymańska, E., & Walecka, I. (2021). The role of epigenetic factors in psoriasis. International Journal of Molecular Sciences, 22(17), 9294.
7. Hahn, M., & Ghoreschi, K. (2017). The role of IL-4 in psoriasis. Expert Review of Clinical Immunology, 13(3), 171-173.
8. Hamza, A., Elwafa, R. A. A., & Omar, S. S. (2021). IL17A (rs2275913 G> A) and IL17F (rs2397084 T> C) Gene Polymorphisms: Relation to Psoriasis Risk and Response to Methotrexate.
9. Huang, T. H., Lin, C. F., Alalaiwe, A., Yang, S. C., & Fang, J. Y. (2019). Apoptotic or antiproliferative activity of natural products against keratinocytes for the treatment of psoriasis. International Journal of Molecular Sciences, 20(10), 2558.
10. Indhumathi, S., Rajappa, M., Chandrashekar, L., Ananthanarayanan, P. H., Thappa, D. M., & Negi, V. S. (2017). T helper-2 cytokine/regulatory T-cell gene polymorphisms and their relation with risk of psoriasis in a South Indian Tamil cohort. Human Immunology, 78(2), 209-215.
11. Kamiya, K., Kishimoto, M., Sugai, J., Komine, M., & Ohtsuki, M. (2019). Risk factors for the development of psoriasis. International Journal of Molecular Sciences, 20(18), 4347.
12. Kim, S. Y., Hur, M. S., Choi, B. G., Kim, M. J., Lee, Y. W., Choe, Y. B., & Ahn, K. J. (2017). A preliminary study of new single polymorphisms in the T helper type 17 pathway for psoriasis in the Korean population. Clinical & Experimental Immunology, 187(2), 251-258.
13. Kimmel, G. W., & Lebwohl, M. (2018). Psoriasis: overview and diagnosis. Evidence-

based psoriasis, 1-16.
14. Li, Y., Wei, C., Xu, H., Jia, J., Wei, Z., Guo, R., & Gao, X. (2018). The immunoregulation of Th17 in host against intracellular bacterial infection. Mediators of inflammation, 2018.
15. Lonnberg, A. S., Skov, L., Skytthe, A., Kyvik, K., Pedersen, O., & Thomsen, S. (2013). Heritability of psoriasis in a large twin sample. British Journal of Dermatology, 169(2), 412-416.
16. Lowes, M. (2014). Suarez-Fari nas M, Krueger JG. Immunology of Psoriasis. Annu Rev Immunol, 32, 227-255.
17. Mills, K. H. (2022). IL-17 and IL-17-producing cells in protection versus pathology. Nature Reviews Immunology, 1-17.
18. Mohamed, N. S., Siddig, E. E., Ahmed, A. E., Albsheer, M., Abdelbagi, H., Ali, E. T., ... & Omer, R. A. (2020). Frequency distribution of IL-17A G197A (rs2275913) and IL-17F A7488G (rs763780).
19. NPF (The National Psoriasis Foundation)(2017). Get the Facts about Psoriasis and Psoriatic Arthritis.
20. Oka, A., Mabuchi, T., Ozawa, A., & Inoko, H. (2012). Current understanding of human genetics and genetic analysis of psoriasis. The Journal of dermatology, 39(3), 231-241.
21. Sanad, E. M., Nazmy, N. N., Abd-El Hamid El Sayed, R., & Hamed, A. M. (2022). Interleukin-17A gene single nucleotide polymorphism and its relation to fungal growth in psoriatic patients: A preliminary study. Journal of Cosmetic Dermatology, 21(7), 3059-3067.
22. Smolnikova, M. V., Freidin, M. B., Barilo, A. A., & Smirnova, S. V. (2019). Analysis of association between cytokine gene polymorphisms and psoriatic disease in Russians of East Siberia.
23. Springate, D. A., Parisi, R., Kontopantelis, E., Reeves, D., Griffiths, C. E. M., & Ashcroft, D. M. (2017). Incidence, prevalence and mortality of patients with psoriasis: a UK population-based cohort study. British Journal of Dermatology, 176(3), 650-658.
24. Tashiro, T., & Sawada, Y. (2022). Psoriasis and Systemic Inflammatory Disorders. International Journal of Molecular Sciences, 23(8), 4457.
25. Zhou, S., & Yao, Z. (2022). Roles of infection in psoriasis. International Journal of Molecular Sciences, 23(13), 6955.