Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 115-123

Role Investigation of Interleukin- IL-21 *rs763780 T/C* Gene Polymorphism with Iraqi Rheumatoid Arthritis Patients

Ahmed H. Mahdi , Da'ad A. Hussain

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Received: 1/6/2022 Accepted: 21/8/2022 Published: December 20, 2022

Abstract: Rheumatoid Arthritis (RA) is a chronic inflammatory condition characterized by autoantibodies development and an elevated spectrum of pro-inflammatory cytokines. This study aims to find a relationship between interleukin -21 *rs682284* gene polymorphism and predisposition to rheumatoid arthritis development in a sample of Iraqi patients. In this study, there were 100 of subjects participated, about 50 of them rheumatoid arthritis (RA) so, represented patient group and the other 50 were apparently healthy who represented the control group. DNA was extracted, then the genotyping polymorphism (rs682284) of the gene Interleukin-21 was genotyped by TaqMan SNPs genotyping method. The genotyping and allele frequencies of IL-21 rs682284 for the two groups appeared that there were significant differences in genotype between patients and controls. The genotyping and allele frequencies of interleukin-21 *rs6822844* G/T for the two groups appeared that there were found to be negative significant relation in genotype between patients and controls. Compared GT genotype between control and patients, heterozygous GT genotype was no associated with significant from controls (X^2=0.782, OR=0.502); and the TT genotype was no associated significant risk for rheumatoid arthritis (X^2=0.821, OR=0.569). In addition, allele frequency for T allele that were no significant differences of allele frequencies of IL-21 rs682284 G/T gene polymorphism with the risk of rheumatoid arthritis in a sample of Iraqi. Moreover, association between the serum IL-21 level and IL-21 rs682284 G/T genotype, patients with RA significantly increased ($P<0.05$) compared to controls. The serum Anti- Cyclic Citrullinated Peptide Antibodies (ACCPA) level and IL-21 rs682284 G/T genotype, patients with RA significantly increased ($P<0.05$) compared to controls.

Keywords: Rheumatoid arthritis (RA), genetic polymorphism, ACCPA and IL-21.

Corresponding author: (Email: ahmed.hasan12009@ige.uobaghdad.edu.iq).

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that mostly affects the joints. It is the most common inflammatory joint condition, characterized by cartilage and bone erosion, resulting to functional decline and disability if left untreated. As a result, cartilage and joint degradation, as well as disability, ensue (1).

Rheumatoid arthritis affects roughly 5 people out of every 1000, and it can cause serious joint damage and disability. Arthralgia, edema, redness, and even a reduction in range of motion are all symptoms of symmetrical joint involvement (2). It affects women twice as much does men, and while it can strike at any age, moreover, it is most common in people over 50 (3). According to that, cytokines play an important role in the pathophysiology of RA, they play a role in the initiation and maintenance of inflammation, making them therapeutic targets (4).

The control of cytokines is imbalanced, resulting in low levels of inhibitory cytokines and increased

levels of pro-inflammatory cytokines, both of which contribute to the chronic inflammatory state. Cell-cell interaction or soluble mediators - cytokines - mediate and determine the systemic response to inflammation and cellular activation. Cytokines form large networks with both synergistic and antagonistic interactions, resulting in both negative and positive effects on target cells (5).

The prognosis of RA is also dependent on early detection and management. The medical history, clinical findings (including imaging modalities), and serological laboratory tests are the three pillars of rheumatological disease diagnosis (6).

Materials and methods

One hundred volunteers were taken in this study. Fifty with RA patients and fifty apparently healthy, who randomly selected between November 2021-February 2022 at the Rheumatology Unit of AL-Hindyia General Hospital in Karbala province.

A questionnaire has been taken from the patients, and the case sheet included age, gender, residence, height, weight, and previous history of the disease. Five ml of peripheral blood from all select subjects through vein puncture by using disposable plastic syringes.

Each blood sample was divided into two (2) ml was placed into EDTA tubes and the remaining three (3) ml pushed slowly into a gel tubes. The blood samples were placed in a cool - box under aseptic conditions and transfer to the laboratory. Serum CRP and RF measured by latex method. The serum ACCPA and IL-21levels were measured by ELISA technique. According to that manufactures instructions, total genomic DNA was isolated from 200µl of whole blood by using Addbio Genomic Kit (Korea). In brief, about 200 µL of whole blood samples were lysed with lysing solution with proteinase K at 56 °C for 10 min. The lysed cells were loaded in the DNA isolation column and centrifuged at 13,000 rpm g for 1 min. Subsequently, the column was washed twice with wash buffer. The membrane-bound DNA was eluted with elution buffer after centrifugation at 13,000 g for 1 min. The isolated genomic DNA was stored at − 20 degrees until further use. DNA concentration was estimated by using Nanodrop.

Genotyping of polymorphism (rs682284) of the *IL-21* gene was done, by using TaqMan SNP genotyping assays. A set of primers was used to amplify specific region within the IL-21 gene. The forward primer 5'-GGCATTTACAGTGGCAACA-3' and the Reverse primer 5'-GCTGGTGTATGCCCTGTCT-3". C Fam-Probe5'-AAGAGTCCTCTATTTTTGC-3'and A Hex-Probe 5'AAGAGTCCTCTCTTTTTGC-3'.

The thermal cycling program was as follows: Carryover prevention in 50 C° for 2 min, followed initial denaturation in 95 C° for 10 min, then 40 cycles of denaturation 95 C° for 30 second and annealing for 1 min (60 C°).

Statistical analysis

The statistical analysis system-SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio in this study.

Results and Discussion

Distribution of RA patients and control group according to anti-cyclic citrullinated peptide antibody (U/ml).

Distribution of rheumatoid arthritis patients and as well as apparently healthy control group was studied according to ACCPA (Table 1). The results showed there was a significant association between the two study groups according to ACCPA level (P<0.01).

Table (1): Distribution of rheumatoid arthritis patients and control group according to anti-cyclic citrullinated peptide antibody

Groups	Mean ± SE	T test	p-value
Patients	13.18 ±1.20	2.583 **	0.0001
Control	6.91 ±0.50		
* (P≤0.05), ** (P≤0.01).			

This result is agreed with (7), they found that anti-CCP is a better diagnostic tool than RF. Anti-citrullinated peptide antibodies (ACCPA) have a sensitivity of 40 to 80 percent, a specificity of 81 to 100 percent, and a good positive and negative predictive value for RA diagnosis (8)..Moreover, the result of this study is agreed with the study of (9) and (10) that reported that ACCPA was significantly higher in RA patients than control groups (P<0.01). However, the result of the present study is agreed with(11).

Anti-CCPA attends to stay fixed or slightly decrease with therapy, also not presence recurrently in another's inflammation or arthritic diseases (12). ACCP also found in many diseases such as psoriasis, idiopathic juvenile arthritis (IJA), and multiple sclerosis along with variation in sensitivity and specificity (13). The anti-CCPA level in serum about many years ago prior to the start of Rheumatoid arthritis act as a good indicator of pre-clinical onset, also can provide information about the initial onset of the sickness. There for it considered a good marker of diagnosis and prognostic of RA (14).

Distribution of rheumatoid arthritis patients and control group according to Interleukin-21 (Pg/ml). Distribution of rheumatoid arthritis patients and as well as apparently healthy control group was studied according to interleukin -21 (Table 2). The results showed there was a significant association between the two study groups according to IL-21 level (P<0.01).

Table (2): Distribution of rheumatoid arthritis patients and control group according to Interleukin-21

Groups	Mean ± SE	T test	p-value
Patients	20.22 ±1.83	3.789 **	0.0001
Control	11.30 ±0.53		
* (P≤0.05), ** (P≤0.01).			

IL- 21 is a type I cytokine produced by activated CD4+ T cells, including T helper Th1, Th2, natural killer T (NKT), Th17 and follicular Th cells (15). IL-21 plays a vital role in the regulation of both innate and adaptive immune systems (16).

Notably, IL- 21 controls the differentiation of Th17 cells, B cell activation, and immunoglobulins

production (17). The role of IL-21 in the pathogenesis of RA is poorly understood. Elevated levels of IL-21 has been demonstrated in the synovial tissue of RA patients (18).

In the present investigation, we observed a significantly elevated plasma IL-21 in Iraqi patients with RA compared to healthy controls. These results are agreed with previous reports ((19). Similarly, in a longitudinal study in patients with early-stage of rheumatoid arthritis, IL-21 level was upregulated in diseased subjects compared to controls (20).

In comparison with healthy control, RA patients have obviously elevated frequencies of circulating naive B cells, activated B cells, and Tfh cells and serum IL-21 levels (21). All of these findings, including our results, indicated the possible function of IL-21 in the advancement of RA pathogenesis.

Interleukin-21 has characterized it as an important molecule in the development and progression of RA, because it not only favors the proliferation and activation of immune cells and fibroblast-like synoviocytes but also promotes the humoral response (22).

Other autoimmune diseases, such as systemic lupus erythematosus and Sjögren's syndrome, and inflammatory pathologies have been associated with high levels of IL-21(23).

Genotype distribution and allele frequency of rs6822844 G>T in rheumatoid arthritis patients and control groups

The genotype and allele frequencies of the IL-21 rs682284 G>T for the two study groups (controls and patients) are shown in table (3). All genotype frequencies of the control group and patients group confirmed to the Hardy-Weinberg equilibrium (HWE).

Results from table (3) show that the genotype and allele frequencies of IL-21 rs682284 G>T for the two study groups appeared that there were no significant differences between rheumatoid arthritis patients and control group. Compared TT genotype between control and patients, homozygous TT genotype was not associated with increased risk for rheumatoid arthritis TT (X^2= 0.821, OR =0.569); and the heterozygous GT genotype was not significant differences between patients and controls (X^2= 0.782, OR =0.502).

The data of allele frequencies of point mutation on IL-21 rs682284 G>T gene polymorphism in two study groups (control and patients) are presented in table (3).

For the patients group the allele frequency of (G) 0.76 %, but (T) allele frequency 0.24 % according to Hardy-Weinberg equation, while for control groups the allele frequency of (G) was 0.84 %, but (T) allele 0.16 % according to Hardy-Weinberg equation Table (3) shows that were no significant differences of allele frequencies of IL-21 rs682284 G>T gene polymorphism with the risk of rheumatoid arthritis in a sample of Iraqi.

The previous results agreed with(24), They investigated there had been decreased relationships between RA risk and IL-21 rs6822844 polymorphism in both Asians and, Caucasians (T-allele ***versus*** G-allele) Similar results, Bayesian hierarchical meta- analysis analysis did not support the significant association of IL21 gene rs6822844 SNP with RA risk. Also these result agreed with (25), study that found the IL-21 (rs6822844 G>T, rs6840978 C>T) and IL-21R (rs2285452 G>A) gene polymorphisms are not risk loci for RA susceptibility, whereas the IL-21 rs2221903 polymorphism is associated with

disease activity. Probably the differences in IL-21 synthesis associated with this polymorphism or linkage with other gene polymorphisms may influence the disease activity in RA patients.

However, this hypothesis requires further investigation. The rs6822844 polymorphism was assessed by those three studies-one (26), reported a strong

Table (3): Genotype distribution and allele frequency of rs6822844 G>T in patients and control groups

Genotype rs682284 G>T	Patients No. (%)	Control No. (%)	Chi-Square (χ^2)	P-value	O.R
GG	35 (70.00%)	41(82.00%)	1.58 NS	0.394	Ref. =1
GT	6 (12.00%)	2 (4.00%)	0.782 NS	0.671	0.502
TT	9 (18.00%)	7(14.00%)	0.821 NS	0.603	0.569
Total	50 (100%)	50 (100%)			
Allele	Frequency				
G	0.76	0.84			
T	0.24	0.16			
* (P≤0.05), ** (P≤0.01), NS: Non- Significant.					

Association with RA and another one(27), reported no association (however, none of these studies specified the tested genotype. The third study (28), they demonstrated that the TT genotype of this polymorphism associated with decreased susceptibility to Rheumatoid arthritis.. In contrast**,**(29)**,** they found if individual carries the IL-*21* rs6822844 T-allele, he/she may have a lower risk to become RA patient than G-allele, in contrast, G-allele carries may have a higher risk to become RA.

Through detected this polymorphism, we may know the susceptibility of RA for one person in advance, which may be helpful or make sense in the future. However, results have been controversial (30). Detected a decrease in frequency of the rs6822844 T-allele in RA (14.1%), and demonstrated significant association between this polymorphism and RA susceptibility (OR = 0.72, 95% CI = 0.61–0.86, P < 0.001). Moreover, (31), showed a protective effect of the minor T-allele (OR = 0.39, 95% CI = 0.26–0.57), whereas the major G-allele appeared to be a risk susceptibility (OR = 2.57, 95% CI = 1.74–3.83, P < 0.001).

Among the different polymorphisms located in the IL2-IL21 region at 4q27, the rs6822844G/T polymorphism was found to be the most significantly associated with autoimmune disease susceptibility, including RA (32). To the best of our knowledge, rs6822844 is in a noncoding polymorphism located between IL21 gene (upstream) and IL2 (downstream) with no molecular function identified.

However, this polymorphism may play a role in autoimmunity by modulating the gene expression of these two genes or by being in linkage disequilibrium with a causative mutation. Interestingly, the neighboring sequences between up- and downstream for rs6822844 show strong homology with mature microRNA (33). MicroRNAs are post-transcriptional regulators that bind to complementary sequences in the 3′ UTR of target mRNAs, usually resulting in gene silencing inhibiting their translation (34).

The major allele G of the IL2-IL21 rs6822844 polymorphism is conserved in all microRNA precursor hairpin structures. Therefore, it is possible that the mutation might abolish microRNA production, altering the expression of the genes regulated by this microRNA (35).

Serum IL-21 Level and Its association with the IL-21 rs6822844 G>T genotype

Given the observed notable association between the IL-21 rs682284G>T genotype and rheumatoid arthritis risk, further investigation of the serum IL-21 levels in patients and control with Rheumatoid arthritis as well as the potential regulatory effects of the IL-21 rs682284G>T genotype and rheumatoid arthritis risk, further investigation of the serum IL-21 levels IL-21 rs682284G>T genotype and rheumatoid arthritis risk, further investigation of the serum IL-21 levels. Serum IL-21 level and its association with IL-21 rs682284G>T genotype presented in (Table 4).

The present study show the serum IL-21 levels were examined by ELISA in 50 rheumatoid arthritis patients and 50 apparently healthy control. The serum IL-21 of patients with RA significantly increased compared to controls ($P<0.01$). Results from table (4) show that the P-value of the association between serum IL-21 level and IL-21 rs682284G/T genotype ($P<0.01$). The T allele- carrying patients had a higher serum IL-21than the non-carries ($P<0.01$). z

The rs6822844 G/T polymorphism was discovered to be the most strongly related with autoimmune disease susceptibility, including RA, among the many polymorphisms detected in the IL2-IL21 area at 4q27. To the best of our knowledge, rs6822844 is an undiscovered molecular function in a noncoding polymorphism located between IL21 (upstream) and IL2 (downstream). However, This polymorphism might be involved in the development of autoimmunity by affecting the gene expression of these two genes or by being in linkage disequilibrium with a causative mutation (36). It's intriguing to observe that mature microRNA and the neighboring rs6822844 sequences share a lot of similarities (37) and (33).

Usually leading to gene silence and blocking their translation, microRNAs are post-transcriptional regulators that bind to complementary regions in the 3 UTR of target mRNAs (34). The dominant allele G of the IL21 rs6822844 polymorphism is preserved in all microRNA precursor hairpin structures. Because of this, it is likely that the mutation will cause microRNA production to cease, altering the expression of the genes that this microRNA controls(24).

Serum ACCPA level and Its association with the IL-21 rs682284 G/T genotype.

The distribution of patients (Rheumatoid Arthritis) as well as apparently healthy control was studied ACCPA and its association with the IL-21 rs682284 G/T Genotype (Table 5). The results showed that there was significant association between ACCPA level with -21 rs682284 G/T Genotype.

Results from table (5) show that the P-value of the genotype of IL-21 rs682284 G/T Genotype gene polymorphisms in the two study groups patients and control has mean differences between GG, GT, TT genotype with anti-ccp level, P-value for association GG genotype with ACCPA level in two study groups it was (0.047), GT (0.0001), TT (0.0093).

So there is a significant difference of IL-21 rs682284 G/T Genotype with ACCPA level.

According to what was mentioned previously, the relationship between the level of interleukin-21 and the IL-21 rs682284 G/T genotype is a positive relationship, and as it was also noted that the relationship between this level of interleukin 21 and ACCP is also a positive relationship, so it is likely or certain that the relationship between the ACCP and IL-21 rs682284 gene polymorphisms is also a positive relationship.

Table (5): Serum ACCPA level and its association with the IL-21 rs682284 G/T Genotype

Genotype rs682284 G/T	ACCP		P-value
	Patients	Control	
GG	12.68 ±1.41	7.06 ±0.76	0.047 *
GT	17.55 ±4.59	6.31 ±0.74	0.0001 **
TT	12.39 ±2.34	6.91 ±0.69	0.0093 **
* (P≤0.05), ** (P≤0.01).			

Conclusion

The ACCPA level and IL-21 level results revealed a significant differences (P<0.01) association between the two study groups in ACCPA level, the mean ± SE serum levels of ACCPA in rheumatoid arthritis patients were significantly higher as compared to apparently healthy control (13.18 ±1.20 versus 6.91 ±0.50 U/ml), while in IL-21 level, the mean ± SE serum levels of IL-21 in rheumatoid arthritis patients were significantly higher as compared to apparently healthy control (20.22 ±1.83 versus 11.30 ±0.53).

The genotyping and allele frequencies of interleukin-21 rs6822844 G/T for the two groups appeared that there were found to be negative significant relation in genotype between patients and controls.

Compared GT genotype between control and patients, heterozygous GT genotype was no associated with significant from controls (X^2=0.782, OR=0.502); and the TT genotype was no associated significant risk for RA (X^2=0.821, OR=0.569). In addition,

allele frequency for T allele that were no significant differences of allele frequencies of IL-21 rs682284 G/T gene polymorphism with the risk of rheumatoid arthritis in a sample of Iraqi. Moreover, association between the serum IL-21 level and IL-21 rs682284 G/T genotype, patients with RA significantly increased (P<0.05) compared to controls. The serum ACCPA level and IL-21 rs682284 G/T genotype, patients with RA significantly increased (P<0.05) compared to controls.

The conclusion obtained from this study that serum ACCPA determination was with important value for diagnosis of rheumatoid arthritis, and increase IL-21 play an important role in the development risk of RA.

References

1. Jebur, M. M.; Al-qaisi, A. H. J. and Harbi, N. S. (2022). Evaluation Serum Chemerin and Visfatin Levels with Rheumatoid Arthritis: Possible Diagnostic Biomarkers. International Journal Current Research Review 14(02): 42.
2. Guo, Q.; Wang, Y.; Xu, D.; Nossent, J.; Pavlos, N. J. and Xu, J. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Research, 6(1): 1–14.
3. van der Woude, D. and van der Helm-van, A. H. M. (2018). Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Practice and Research Clinical Rheumatology, 32(2): 174–187.

4. Noack, M. and Miossec, P. (2017). Selected cytokine pathways in rheumatoid arthritis. Seminars in Immunopathology, 39(4): 365-383.
5. Mackey, K.; Ayers, C. K.; Kondo, K. K.; Saha, S.; Advani, S. M.; Young, S., *et al*. (2021). Racial and ethnic disparities in COVID-19–related infections, hospitalizations, and deaths: a systematic review. Annals of Internal Medicine, 174(3): 362–373.
6. Alm, L. M.; Fountain, D. L.; Cadwell, K. K.; Madrigal, A. M.; Gallo, G. and Poorafshar, M. (2018). The performance of anti-cyclic citrullinated peptide assays in diagnosing rheumatoid arthritis: a systematic review and meta-analysis. Clinical Experimental Rheumatology, 36: 144-152.
7. Van Venrooij, W. J.; Van Beers, J. J. B. C.; and Pruijn, G. J. M. (2008). Anti- CCP antibody, a marker for the early detection of rheumatoid arthritis. Annals of the New York Academy of Sciences, 1143(1): 268–285.
8. Hinks, A.; Marion, M. C.; Cobb, J.; Comeau, M. E.; Sudman, M.; Ainsworth, H. C., *et al*. (2018). Brief Report: The Genetic Profile of Rheumatoid Factor–Positive Polyarticular Juvenile Idiopathic Arthritis Resembles That of Adult Rheumatoid Arthritis. Arthritis and Rheumatology, 70(6): 957-962.
9. Eker, Y. Ö.; Pamuk, Ö. N.; Pamuk, G. E.; Dönmez, S. and Çakır, N. (2014). The Frequency of anti-CCP antibodies in patients with rheumatoid arthritis and psoriatic arthritis and their relationship with clinical features and parameters of angiogenesis: A comparative study. European Journal of Rheumatology, 1(2): 67.
10. Alta'ee, A. H. and Alrubiae, S. (2017). Serum Interleukin-6 and Gene Polymorphisms in Rheumatoid Arthritis Patients in Babylon Province, Iraq. International Journal of ChemTech Research, 10(2): 662-669.
11. Beyazal, M. S.; Devrimsel, G.; Cüre, M. C.; Türkyılmaz, A. K.; Çapkın, E. and Karkucak, M. (2017). Serum Levels of IL-17, IL-6, TNF-α and disease activity in patients with rheumatoid arthritis. Aktuelle Rheumatologie, 42(04): 323–328.
12. Weisman, M. H.; Moreland, L. W.; Furst, D. E.; Weinblatt, M. E.; Keystone, E. C.; Paulus, H.E., *et al*. (2003). Efficacy, pharmacokinetic, and safety assessment of adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, in adults with rheumatoid arthritis receiving concomitant methotr-exate: a pilot study. Clinical Therapeutics, *25*(6): 1700–1721.
13. Masson-Bessière, C.; Sebbag, M.; Girbal-Neuhauser, E.; Nogueira, L.; Vincent, C.; Senshu, T., *et al*. (2001). The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the α-and β-chains of fibrin. The Journal of Immunology, 166(6): 4177–4184.
14. Mimori, T. (2005). Clinical significance of anti-CCP antibodies in rheumatoid arthritis. Internal Medicine, 44(11): 1122–1126.
15. Asao, H.; Okuyama, C.; Kumaki, S.; Ishii, N.; Tsuchiya, S.; Foster, D., *et al*. (2001). Cutting edge: the common γ-chain is an indispensable subunit of the IL-21 receptor complex. The Journal of Immunology, 167(1): 1–5.
16. John, S. Y.; Cox, M. A. and Zajac, A. J. (2010). Interleukin-21: a multifunctional regulator of immunity to infections. Microbes and Infection, 12(14–15): 1111–1119.
17. Sarra, M. and Pallone, F. (2009). Interleukin-21 in T cell-mediated diseases. Discovery Medicine, 8(42): 113–117.
18. Kwok, S.; Cho, M.; Park, M.; Oh, H.; Park, J.; Her, Y., *et al*. (2012). Interleukin- 21 promotes osteoclastogenesis in humans with rheumatoid arthritis and in mice with collagen- induced arthritis. Arthritis and Rheumatism, 64(3): 740–751.
19. Xing, Rui, Sun, L.; Wu, D.; Jin, Y.; Li, C.; Liu, X. and Zhao, J. (2018). Autoantibodies against interleukin-21 correlate with disease activity in patients with rheumatoid arthritis. Clinical Rheumatology, *37*(1): 75–80.
20. Agonia, I.; Couras, J.; Cunha, A.; Andrade, A. J.; Macedo, J. and Sousa-Pinto, B. (2020). IL-17, IL-21 and IL-22 polymorphisms in rheumatoid arthritis: *A systematic review and meta-analysis.* Cytokine, 125: 154813.
21. Long, D.; Chen, Y.; Wu, H.; Zhao, M. and Lu, Q. (2019). Clinical significance and immunobiology of IL-21 in autoimmunity. Journal of Autoimmunity, *99*: 1–14.
22. Dinesh, P. and Rasool, M. (2018). Multifaceted role of IL- 21 in rheumatoid arthritis: Current understanding and future perspectives. Journal of Cellular Physiology, 233(5): 3918–3928.
23. Wang, H.X.; Chu, S.; Li, J.; Lai, W.N.; Wang, H.X.; Wu, X.J., *et al*. (2014). Increased IL-17 and IL-21 producing TCRαβ+ CD4− CD8− T cells in Chinese

systemic lupus erythematosus patients. Lupus, *23*(7): 643–654.
24. Ren, K.; Tang, J.; Nong, L.; Shen, N. and Li, X. (2019). Association between interleukin-21 gene rs6822844 polymorphism and rheumatoid arthritis susceptibility. Bioscience Reports, 39(8): BSR20190110.
25. Malinowski, D.; Paradowska-Gorycka, A.; Safranow, K. and Pawlik, A. (2017). Interleukin-21 gene polymorphism rs2221903 is associated with disease activity in patients with rheumatoid arthritis. Archives of Medical Science, 13(5): 1142–1147.
26. Maiti, A. K.; Kim- Howard, X.; Viswanathan, P.; Guillén, L.; Rojas- Villarraga, A.; Deshmukh, H., *et al*. (2010). Confirmation of an association between rs6822844 at the Il2–Il21 region and multiple autoimmune diseases: evidence of a general susceptibility locus. Arthritis and Rheumatism: *Official* Journal of the American College of Rheumatology, 62(2): 323-329.
27. Daha, N.A.; Kurreeman, F.A.S.; Marques, R.B.; Stoeken- Rijsbergen, G.; Verduijn, W.; Huizinga, T.W.J., *et al*. (2009). Confirmation of STAT4, IL2/IL21, and CTLA4 polymorphisms in rheumatoid arthritis. Arthritis & Rheumatism, 60(5): 1255–1260.
28. Teixeira, V. H.; Pierlot, C.; Migliorini, P.; Balsa, A.; Westhovens, R.; Barrera, P., *et al*. (2009). Testing for the association of the KIAA1109/Tenr/IL2/IL21gene region with rheumatoid arthritis in a European family-based study. Arthritis Research and Therapy, 11(2): 1–7.
29. Yu, M.; Hou, J.; Zheng, M., Cao, Y.; Alike, Y.; Mi, Y., *et al* J. (2020). IL-21 gene rs6822844 polymorphism and rheumatoid arthritis susceptibility. *Bioscience Reports*, 40(1).
30. Zhernakova, A.; Alizadeh, B. Z.; Bevova, M.; van Leeuwen, M. A.; Coenen, M. J.; Franke, B., *et al*. (2007). Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. The American Journal of Human Genetics, *81*(6): 1284-1288.
31. Louahchi, S.; Allam, I.; Raaf, N.; Berkani, L.; Boucharef, A.; Abdessemed, A., *et al*. (2016). Association of rs6822844 within the KIAA1109/TENR/IL2/IL21 locus with rheumatoid arthritis in the Algerian population. Hla, 87(3): 160–164.
32. Van Heel, D. A.; Franke, L.; Hunt, K. A.; Gwilliam, R.; Zhernakova, A.; Inouye, M., *et al*. (2007). A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nature Genetics, 39(7): 827–829.
33. Cai, X.; Lu, S.; Zhang, Z.; Gonzalez, C. M.; Damania, B. and Cullen, B. R. (2005). Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proceedings of the National Academy of Sciences, 102(15): 5570–5575.
34. Davidson- Moncada, J.; Papavasiliou, F. N. and Tam, W. (2010). MicroRNAs of the immune system: roles in inflammation and cancer. Annals of the New York Academy of Sciences, 1183(1): 183-194.
35. Rodríguez-Rodríguez, L.; Castañeda, S.; Vázquez-Rodríguez, T. R.; Morado, I. C.; Gómez-Vaquero, C.; Marí-Alfonso, B., *et al*. (2011). Role of the rs6822844 gene polymorphism at the IL2-IL21 region in biopsy-proven giant cell arteritis. Clinical and Experimental Rheumatology-Incl Supplements, 29(1): S12.
36. Festen, E.A.; Goyette, P.; Scott, R.; Annese, V.; Zhernakova, A.; Lian, J., *et al*. (2009) Genetic variants in the region harbouring IL2/IL21 associated with ulcerative colitis. Gut. 58: 799-804.
37. Houbaviy, H. B.; Murray, M. F. and Sharp, P. A. (2003). Embryonic stem cell-specific MicroRNAs. Developmental Cell, 5(2): 351-358.
38. Rasheed, M. N., Hasan, O. M., & Mahmood, A. S. (2015). Association of glutathione S-transferase (GSTM1, T1) gene polymorphisms with type 2 diabetes mellitus (T2DM) in the Iraqi patients. Iraqi Journal of Biotechnology, 14(1).