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Original Article

Association of Interleukin-6 gene polymorphisms with etanercept response in Iraqi patients with rheumatoid arthritis

Dhulfiqar Nidhal Alhilali[1]*, Samer Imad Mohammed[1], Faiq Isho Gorial[2]

[1]Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq. [2]College of Medicine, University of Baghdad, Baghdad, Iraq.

Correspondence: Dhulfiqar Nidhal Alhilali, Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq. thulfekarnkazam@copharm.uobaghdad.edu.iq

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ABSTRACT

The present study aimed to examine the role of Interleukin-6 (IL-6) promoter polymorphisms in the response to Etanercept (ETN) treatment in a group (Cohort) of Iraqi RA patients. Ninety active RA patients were chosen and studied at baseline, three to six months post-ETN therapy. The experiment was carried out in the Rheumatology unit at Baghdad Teaching Hospital in Medical City, Baghdad/Iraq. Based on the Disease Activity Score 28-Erythrocyte Sedimentation Rate (DAS28-ESR), the patients were categorized into responders and non-responders. The Results revealed that at baseline, responders were significantly younger than non-responders ($P=0.0025$). For the responders group; there were a significant enhancement in DAS28-ESR components ($P<0.001$), in all disease activity parameters included Patient and Provider Global Assessment (PtGA) and (PrGA), Tender Joint Count (TJC), Swollen Joint Count (SJC), Clinical Disease Activity Index (CDAI), DAS28-ESR post treatment, while low or negative reduction recorded for non-responders. In addition, the genetic tests disclosed a significant correlation between IL-6 rs1800795 genotypes and treatment response; the C allele of non-responders was more prominent (28.9% vs. 12.5%, $P=0.0059$), and GG carriers achieved a significantly greater reduction in DAS28-ESR after six months compared to GC and CC carriers ($P<0.05$). No significant linkage was detected for the rs1800796 or rs1800797 polymorphisms. In summary, the IL-6 rs1800795 C allele is associated with increased disease activity and nonresponsiveness to ETN in Iraqi RA patients, whereas the G allele predicts a favorable response. This polymorphism represents a potential biomarker for personalizing anti-TNF therapy.

Keywords: Polymorphism, IL-6, Etanercept, Anti-TNF, Rheumatoid arthritis

Introduction

Rheumatoid arthritis is one of the long-lasting heterogeneous inflammatory auto-immune disorders characterized by continuous synovitis accompanied by stiffness, pain, joint swelling, and destruction with a significant disability in the joints [1, 2]. It impacts approximately 17.6 million of the global population [3] with a higher prevalence among women and elderly or comorbid conditions [4-7].

The pathogenesis of RA involves immune cells stimulation, autoantibodies production, and cytokine release, including both IL-6 and tumor necrosis factor-alpha (TNF-α) [8, 9].

Interleukin-6 exerts a key role in RA pathophysiology; It starts synovitis and systemic inflammation, osteoclastogenesis, and subsequent elevation of acute-phase mediators such as CRP [10, 11].

Rheumatoid arthritis susceptibility and disease activity are diagnosed via different complementary examination approaches, including clinical and laboratory. Tests by measuring ESR, non-specific inflammatory markers like complete blood count (CBC), C-reactive protein (CRP), and specific RA biomarker, including antinuclear antibody (ANA) and anti-citrullinated protein antibodies (ACPA) assay [12, 13].

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Anti-TNF inhibitor, including ETN, is part of biologic disease-modifying antirheumatic drugs (bDMARDs), manufactured and widely used to manage and treat RA [14]. Etanercept is a soluble receptor fusion protein that binds TNF-α and lymphotoxin, thereby diminishing inflammatory responses, reducing disease activity, and improving RA symptoms [15-17]; however, 30-40% exhibit an inadequate response and are classified as non-responders [18, 19].

According to the clinical studies, ETN decreases TNF-α, IL-6 levels, and DAS28-ESR score. Within several weeks of establishing treatment, progress further through the subsequent weeks [20-23].

In this disease, the genetic polymorphisms within the promoter of TNF-α and interleukins such as IL-6 and IL-1 genes are hypothesized to affect predisposition to RA and other auto-immune diseases and also influence patient response to anti-TNF biologics [24-30].

The genetic analysis identified several Nucleotide Polymorphisms (SNPs); IL-6 rs1800795 has been linked to increased IL-6 production and RA susceptibility [31, 32]. Other promoter variants, such as rs1800796 and rs1800797, have also been implicated in the response of RA patients to anti-TNF agents [33]. The genetic variations in IL-6 could modulate response to TNF inhibitors like ETN [34].

Given the above implications, the goal of this study is to examine the possible association between IL-6 promoter SNPs and response to ETN in Iraqi RA patients, comparing genetic profiles between responders and non-responders to improve clinical outcomes in RA.

Materials and Methods

Study population and design

A cohort (n=90) of RA patients (82 females and eight males, mean age 51.34 ± 10.51 years) was recruited from the Clinic of Rheumatology-Baghdad Teaching Hospital in Medical City, Baghdad, Iraq. The experiment extended from March 2024 to March 2025. All patients were diagnosed depending on the RA-ACR/EULAR classification criteria (2010). The study was approved by the ethical committee of the College of Pharmacy/University of Baghdad, Registration number (RECAUBCP123202406150H). Following treatment with ETN (Altebrel®; AryoGen Pharmed, Iran), study participants were categorized as either responders or non-responders based on ACR/EULAR criteria (2010).

Inclusion criteria

All RA patients were Iraqi adults (Over 18 years), the severity of RA ranged from moderate to severe active (DAS28-ESR > 3.2), who had been receiving a consistent regimen of 50 mg of ETN weekly for at least six months.

Exclusion criteria

Patients who lost follow-up or were unable to comply with the study duration, Patients with missed doses (by measuring adherence through the medication possession ratio (MPR) of 85%), and patients with missing data.

Treatment protocol and response assessment

Altebrel® was administered subcutaneously to the RA patient (50 mg/mL) weekly for six months. Treatment response was measured at different stages, including baseline, three to six months post-induction of treatment.

Depending on the response criteria of EULAR, patients were subdivided into two groups: Responders: Patients who achieved a reduction in DAS28 to < 5.1 with a change (ΔDAS28) of > 0.6. The non-responders were defined as patients who failed to achieve the above criteria (DAS28 remained ≥ 5.1 with ΔDAS28 < 0.6).

Sample collection

Peripheral blood samples (10 mL) were collected from each patient (6 mL) used for CBC and ESR assessments, and the other (4 mL) was collected in K3 EDTA tubes for DNA extraction.

Laboratory analyses

Genotyping of IL-6 promoter polymorphisms

G-spin™ Total DNA Extraction Kit (Promega/USA) was used for genomic DNA extraction according to the manufacturer's protocol. The target promoter region was amplified by PCR (Thermal Cycler, BioRad, USA) using four specific primers , which were designed by the primer BLAST option in NCBI. The 20 µL PCR reaction mixture contained 1 µL of each primer (10 pmol/µL), 2 µL of DNA template, 10 µL of PCR Master Mix, and 6 µL of nuclease-free water. The primer used were:

IL-6-F1:CCTCTAAGTGGGCTGAAGCA,

IL-6-R1:ACTCATGGGAAAATCCCACA,

IL-6-F2: GTAAAACGACGGCCAGTCAGTGAAACAGTGGTGAAGA,

IL-6-R2:CAGGAAACAGCTATGACCTTGTGGAGAAGGAGTTCATAG

The PCR cycling reaction conditions were used according to Qin *et al*. [35]. Subsequently, PCR products were sent for Sanger sequencing (Macrogen, Korea) for definitive genotype identification.

Measurement of erythrocyte sedimentation rate (ESR)

ESR was determined using the modified Westergren method.

Statistical analysis

Genotype and allele frequencies will be compared between responders and non-responders using Chi-square (χ^2) or Fisher's exact tests. Mean ± standard deviation was compared using a t-test and LSD depending on data distribution. The association between genotypes and treatment response was evaluated using logistic regression, adjusted for potential confounding variables. Paired t-tests and Welch's t-test for unequal variances were used to analyze the differences within and between groups, respectively, at different time points. Hardy–Weinberg expectations were used to ensure the random distribution of. The 95 % confidence intervals (CI) and Odds ratios (OR) were utilized to calculate the minor allele. Welch's ANOVA (or Welch t-test for two-group comparisons) was used; otherwise, one-way ANOVA was also applied.

Results and Discussion

Baseline characteristics values in responders and non-responder RA patients

The baseline features of all patients, before the onset of ETN injection, are summarized in **Table 1**. A significantly higher mean age (P=0.0025) was observed among non-responders (54.8 ± 5.3 years) compared with responders (48.8 ± 12.5 years), suggesting that younger patients were more likely to respond to ETN treatment. In addition, no significant variances were noted between the same groups regarding parameters: Age of disease onset (P=0.18), disease duration (P=0.0986), or baseline disease activity (P=0.94).

Table 1. Baseline Traits of Rheumatoid Arthritis Patients Receiving Etanercept According to Treatment Response

Parameters	Responders N=52	Non- Responders N=38	P-value
Age (year)	48.79 ± 12.53	54.84 ± 5.25	**0.0025 ****
Age of onset (year)	35.29 ± 10.07	38.11 ± 9.64	0.1828
Sex (female/total)	46 / 52 (88.5%)	36 / 38 (94.7%)	0.4594
Disease duration	13.50 ± 8.58	16.74 ± 9.41	0.0986 †
disease activity (High/total)	27/52 (51.9%)	20/38 (52.6%)	0.94

†p < 0.1, *P<0.05, **P<0.01

The current results recorded that more than 94% of the non-responders group was female vs. 85% for the responders' group, without a significant difference.

The significant correlation between non-responders and responders regarding age suggests that this demographic factor substantially influences good and poor responses to ETN. This outcome was consistent with the Bordean *et al*. [36] study, which showed an association between some parameters, including age and primary and secondary non-response status to ETN therapy. The same finding was reported by Khafagi *et al*. [37], who concluded that increasing age and earlier age at onset at baseline were predictors of failure to respond to TNF inhibitor agents. Aghdashi *et al*. [38] found age to be a poor predictor of anti-TNF treatment response, whereas Lauridsen *et al*. [39] found no significant association at 12-month follow-up. However, their findings indicated that patient over 50 years was associated with a higher risk of treatment discontinuation, particularly among patients under 50 years of age.

Although there was a difference in sex distribution between the responders and non-responders' groups, with a higher proportion of female patients in the non-responders than responders, the lack of statistical significance suggests that sex-specific factors didn't predict response. Same results were observed by Jassim [40], and Lauridsen *et al*. [39] reported that gender did not predict response to anti-TNF therapy. In contrast to the present findings, the studies by Carmona *et al*. [41] indicate that sex differences can influence both RA and treatment outcomes. Lauridsen *et al*. [42, 43] postulated that females had a lower probability than males of achieving a better therapeutic response at 4 and 12 months after initiating Anti-TNF therapy. The gender variance includes hormonal impact on immune response and reactivity, differences in drug metabolism, or differences in the pattern of pain perception that may confound the assessment of treatment response [44, 45]. This lack of significance in the present study is likely due to the wide confidence intervals and high standard error observed between the cohorts.

The other parameters, age at onset, disease duration, and disease activity, showed no significant effect on treatment response (P>0.05). These observations were consistent with Yoo *et al*. [46], who didn't report any significant variation in the baseline traits among both groups before inducing therapy.

Changes in clinical and laboratory parameters after ETN treatment

(**Table 2)** summarizes the temporal evolution of clinical and laboratory parameters after initiation of ETN treatment in RA patients.

Initially, no significant variances were detected between the identified groups in terms of SJ, ESR, CDAI, or DAS28-ESR, confirming pre-treatment comparability. Only the mean TJ in non-responders was significantly higher than that in non-responders (P = 0.007).

After 3 months, responders showed a statistically significant reduction in swollen and tender joint counts, ESR, CDAI, PtGA, PrGA, and DAS28-ESR (all P<0.001), whereas non-responders showed no or only slight improvement.

Improvement among responders persisted and deepened at 6 months, with the mean ΔDAS28-ESR decreasing from 5.16 ± 0.80 to 3.43 ± 0.76 (P<0.001), while non-responders showed stable or higher disease activity scores (5.14 ± 1.04).

The mean ΔDAS28-ESR decline was also significantly greater in responders at 3 and 6 months (1.73 ± 0.83 vs. -0.35 ± 0.65, P < 0.001).

Table 2. Longitudinal Changes in Clinical and Laboratory Parameters of Rheumatoid Arthritis Patients Before and After Etanercept Treatment According to Response Status

Parameters	Responders N=52			Non- Responders N=38			Within-group p-Value	Between groups p-Value
	Pre-treatment	3 months post-treatment	6 months post-treatment	Pre-treatment	3 months post-treatment	6 months post-treatment		
Number of SJ	3.35±2.50	1.65±2.07 a	0.60±0.80 a,b	3.21±3.01	2.71±3.07	4.18±4.09 b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.111, 6m=0.120	Pre=0.822, 3m=0.071, 6m=<0.001
Number of TJ	6.31±4.46	3.10±2.73 a	1.31±1.15 a,b	4.13±3.08	4.53±4.32	6.26±4.73 a,b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.485, 6m=<0.001	Pre=0.007, 3m=0.078, 6m=<0.001
ESR (mm/h)	40.27±15.78	31.87±19.00 a	26.06±17.20 a,b	41.55±21.6	37.74±20.6a	40.03±23.7b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.009, 6m=0.416	Pre=0.757, 3m=0.172, 6m=0.003
CDAI	20.83±6.86	13.85±5.83 a,b	9.81±3.18 a,b	18.34±7.29	17.89±9.14 b	22.03±9.77 a,b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.611, 6m=<0.001	Pre=0.106, 3m=0.020, 6m=<0.001
PtGA	6.15±1.29	5.10±1.24 a,b	4.58±1.38 a,b	6.00±1.39	5.97±1.79 b	6.42±1.70 b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.922, 6m=0.153	Pre=0.595, 3m=0.012, 6m=<0.001
PrGA	5.02±1.02	4.00±1.25 a,b	3.33±1.35 a,b	5.00±1.21	4.68±1.42 b	5.16±1.79 b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.090, 6m=0.559	Pre=0.937, 3m=0.020, 6m=<0.001
DAS28-ESR	5.16±0.80	4.10±0.91 a,b	3.43±0.76 a,b	4.79±0.98	4.66±1.04 b	5.14±1.04 a,b	R: 3m=<0.001, 6m=<0.001 N: 3m=0.254, 6m=0.002	Pre=0.064, 3m=0.010, 6m=<0.001
DAS28-ESR Reduction value	-	1.06±0.96 b	1.73±0.83 b	-	0.13 ±0.68	-0.35±0.65	<0.001 b	Pre=N/A, 3m=<0.001 6m=<0.001

Data are expressed as mean ± standard deviation (SD). Within-group changes over time (baseline → 3 months → 6 months) were analyzed using paired t-tests; between-group differences (responders vs non-responders) at each time point were assessed using Welch's t-test for unequal variances. Superscript **a** denotes a statistically significant difference compared with baseline within the same group ($p < 0.05$). Superscript **b** denotes a statistically significant difference between responders and non-responders at the same time point ($p < 0.05$). Baseline DAS28-ESR scores did not differ significantly between responders and non-responders (5.16 ± 0.80 vs 4.79 ± 0.98, $p = 0.064$), confirming group comparability prior to treatment. Superscript b therefore applies only to the 3- and 6-month columns and ΔDAS28-ESR values where significant divergence occurred. All statistical tests were two-tailed; $p < 0.05$ was considered statistically significant.

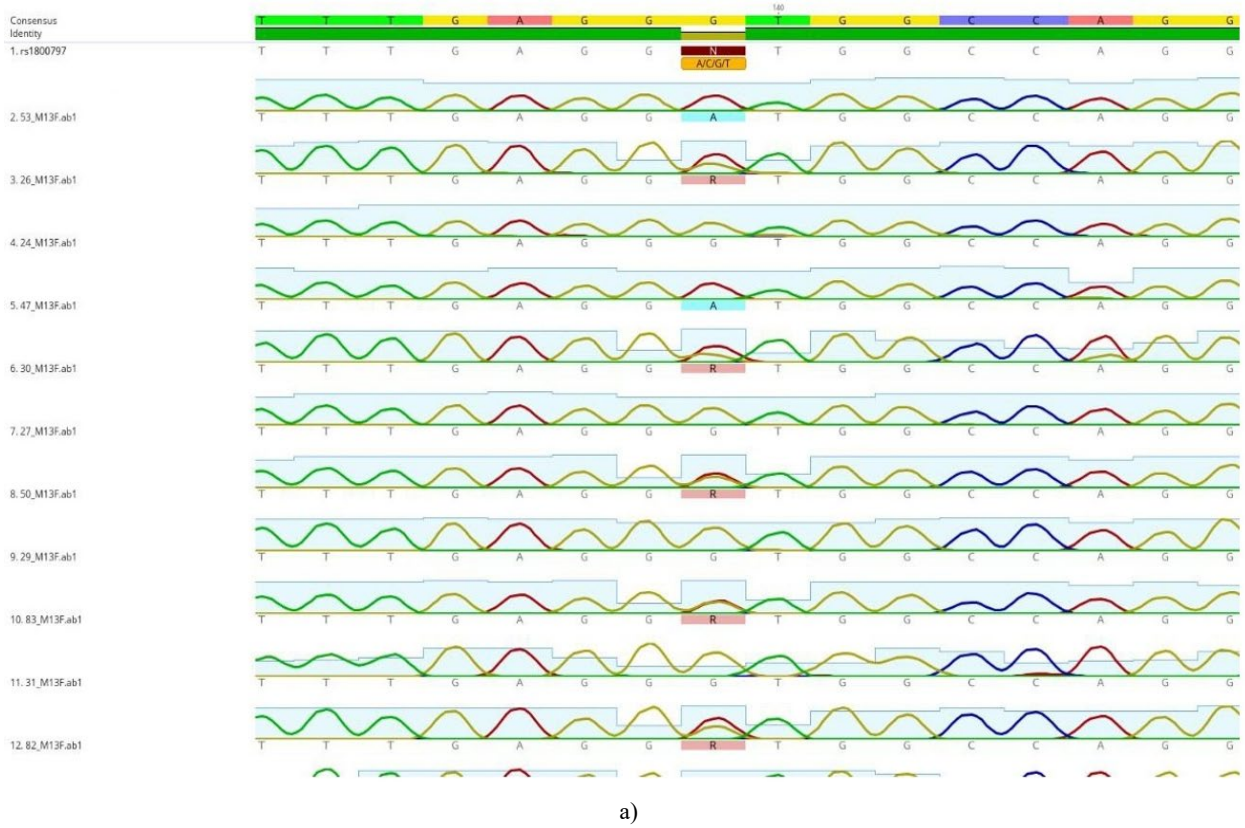
The significant reduction in core disease activity measures (SJC and TJC, CDAI, and DAS28) exclusively in the responder group after six months of therapy compared to pre-treatment and non-responders robustly validates the clinical efficacy of ETN for a subset of patients. These findings agreed with those of Aghdashi *et al*. [38], who reported that ESR, TJC, and DAS28 decreased significantly in RA patients after several months of post-ETN treatment. Su *et al*. [47] also identified that the therapeutic regimen elicited a rapid and substantial response, with all measured indices of disease activity, including DAS28, ESR, CRP, TJC, and SJC, demonstrating significant improvement. Conversely, the non-responder group exhibited negligible improvement or a statistically significant deterioration in clinical parameters following ETN treatment.

A high number of RA patients did not respond to these medicines as expected, and remission frequency is still less than 50% in RA patients because the lower ETN levels were associated with non-response [38].

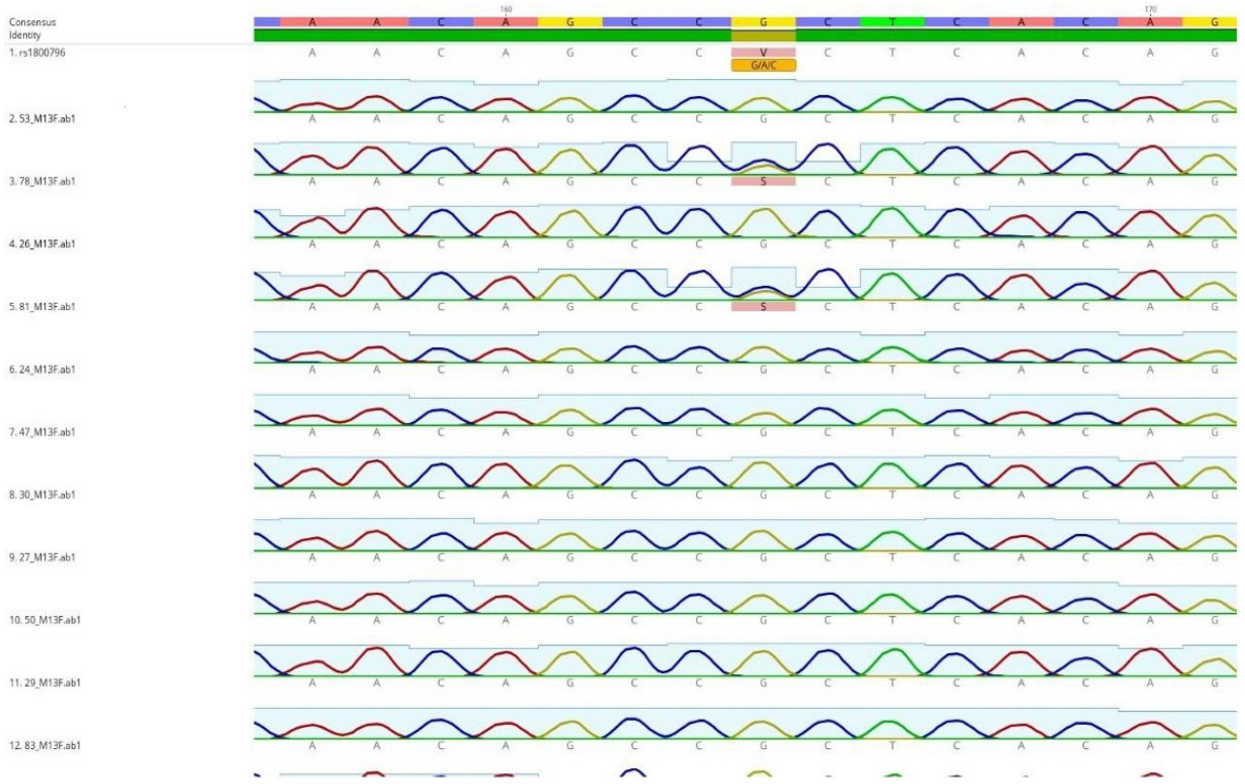
Amplification of the specific promoter region of the IL-6 gene

Amplification of the promoter regions for IL-6 via PCR yielded specific and distinct amplicons corresponding to the expected

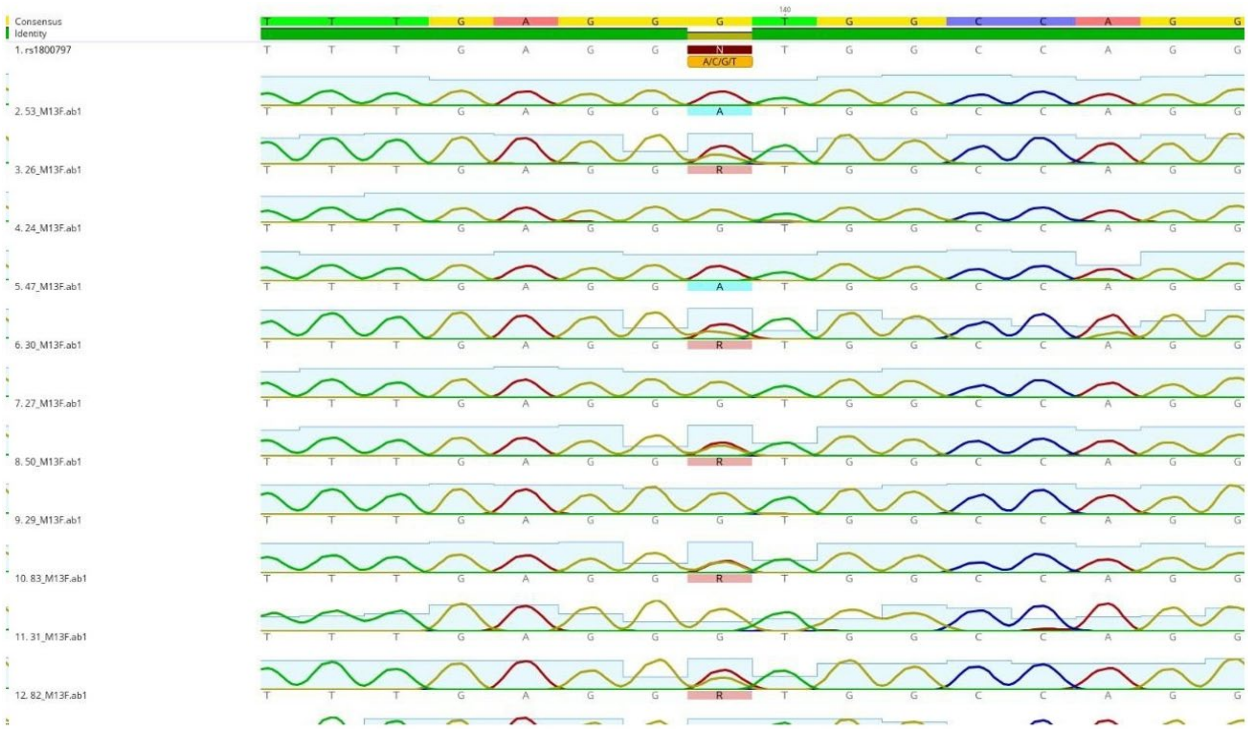
sizes for each primer set. Amplification of primers revealed products of 653 base pairs (bp) for fragments one and two, respectively. The genetic analysis of the IL-6 promoter regions in RA patients revealed a total of three SNPs within the 3 untranslated region (3 UTR): these SNPs, rs1800795 (-174 G>C), rs1800796 (-572G>C), and rs1800797 (-597G>A) **(Figure 1)**.



a)



b)



c)

Figure 1. Genotyping of the IL-6 rs1800795, rs1800796, and rs1800797 polymorphisms was performed via Sanger sequencing.

Prevalence of IL-6 genotypes polymorphism in RA patients

A study of IL-6 gene polymorphisms, particularly variants located at positions in the 5'-flanking region, established a significant association with susceptibility to RA [48] and other diseases [49].

(**Table 3)** presents the observed genotypic and allelic frequencies of the investigated IL-6 SNPs; the genotypic distribution of the IL-6 rs1800795 (-174 G>C) variant in an Iraqi cohort with RA showed a predominance of the G allele. The GG genotype was observed with the greatest frequency, followed by the GC genotype, while the CC homozygous genotype was infrequent. This outcome is in agreement with a previous study by Shafia *et al*. [50], which reported a higher prevalence of the G allele. The genotyping analysis aligned with Ibrahim *et al*. [51] study showed that the -174 GG genotype was prominent than GC, but the results did not support the role of IL-6 promoter -174 GG genotype as a risk factor for RA since the frequency did not statistically differ from healthy controls.

IL-6 is the most prevalent cytokine detected in the serum and synovial fluid of RA patients, and its concentration is a reliable biomarker of disease severity [52]. Evidence suggests a significant role for the IL-6 -174 GG polymorphism in RA. Severity and the G allele are associated with accelerated radiologic damage [53, 54].

Evidence through meta-analysis demonstrates a significant association between the IL-6 -174 SNP and an increased risk of developing RA in a pooled population analysis. This risk was found to be significantly more pronounced in Asian populations compared to other ethnicities [55].

Similarly, the significant associations observed for IL-6 rs1800796 (-572G>C) and rs1800797 (-597G>A) further implicate genetic regulation of IL-6 production in RA predisposition. A meta-analysis of Zhang *et al*. [56] concluded that the IL-6 -572 G allele and GG genotype are significant risk factors for developing RA, especially among Asians. In contrast, investigation by Lo *et al*. [57] revealed no significant association between the IL6-572 G/C polymorphism and RA. Regarding rs1800797, the G allele, GG, with GA genotypic frequencies were significantly higher in RA patients. The present results partially agreed with a meta-analysis of Azizi *et al*. [58], which found the genotypic frequencies of the rs1800797 variant had a significantly elevated risk of RA; however, GA and AA were at a higher risk than GG. While no correlation was found between rs1800797 (-597G>A) genotypes and RA susceptibility, RA was reported by Hao *et al*. [59].

Table 3. Genotypic and allelic frequencies of IL-6 gene loci and their Hardy–Weinberg equilibrium (HWE) status

Locus	Genotypes	Observed n	Frequency %	Allele	Allele Frequency %	HWE P-Value	HWE Interpretation
IL-6: rs1800795 G>C	GG	59	66	G	81	0.69	In Equilibrium [a]
	GC	27	30	C	19		
	CC	4	4				
IL-6: rs1800796 G>C	GG	84	93	G	97	0.74	In Equilibrium [a]

	GC	6	7	C	3		
	CC	0	0				
	GG	53	59	G	77		
IL-6: rs1800797 G>A	GA	33	37	A	23	0.69	In Equilibrium [a]
	AA	4	4				

HWE: Hardy–Weinberg equilibrium. "**a**": Exact test for HWE applied when expected genotype counts < 5. All genotypes of the studied loci conformed to Hardy–Weinberg expectations (P>0.05), indicating random allele distribution and the absence of genotyping bias or population substructure effects within the sample.

This study was predicated on the hypothesis that genetically determined variation in IL-6 production may influence differential responses to therapy, suggesting that functional IL-6 polymorphisms could serve as predictive biomarkers. Previous research has implicated the G allele of the IL-6 promoter polymorphism rs1800795 (-174 G>C) in a favorable outcome following anti-TNF treatment [60]. Similar studies by Jancic *et al*. [61] and Davila-Fajardo *et al*. [62] reported the same findings, showing that disease activity and clinical response to ETN were influenced by the (–174G>C) IL-6 SNP in RA patients. Specifically, the GG carriers were better responding to ETN, unlike the GC or CC genotype carriers.

Table 4. Comparison of IL-6 genotypes and allele distributions between responders and non-responders to etanercept therapy

SNP	Genotypes	Responders n=52 (%)	Non-Responders n=38 (%)	P-Value	OR (95% CI)
	GG	39 (75.0%)	20 (52.6%)		
	GC	13 (25.0%)	14 (36.8%)	**0.016 ***	P<0.05
IL-6: rs1800795 G>C	CC	0 (0%)	4 (10.5%)		
	G	91 (87.5%)	54 (71.1%)	**0.0059 ****	2.85
	C	13 (12.5%)	22 (28.9%)		(1.33–6.12)
	GG	48 (92.3%)	36 (94.7%)		
	GC	4 (7.7%)	2 (5.3%)	1	
IL-6: rs1800796 G>C	CC	0 (0%)	0 (0%)		
	G	100 (96.2%)	74 (97.4%)	0.977	0.68
	C	4 (3.8%)	2 (2.6%)		(0.12–3.79)
	GG	29 (55.8%)	24 (63.2%)		
	GA	19 (36.5%)	14 (36.8%)	0.209	
IL-6: rs1800797 G>A	AA	4 (7.7%)	0 (0%)		
	G	77 (74.0%)	62 (81.6%)	0.234	0.64
	A	27 (26.0%)	14 (18.4%)		(0.31–1.33)

Chi-square test was applied for 2 × 3 genotype comparisons, whereas Fisher's exact test was used for 2 × 2 allele comparisons or whenever any expected cell count was < 5. When OR > 1 indicates increased odds of non-response; OR < 1 indicates a protective (response-favoring) effect. *P< 0.05, **P< 0.01

Because of the ability of IL-6 to induce an inflammatory reaction and activate inflammatory cells (Myeloid cells and lymphocytes), causing an increased inflammatory process, it has been noticed that the suppression (by neutralization) of the TNF-α results in a reduction of the inflammatory cascade [63].

On the other hand, the analysis of the other IL-6 promoter loci, rs1800796 (G>C) and rs1800797 (G>A), revealed no statistically significant variance (p > 0.05) in either genotypic or allelic frequencies between responders and non-responders to ETN therapy **(Table 4)**. These results help refine the genetic landscape of treatment response, indicating that not all IL-6 gene variants carry equivalent predictive power.

Schotte *et al*. [33] also reported that there were no significant divergences between the genotypes of IL-6 SNPs (-597 and -572) and response to ETN; however, the G allele appeared to be more frequent in patients with a good or moderate response.

The correlations between the IL-6 genotypes and the difference in DAS28-ESR over six months post Etanercept treatment

The results revealed that only rs1800795-174 G>C showed a reduction in DAS28-ESR score. The GG genotype was related to a superior improvement in disease activity over six months. As shown in **Table 5**, the majority of differences in DAS28-ESR change post 6 months of ETN treatment among genotypes of other variants were not significant.

Post-hoc Games–Howell analysis demonstrated that patients carrying the GG genotype of rs1800795 achieved markedly greater improvement in DAS28-ESR compared with GC (P= 0.022) and CC (P= 0.002) carriers; a smaller yet significant difference was also found between GC and CC (P= 0.044).

(Table 5) demonstrated that genotype-dependent effect on the reduction of disease activity, as measured by the change in DAS28-ESR over six months.

Regarding IL-6 rs1800795 (G>C) SNP, the key finding demonstrates that the G allele (GG genotype) experienced a significantly greater improvement in disease activity compared to both heterozygotes (GC) and homozygotes for the C allele (CC). The declining pattern in mean ΔDAS28-ESR across the genotypes led to speculation that the refractory effect of the C

allele to ETN therapy, where the G allele is linked with a more favorable response to ETN. The present experiment appeared to be of clinical interest as it positions rs1800795 as a potential

Table 5. Effect of IL-6 Gene Polymorphisms on 6-Month Change in Disease Activity (ΔDAS28-ESR) Among Rheumatoid Arthritis Patients Receiving Etanercept

Locus	Genotypes	Observed n	ΔDAS28-ESR mean ± SD	P-Value	Effect size (ω^2)	Post-hoc (Games–Howell)
IL-6: rs1800795 G>C	GG	59	1.15 ± 1.11 a	**0.004 ****	0.098	GG vs GC p=0.022
	GC	27	0.40 ± 1.46 b			GG vs CC p=0.002
	CC	4	−0.43 ± 0.49 b			GC vs CC p=0.044
IL-6: rs1800796 G>C	GG	84	0.87 ± 1.32	0.31		NS
	GC	6	0.60 ± 0.51			
IL-6: rs1800797 G>A	GG	53	0.80 ± 1.05	0.523		NS
	GA	33	0.85 ± 1.62			
	AA	4	1.56 ± 0.79			

ΔDAS28-ESR: higher positive values indicate greater clinical improvement after etanercept therapy. Reported p-values correspond to the omnibus (overall) test of group mean differences. The effect size (ω^2) represents the proportion of total variance in ΔDAS28-ESR explained by the genotype, interpreted as small (≈0.01), medium (≈0.06), or large (≈0.14). Superscript letters (a, b) denote statistically distinct groups: means that share the same letter do not differ significantly ($p > 0.05$), while those with different letters differ significantly ($p < 0.05$). NS: not significant; * $p < 0.05$; ** $p < 0.01$.

predictive biomarker for treatment selection by ETN. For instance, Schotte *et al*. [33] observed that the -174G allele DAS-ESR showed a significant reduction in comparison with the C allele; it was associated most closely with a favorable response, and the C allele with a poor predicted response. Kamal *et al*. [34] showed that the IL-6 rs1800795 C allele was identified with reduced response to TNF inhibitors; in contrast, the G allele was linked with a significant reduction of disease activity (DAS28), classified as good or moderate at one year. In opposition, the other IL-6 SNPs that were investigated in this study, rs1800796 (G>C) and rs1800797 (G>A), showed no significant correlations with the changes in disease activity. The lack of association for rs1800796 is specifically noteworthy and in accordance with the work of Ad'hiah *et al*. [64], who also found no significant differences between this specific polymorphism and IL-6-related phenotypes in RA patients.

Genotypic effect on the IL-6 expression and its blood level in RA patients may affect disease activity. The IL-6 rs1800795 C allele is correlated with higher IL-6 levels and, consequently, disease activity. Mohammed *et al*. [65] reported that many TNF inhibitors, including ETN, reduce plasma IL-6 levels and DAS28 during maintenance therapy in RA patients after 14 weeks post-therapy.

The results of Warjukar *et al*. [66] revealed a substantially significant positive association between IL-6 levels and DAS28 ($P<0.001$), indicating that IL-6 decreases with the reduction of DAS28 after anti-TNF therapy. Wielińska *et al*. [67] recorded the same data, who found that CC homozygous of rs1800795 possess RA patients who were characterized with the highest average concentrations of this pro-inflammatory cytokine.

In contrast, the other IL-6 polymorphisms investigated, rs1800796 and rs1800797, showed no significant correlation with changes in disease activity. It aligns with Ad'hiah *et al*. [64] study, which also found no significant correlation between this specific SNP and IL-6-related phenotypes in RA patients, and others in the same gene region may not directly modulate the clinical efficacy of TNF-α inhibitor therapy. Schotte *et al*. [33] also reported that SNPs rs1800796 and rs1800797 were not related to a significant change in DAS after ETN treatment. Hao *et al*. [59] indicated that there was a marginal association between rs1800797 (-597G>A) and also rs1800796 (-572 G>C) and susceptibility to RA, and not associated with DAS-ESR reduction.

Conclusion

These results indicated that age may be a predictive demographic factor for response to ETN, although the cause remains unclear. The experiment highlighted an ETN efficacy in reducing RA disease activity among responders' patients by decreasing TJC, SJC, CDAI, ESR, PtGA, PrGA, and DAS28-ESR at two time points (three- and six-month intervals) following treatment initiation. Non-responders exhibited minimal to no reduction in these measures.

Evaluation of the baseline characteristic revealed that TJ was higher in future non-responders than in the responsive cohort. Furthermore, the C allele of the IL-6 -174G>C locus was related to both increased disease activity and poor response to ETN at the six-month post-onset of therapy, unlike the G allele, suggesting the IL-6 -174G>C locus may serve as a valuable biomarker for poor response to ETN therapy. This IL-6-174 G>C locus represents a marker for personalizing anti-TNF (ETN) therapy, optimizing treatment efficacy, and improving clinical outcomes.

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