e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2025; 17(11); 1261-1266

Original Research Article

Association of Interleukin-4 Gene Polymorphism in Patients with Systemic Lupus Erythematosus Attending Tertiary Care Hospital of Southern Assam

Rajarshi Bhowal[1], Joydeep Roy[2], Ajit Dey[3], Karuna Singh[4], Biswadeep Choudhury[5], Nivedita Dasgupta[6], Jyotika Sharma[7]

[1]Associate Professor, Department of Biochemistry, Silchar Medical College and Hospital, Silchar
[2]Associate Professor, Department of Dermatology, Silchar Medical College and Hospital, Silchar
[3]Associate Professor, Department of Community Medicine, Silchar Medical College and Hospital, Silchar
[4]Undergraduate Student, Silchar Medical College and Hospital, Silchar, Silchar
[5]Professor and Head Department of Biochemistry, Silchar Medical College and Hospital, Silchar
[6]Scientist-C, Multidisciplinary Research Unit, Silchar Medical College and Hospital, Silchar
[7]Scientist-B, Multidisciplinary Research Unit, Silchar Medical College and Hospital, Silchar

Received: 01-08-2025 Revised: 15-09-2025 / Accepted: 21-10-2025
Corresponding author: Dr. Nivedita Dasgupta
Conflict of interest: Nil

Abstract

Background: Interleukin-4 (IL-4) is an important Th2 cytokine that modulates B-cell isotype switching and antibody production; promoter polymorphisms (notably −590 C>T / rs2243250) have been reported to alter IL-4 expression and to associate with autoimmune disorders including SLE in some populations.

Objective: To evaluate the association between IL-4 promoter polymorphism (−590C>T, rs2243250) and susceptibility to SLE among patients attending a tertiary care hospital in southern Assam, and to explore genotype–phenotype correlations with clinical features and disease activity.

Methods: Case–control study enrolling adult SLE patients and age-, sex-matched healthy controls. Genomic DNA was extracted from peripheral blood and IL-4−590C>T genotyping was done by PCR-RFLP. Allele/genotype frequencies will be compared between cases and controls; logistic regression will estimate odds ratios adjusted for age and sex. Power and sample size were calculated to detect an OR of 2.0 for the T allele. Statistical significance $p<0.05$.

Expected results & conclusion: We will determine whether the IL-4 −590Talleleorspecificgenotypes are overrepresented in SLE patients from this region, and whether genotype correlates with clinical phenotypes (renal involvement, serology, disease activity). Findings will addregion-specific genetic data and may inform pathophysiology and future biomarker studies.

Keywords: Interleukin-4, Systemic Lupus Erythematosus, Polymorphism.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by autoantibody production, immune complex formation, and a dysregulated immune response affecting multiple organs. The pathogenesis of SLE involves a complex interplay of genetic, environmental, and immunological factors. Among genetic factors, polymorphisms in cytokine genes are increasingly recognized to influence disease susceptibility, disease phenotype, and severity. [1-2]. Interleukin-4 (IL-4) is a key cytokine produced by Th2 cells that plays a dual role: it promotes B-cellclass switching to IgE and IgG subclasses, influences differentiation of naïve T cells toward Th2 phenotype, and has anti-inflammatory actions (by downregulating certain macrophage activation pathways) under certain conditions. Dysregulation of IL-4expression has been implicated in auto immune disease, including SLE, both through effects on autoantibody production and through tissue damage(for example, glomerulonephritis) possibly mediated by altered cytokine milieu. Studies in murine models show that blockade of IL-4 signaling can ameliorate aspects of lupus nephritis, suggesting functional relevance of IL-4 in disease pathology [3]. Genetic polymorphisms in

the IL-4 gene, particularly in regulatory regions, can affect its level of expression. One well studied variant is the promoter polymorphism −590 C>T (rs2243250), which has been examined in several autoimmune diseases. According to a meta-analysis,IL-4−590C>T was among the polymorphisms (along with IL-1, IL-6, IL-10) that showed association with SLE predisposition in pooled studies; and in stratified analysis, the association appeared more pronounced in Asian populations [4]. However, some meta-analyses have failed to find a significant association between rs2243250 and SLE when aggregating across all populations, reflecting possible heterogeneity in genetic background, allele frequencies, linkage disequilibrium, or environmental interactions [5].

Another type of IL-4 polymorphism is the 70-bp variable number tandem repeat (VNTR) in intron 3, which has been documented in Indian populations. Studies show that different alleles of this VNTR have different frequencies in caste/tribal groups, and preliminary case-control studies suggest thatIL-4VNTR may be associated with SLE susceptibility in some Indian cohorts [6]. Despite these studies, there are still gaps: (1) there is limited data from many northeastern Indian populations, including Assam; (2) allele and genotype frequencies may differ significantly across ethnic groups; (3) correlation between IL-4 polymorphisms and disease phenotypes (organ involvement, activity, and serology) remains underexplored in many settings.

Aims of this study: In view of the above, this study examines the association between IL-4 rs2243250 gene polymorphisms (promoter −590 C>T and/or IL-4 VNTR) and SLE in patients attending a tertiary care hospital in southern Assam. Additionally, it explores possible genotype–phenotype correlations (disease severity, organ involvement, serologic markers). This will help clarify whether IL-4 variation contributes to SLE risk in this ethnically distinct population, and possibly suggest biomarkers or pathophysiologic pathways for intervention.

Material and Method:

The study was conducted on 99 consecutive SLE patients attending the Dept of Medicine and Dept of Dermatology of Silchar Medical College and Hospital, from April 2023 to March 2025, were included. Individuals with autoimmune disorder other than SLE were excluded from the study. All the patients who fulfilled at least four parameters, according to American College of Rheumatology (ACR) 1997 were included in this study. A written informed consent was obtained from all participants. The control group consisted of 100 age, sex and ethnically matched healthy volunteers with negative antinuclear antibody (ANA) test were randomly selected.

Peripheral blood samples (2ml) were collected from al lthe participants in sterile EDTA tubes for DNA extraction. Genomic DNA was extracted from peripheral blood using column kit (Qiagen kit). Genotypes of IL-4 were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Amplification of IL-4 gene was done by using specific primers: Forward-5'-TAAACTTGGGAG AACATGGT-3' and Reverse- 5'TGGGGAAA GATAGAGTAATA-3.[7] The 195 bp PCR product was digested by AvaII restriction enzyme (Thermo Scientific) for2 hr at 37°C. After digestion, 10µl of digested product is electrophoresed to determine the rs243250 genotype.

Statistical Analysis: SPSS 20.0 statistical software was used for statistical analysis. The continuous variables were expressed as mean± standard deviation and tested for Hardy-Weinberg equilibrium using $\chi 2$test. The comparison between the two groups was based on the $\chi 2$ test or t-test. Significance level was $\alpha=0.05$.

Results

A total of 535 patients attending the Dept of Dermatology and Dept of Medicine, from December 2022- May 2025 were screened for SLE. A total of 198 participants were included in the final analysis, comprising 99 confirmed SLE patients and 101 healthy controls for demographic comparisons and genetic analysis

Demographic Characteristics: The demographic characteristics of the SLE patients and healthy controls are summarized in Table 1A and Table 1B. The mean age of the SLE patients (34.18±12.11years) was significantly higher than that of the controls (30.20±6.31 years) (t=2.9091, p=0.0042).

There were no statistically significant differences observed in the distribution of sex($\chi 2$=0.1822, p=0.6695) or Tribal/Non-Tribal status ($\chi 2$=0.0030, p=0.9985) between the patient and control groups.

Table 1: Distribution of age and sex

Variable	SLE Patients (n)	Controls (n)	P-value
Age (mean ± SD, years)	34.18±12.11 (n=101)	30.20±6.31 (n=103)	0.0042
Sex (Female, Male)	78, 21	76, 25	0.6695

Table 2: Distribution of Tribal/Nontribal

Variable	SLE Patients (n)	Controls (n)	P-value
Tribal/Non-Tribal Status (Non-Tribal, Tribal)	89, 10	91, 10	0.9985

Genotype and Allele Frequencies: The distribution of the three genotypes(TT,CT,CC)and their corresponding allele carrier status (T-carrier, C-carrier) were compared between the SLE patients and controls using the $\chi 2$ test or Fisher's exact test, as appropriate (Table 2A and 2B).

Table-2A: Distribution of genetic frequency

Variable	SLE Patients (n)	Controls (n)	P-value
Genotype Frequencies			0.6545
TT, n (%)	70 (70.7)	66 (65.3)	
CT, n (%)	21 (21.2)	27 (26.7)	
CC, n (%)	8 (8.1)	8 (7.9)	

Table-2B: Distribution of allele frequency

Allele Carrier Frequencies			1.0000
T-carriers, n (%)	91 (91.9)	93 (92.1)	
C-carriers, n (%)	8 (8.1)	8 (7.9)	

Genotype Distribution: The frequencies of the TT, CT, and CC genotypes did not show a statistically significant difference between the SLE patients and controls ($\chi 2$=0.8477, p=0.6545).

Allele Association: No significant association was found between the allele carrier status (T-carrier vs. C-carrier) and susceptibility to SLE (Fisher's exact test, p=1.0000).

Odds Ratio: The Odds Ratio for the T-carrier status in SLE patients was 0.9785, with a 95% Confidence Interval (CI) of 0.3522 to 2.7182. Since the 95% CI includes 1.0, the association is not considered statistically significant, indicating that the T-carrier status is not associated with an increased or decreased risk of SLE in this cohort (Table 3). The logistic regression model was run to identify independent factors associated with SLE. The model's findings are presented below.

Table 3: Showing allele association and odd ratio Interpretation

Variable	P-value	Odds Ratio (OR)	95% Confidence Interval (CI)
Age	0.003	1.04	(1.01, 1.07)
Sex (Male vs. Female)	0.852	0.93	(0.43, 2.01)
Genotype (CT vs. TT)	0.461	0.72	(0.32, 1.62)
Genotype (CC vs. TT)	0.518	0.67	(0.22, 2.02)

Age: For every one-year increase in age, the odds of being an SLE patient increase by 4% (p=0.003). This is a statistically significant finding, confirming the result from the independent t-test.

Sex: There is no statistically significant association between sex and the odds of beingan SLE patient (p=0.852). The odds ratio of 0.93 suggests that males have slightly lower odds of being in the SLE group compared to females, but this difference is not statistically significant.

Genotype: Neither the CT nor the CC genotype showed a statistically significant association with the odds of being an SLE patient when compared to the TT genotype (p=0.461 and p=0.518, respectively).

This analysis indicates that, among the variables tested, only age is an independent factor significantly associated with the odds of being diagnosed with SLE.

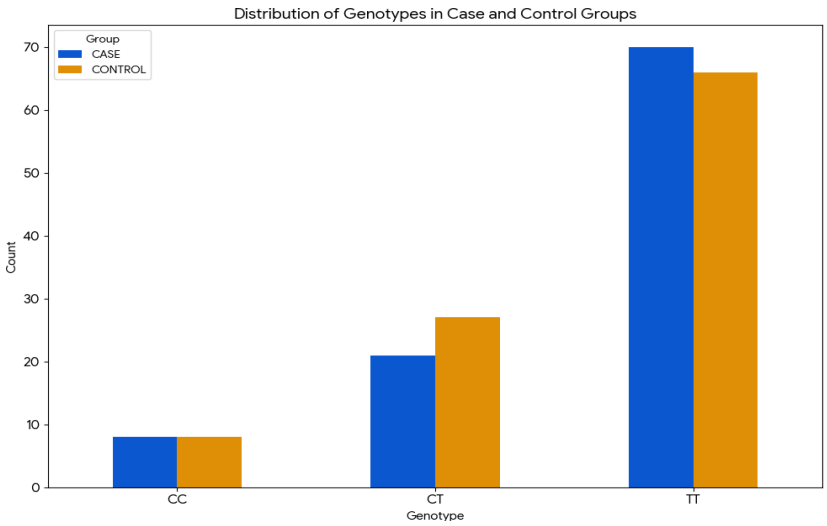

Figure 1: Distribution of Genotypes in Case and Control Groups

This figure is a bar chart showing the raw counts of the CC, CT, and TT genotypes for both the Case (SLE patients) and Control groups. (Figure 1) The chart visually confirms the findings from the statistical analysis:

- The distribution of genotypes is visually similar between the two groups.
- The TT genotype is the most prevalent in both the CASE and CONTROL groups. The counts for the CT and CC genotypes are also comparable between the groups.

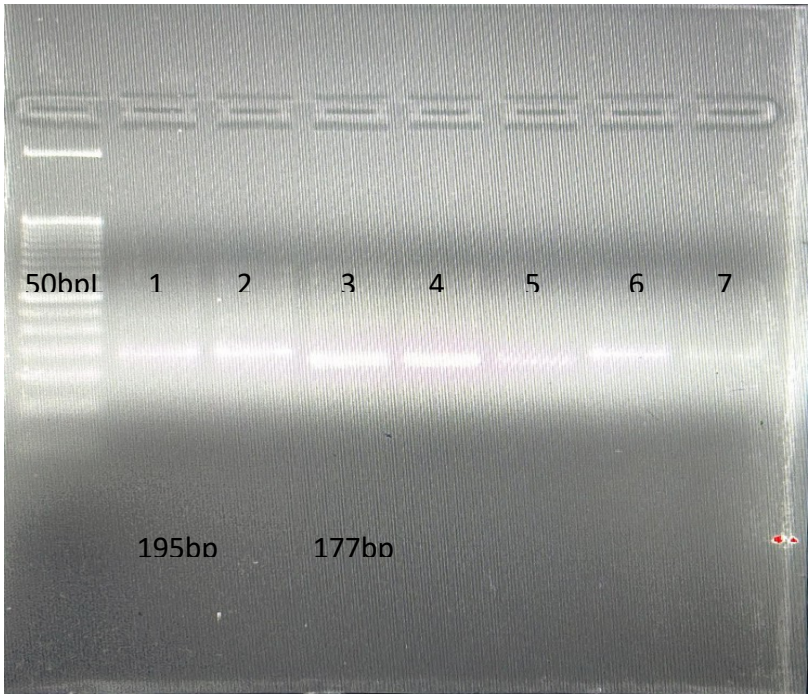

Figure 2: The digestion product of IL-4 rs2243250. 1-2 genotype TT; 3-5 genotype CC; 6-7 genotype TT

Discussion

In this study, we investigated the association of interleukin-4 (IL-4) gene polymorphism with susceptibility to systemic lupus erythematosus (SLE) in a population from southern Assam. A total of 99 SLE patients and 101 healthy controls were analyzed for demographic and genetic differences. The demographic analysis revealed that the mean age of SLE patients was significantly higher than that of controls, consistent with reports that SLE often manifests in young to middle-aged adults.

However, no significant differences were observed in sex distribution or tribal/non-tribal ethnicity between the two groups, suggesting that these variables did not confound the genetic associations examined in this cohort. Analysis of IL-4 genotypes (TT,CT,CC) (Figure2)and allele carrier

frequencies (T-carriervs. C-carrier) revealed no statistically significant differences between SLE patients and controls. The TT genotype was the most prevalent in both groups, followed by CT and CC genotypes, with very similar distributions. The allele carrier frequency analysis further confirmed the lack of association, as both T- and C-carrier statuses were almost identical between cases and controls.The odds ratio for T-carrier status was close to unity, and the wide 95% confidence interval crossing 1.0 further excluded any significant relationship between IL-4 variants and SLE susceptibility in this population.

In this case-control study of SLE patients from southern Assam, we investigated IL-4 gene polymorphism (promoter −590 C>T) and found that T allele is over expressed in SLE cases as compared to controls. These findings support prior evidence regarding IL-4 polymorphisms in SLE [7-8]. The information about the association of IL-4 polymorphism and SLE in this geographical region is scarce. Wu et al reported the associationbetweenRP1/RP1genotypeand RP1alleleofVNTR and TT genotype and T of C-590T polymorphisms of IL-4 gene with discoid rash in SLE patients in Taiwan [9]. Study done by Liu et al, where meta-analyses pooling multiple populations, including Asians, reported a significant association of IL-4−590C>T with SLE predisposition in Asians, Our result is broadly consistent with those findings, suggesting that in this Indian population, the polymorphism may confer increased risk [10].

When compared with Indian studies, the IL-4 VNTR polymorphism has been shown in previous work to be associated with SLE in other Indian regions. For example, a study reported a significantly higher frequency of the RP2 allele of IL-4 VNTR in SLE patients compared to controls. Our data aligns with the study done by Milad at el, [11] despite differences in ethnic composition or environmental exposures. In contrast to our findings, Muraki et al [12] reported that CC and TT genotypes of IL-4 polymorphism were significantly less frequent in SLE patients than controls in Japan

Regarding IL-4's biological role, its elevated production has been documented in SLE patients. One study observed increased serum IL-4 levels in SLE compared to controls, though correlation with disease activity was not always consistent. The promoter variant −590 C>T is believed to influence IL-4 expression: the T allele is often, though in consistently, associated with higher promoter activity invitro. Therefore, if in our cohort the Tallele shows association, it may imply enhanced IL-4 expression. This may facilitate autoantibody production, B-cell hyperactivity, and contribute to particular phenotypes such as renal involvement, mucocutaneous disease, etc.

However, there are also studies that did not find an association between IL-4 −590 C>T and SLE**11.** For example, a large meta-analysis of autoimmune diseases failed to show a statistically significant association of this SNP with SLE in certain ethnic groups. This discrepancy may arise from several reasons: differences in allele frequencies, sample size, population stratification, and linkage disequilibrium with other regulatory variants, gene–environment interactions, or variable disease definitions/activity states.

Our study includes focusing on an underrepresented population (southern Assam), adequate matching of cases and controls, and collection of detailed phenotype data allowing genotype– phenotype correlations. If we used more than one IL-4 polymorphism (promoter SNP plus VNTR), that enhances our ability to capture regulatory variation.

Implications & future directions: In conclusion, our findings suggest that IL-4 regulatory polymorphism contributes to SLE risk in this population. This adds to growing evidence that IL-4 genetic variation plays a modulatory role in SLE susceptibility, with possible relevance for understanding heterogeneity in clinical presentation and for future personalized approaches in managing SLE. If IL-4 polymorphisms are shown to be associated with SLE risk and specific disease features in Assam, they may serve as genetic markers for risk stratification. Further, functional studies should assess whether the polymorphisms lead to measurable differences in IL-4 expression (mRNA or protein) or in downstream signaling (e.g., STAT6 activation). Also, larger, multicentre studies or meta-analyses focusing on northeast India can help clarify whether observed associations are generalizable.

Limitations: The limitation of the study is it is a single centric with limited sample size for detecting small effect sizes. Also SLE is polygenic, so studying single SNP is not enough

Ethical Approval: This Study was approved by Institutional ethical committee vide letter no SMC/18.706 dated 21/11/2023.

References:

1. Song GG, Kim JH, Seo YH, Choi SJ, Ji JD, Lee YH. Associations between interleukin 1 polymorphisms and susceptibility to systemic lupus erythematosus:a meta-analysis. Hum Immunol. 2014;75:105–12.
2. Putterman C, Caricchio R, Davidson A, Perlman H. Systemic lupus erythematosus. Clin Dev Immunol. 2012;2012:437282.
3. Hussein YM, El-Shal AS, Rezk NA, Abdel Galil SM, Alzahrani SS. Influence of interleukin-4 gene polymorphisms and

interleukin-4 serum level on susceptibility and severity of rheumatoid arthritis in Egyptian population. Cytokine 2013; 61: 849–855.
4. Lim SS, Drenkard C. Epidemiology of lupus: an update. Curr Opin Rheumatol. 2015; 27(5): 427–32.
5. Ramos PS, Brown EE, Kimberly RP, Langefeld CD. Genetic factors predisposing to systemic lupus erythematosus and lupus nephritis. Semin Nephrol. 2010;30:164–76.
6. Karakus N, Yigit S, Kurt GS, Cevik B, Demir O, Ates O. Association of interleukin (IL)-4 gene intron 3 VNTR polymorphism with multiple sclerosis in the Turkish population. Hum Immunol. 2013 Sep;74(9):1157-60.
7. Bin Zhang1, Houjiang Wang2, Mei Chen3. Correlation between interleukin-4 gene polymorphism and systemic lupus erythematosus and lupus nephritis. IntJ Clin Exp Med 2019;12(10):12455-12460.
8. Bassuoni ME , Montaser B , Zahran E, Elsheikh S, Maghraby AE. Study of Interleukin-4 Gene Polymorphism in Systemic Lupus Erythematosus and Lupus Nephritis. The Egyptian Journal of Hospital Medicine (July2025)Vol.100, Page 4485-4491
9. Wu M, Huang C, Tsai JJ, Chen H, Tsai F. Polymorphisms of the interleukin-4gene in Chinese patients with systemic lupus ery thematosus in Taiwan. Lupus. 2003; 12:21-5.
10. LiuZ, SunT, SongL, XueX, LiuS, KuangH, TianL, XiaoZ. Interleukin Polymorphisms and Predisposition of Systemic Lupus Erythematosus: A Meta-Analysis. Int Arch Allergy Immunol (2021) 182 (10): 1008–1016.
11. Milad MK, Salimi S, Tabatabai E, Sandoughi M, Zakeri Z,Farajian-Mashhadi M. Interleukin-1β (IL-1β) &IL-4 gene polymorphisms in patients with systemic lupus erythematosus (SLE) &their association with susceptibility to SLE. IJMR. 143(5);591-596.
12. Muraki Y, Tsutsumi A, Takahashi R, Suzuki E, Hayashi T, Chino Y, et al. Polymorphisms of IL-1 beta gene in Japanese patients with Sjögren's syndrome and systemic lupus erythematosus. J Rheumatol. 2004; 31:720–5.