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Association of TNFSF15 Gene Polymorphisms (rs4979462, rs6478106 and rs7848647) and mRNA Expression with Susceptibility to Systemic Lupus Erythematosus in the Kashmiri Population

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Abstract

Background: Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by loss of self-tolerance and a complex genetic background. TNFSF15, encoding the cytokine TL1A, plays an important role in immune regulation, but its role in SLE susceptibility remains insufficiently explored in specific ethnic populations. This study evaluated the association of TNFSF15 polymorphisms and mRNA expression with SLE risk and clinical manifestations in the Kashmiri population of North India. ***Material & Methods:*** A hospital-based case-control study was conducted involving 130 SLE patients and 150 age- and sex-matched healthy controls. Genotyping of three TNFSF15 single nucleotide polymorphisms (rs4979462, rs6478106 and rs7848647) was performed, and mRNA expression levels were quantified using qRT-PCR. ***Results:*** Significant associations were observed for rs6478106 and rs4979462. The rs6478106 TT genotype was significantly associated with increased SLE risk (OR = 4.0; $P < 0.05$) and correlated with anti-dsDNA positivity and photosensitivity. Conversely, the rs4979462 TT genotype exhibited a protective effect against SLE (OR = 0.30; $P < 0.05$). TNFSF15 mRNA expression was significantly upregulated in patients compared to controls. Functional analysis revealed that the rs6478106 TT genotype correlated with higher mRNA expression, whereas the rs4979462 CC genotype was associated with decreased expression ($P = 0.04$), suggesting a regulatory impact of these variants. No significant association was found for rs7848647. ***Conclusion:*** These findings indicate that TNFSF15 genetic variants and elevated expression levels contribute to SLE pathogenesis in the Kashmiri population. The TNFSF15/TL1A axis represents a potential biomarker for disease susceptibility.

Keywords: Systemic lupus erythematosus, TNFSF15, TL1A, Polymorphism, mRNA expression, Kashmiri population.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by a profound loss of immunological self-tolerance. This breakdown leads to the production of pathogenic autoantibodies directed against nuclear and cytoplasmic self-antigens, resulting in widespread tissue and organ damage, particularly affecting the joints, skin, central nervous system, and kidneys1. The pathogenesis of SLE involves a complex interplay where immune complexes, formed by autoantibodies and self-antigens, deposit in tissues to activate the complement system, promote the infiltration of neutrophils and monocytes, and drive the expansion of self-reactive lymphocytes2. Despite significant advances in rheumatology, the precise etiology of SLE remains incompletely understood, although it is widely accepted that disease onset involves a convergence of genetic, epigenetic, and environmental risk factors3. Extensive genome-wide association studies (GWAS) and candidate-gene approaches have identified numerous susceptibility loci, yet much of the "missing heritability" of SLE remains to be elucidated4.

A promising candidate in the study of autoimmune regulation is the Tumor Necrosis Factor Superfamily Member 15 (TNFSF15), also known as TNF-like ligand 1A (TL1A) or vascular endothelial growth inhibitor (VEGI). TNFSF15 is a key regulator of vascular homeostasis and inflammatory signalling5. The TNFSF15 gene is located on chromosome 9q32, spanning approximately 17 kb and comprising four exons and three introns6,7. The gene product is predominantly expressed as a membrane-bound homotrimer. While TL1A is constitutively expressed by endothelial cells, it is significantly upregulated in response to tumor necrosis factor-alpha (TNF-α) stimulation. Upon binding to its cognate death receptor 3

(DR3), TL1A activates downstream signaling cascades that modulate both innate and adaptive immune homeostasis8,9.

Crucially, the TNFSF15 locus is highly polymorphic. Genetic variations within this gene have been linked to susceptibility across a spectrum of immune-mediated conditions, including various malignancies9,10, Crohn's disease (CD), and ulcerative colitis (UC)7,11. In the context of SLE, TNFSF15 is hypothesized to facilitate disease progression by promoting the activation and proliferation of T and B cells, thereby sustaining the chronic inflammation and autoantibody production characteristic of the disorder8,12.

Given its central role in immune regulation, it is highly plausible that genetic polymorphisms within TNFSF15 contribute to the dysregulation of inflammatory responses in SLE. However, genetic susceptibility is often population-specific, and to date, no study has examined the influence of TNFSF15 genetic variation on SLE susceptibility in the Kashmiri population of North India. The present study was therefore designed to investigate the association of three specific TNFSF15 polymorphisms—rs7848647 (intronic), rs6478106 (promoter region), and rs4979462 (non-coding regulatory region) – with SLE susceptibility in this distinct ethnic group. Furthermore, we aimed to evaluate the functional relevance of these variants by assessing their impact on TNFSF15 mRNA expression levels.

MATERIALS AND METHODS

Study design and participants: This hospital-based case-control study included 280 participants (130 SLE patients and 150 age and sex-matched healthy controls) collected from 2020 to 2022 was conducted in the Department of Immunology and Molecular Medicine, in collaboration with the Division of Rheumatology, Sher-i-Kashmir Institute of Medical Sciences (SKIMS) Srinagar, Jammu & Kashmir. The study protocol was approved by the Institutional Ethics Committee (IEC/SKIMS RP-67-B/2020). Written informed consent was obtained from all participants prior to enrolment. Patients fulfilling the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for SLE13 were eligible for inclusion. Patients with a prior history of any other autoimmune disease or those receiving immunosuppressive therapy were excluded. The control group comprised individuals attending the same hospital for routine medical examinations who had no history of autoimmune disease. A structured proforma was completed for each participant.

RESULTS

The Demographic Characteristics, Clinical Manifestations, and Laboratory Findings of patients with SLE is shown in Table 1.

Table 1: The Demographic Characteristics, Clinical Manifestations, and Laboratory Findings of patients with SLE (n = 130)

Variables	Category	Number (%)
Age in Years	Age <30 years	71 (54.6)
	Age ≥30 years	59 (45.4)
Gender	Female	126 (96.9)
	Male	4 (3.1)
Duration of Disease	< 5 years	72 (55.4)
	> 5 years	58 (44.6)
Clinical Manifestations	Arthritis	121 (93.1)
	Lupus nephritis	43 (33.1)
	Alopecia	108 (83.1)
	Photosensitivity	95 (73.1)
	Malar rash	92 (70.8)
	Neurological manifestations	89 (68.5)
Features	Oral ulcers	92 (70.8)
	Serositis	20 (15.4)
	Discoid rash	23 (17.7)
Clinical Manifestations	Thrombocytopenia	97 (74.6)
	Proteinuria	45 (34.6)
	CRP	45 (34.6)
	ESR	55 (42.30)
	Complement levels	72 (55.4)
	Anti-ds-DNA antibodies	108 (83.1)
	Anti-smith antibodies	66 (50.8)

CRP= C-reactive protein, ESR= Erythrocyte sedimentation rate

Sample collection/storage: Blood samples from both healthy controls and patients diagnosed with systemic lupus erythematosus (SLE) were collected at Sher-i-Kashmir Institute of Medical Sciences, a tertiary care hospital India. All samples were obtained in EDTA-containing vials (200µL of 0.5M EDTA, pH 8.0). Following collection, samples were immediately stored at −20°C for subsequent DNA isolation, while a subset was preserved at −80°C for RNA extraction.

Genotyping by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP): Genomic DNA was isolated from peripheral blood samples using the phenol-chloroform extraction method [12]. The quality and quantity of the extracted DNA were determined by using UV spectrophotometry (NanoDrop; Eppendorf AG, Hamburg, Germany). Primers for TNFSF15 SNPs (rs4979462, rs6478106 and rs7848647) were designed using IDT software. The polymerase chain reaction (PCR) was carried out using a thermal cycler (Applied Biosystems, USA) on a reaction mixture of 25 µL containing 50 ng of genomic DNA template, 200 µM of each dNTP (0.2 µM of each primer (forward and reverse), 1.5 mM MgCl2 (KAPA biosystem) and 1 U of Taq DNA polymerase (KAPA Taq). The thermal cycling programme comprised an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing for 30s (at the temperatures specified in Table 2), and extension at 72°C for 30s, with a final extension at 72°C for 5 min. Amplified products were verified on 2% agarose gels stained with ethidium bromide. Table 2 showcases the primer sequences, PCR thermal cycling conditions, restriction enzyme utilized, and the resultant banding pattern generated in RFLP. The technique of restriction fragment length polymorphism was adopted to detect the amplified products in order to determine genotype. The 416bp, 256bp and 408bp PCR product of TNFSF15- rs7848647, rs6478106 and rs4979462 respectively were subjected to digestion using 2U restriction endonucleases CViQI, Eco53KI, and MScI (Thermofisher Scientific) respectively and incubated at 37°C for 16 hrs. The process of electrophoresis was employed to separate the DNA fragments that had undergone digestion, with the aim of achieving optimal resolution using a 3% agarose gel. The identification of genotypes for the three SNPs were performed through the detection of distinct band sizes represented in Fig. 1.

Table 2: Primers and restriction enzymes used in genotyping of TNFSF15 gene

Gene	Primer sequence (*5′ -3′*)	T_m (° C)	Amplicon size (bp)	Restriction enzyme	RFLP Product Size (bp)
TNFSF15 (1)	F: ACAGAGGAGCTAGGAAGATG	58.8	416	CViQI	Wild (TT)-416bp
rs7848647	R: TCCTGGCTCTACCACTTG				Homozygous (CC)-293bp, 123bp
					Heterozygous (CT)-416bp, 293bp, 123bp
TNFSF15 (2)	F: TCCTGGCTCTACCACTTG	57.7	256	Eco53KI	Wild (CC)-256bp
rs6478106	R: AGACGTTCTGACTACTATTCC				Homozygous (TT)-148bp, 108bp
					Heterozygous (CT)-256bp, 148bp, 108bp
TNFSF15 (3)	F: AAGGGCTCTCAGACATCATC	58.8	408	MScI	Wild (TT)-408bp
rs4979462	R: TCAAAGCATAGACACCACAAG				Homozygous (CC)-309bp, 99bp
					Heterozygous (CT)-408bp, 309bp, 99bp

TNFSF15: Tumor necrosis factor superfamily member 15

Quantitative Real-Time PCR: Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) using TRIzol reagent (Invitrogen, USA). RNA integrity was verified on 1% agarose gels and quantified by the 260/280nm absorbance ratio. Prior to complementary DNA (cDNA) synthesis, extracted RNA was treated with DNase I to remove any residual genomic DNA. cDNA was synthesised using a first-strand cDNA synthesis kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The IDT software was used for designing primers for TNFSF15 (forward, 5′-GCAGGACTCACCACATACC-3′; reverse, 5′-CCTTGGCTTAT-CTCCGTCTG-3′) with 139bp and Glyceraldehyde 3-phosphate dehydrogenase (forward, 5′-AGAAGGCTGGCTCATTTG-3′; reverse, 5′-AGGGGCCATCCACAGTTC-3′) with 260bp Fig. 3. Quantitative real-time Polymerase chain reaction (qRT-PCR) was performed using the Rotor-Gene Q system (Qiagen) with Maxima SYBR Green qPCR Master Mix (2x) (ThermoFisher Scientific). The outcome of the

experiment was standardized to the internal control gene GAPDH. The thermal cycling programme comprised initial activation at 95°C for 5 min, the mixture was subjected to 40 amplification cycles, consisting of three stages: denaturation at 95°C for 10 s, annealing at 60°C (for TNFSF15) for 30 s, and extension at 72°C for 30 s thereby accomplishing the qRt-PCR method. All amplified products were verified on 2% agarose gels in order to confirm the integrity of the desired product. Relative quantification was performed using the Livak method (2-ΔΔCt), with GAPDH functioning as the reference gene. The computation of the ΔCt for TNFSF15 was carried out through the subtraction of its Ct value from the Ct value of GAPDH (ΔCt= Ct TNFSF15-Ct GAPDH).

Statistical Analysis: Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using Fisher's exact test. The distribution of clinical manifestations across genotypes was evaluated using the chi-square test. Means and standard deviations were compared using the unpaired Student's t-test. Hardy-Weinberg equilibrium (HWE) was assessed for each SNP in the control population. All reported P-values were two-tailed, and a P-value < 0.05 was considered statistically significant.

Patient characteristics: Baseline demographic characteristics of SLE patients are presented in Table 1. All 130 patients were positive for ANA and were receiving hydroxychloroquine and less than 20mg of prednisolone/day. The cases included 4(3.07%) males and 126(96.92%) females with mean age of 30.43±9.44 years. The control group had 9(6%) males and 141(94%) females with mean age of 31.3±10.2 years. The age and gender distributions were not significantly different among cases and controls, suggesting an adequate frequency matching.

Genotyping: All samples included in the present study were genotyped for three TNFSF15 polymorphisms: rs4979462 located in a non-coding regulatory region, rs6478106 located in an upstream/promoter regulatory region, and rs7848647 located in an intronic regulatory region. The genotype and allele frequencies of the control group were found to be in agreement with Hardy–Weinberg equilibrium ($p > 0.05$), indicating that the studied population was genetically representative.

Among the investigated SNPs, rs4979462 and rs6478106 demonstrated significant associations with SLE susceptibility, whereas rs7848647 showed no significant association as shown in Table 3. For rs4979462, the TT genotype was significantly less frequent in SLE patients compared with healthy controls (38.46% vs. 68%), suggesting a protective effect against SLE (OR = 0.30, 95% CI: 0.18–0.49, $p < 0.0001$). Likewise, the dominant model (CT+TT) was significantly associated with decreased disease susceptibility (OR = 0.34, 95% CI: 0.20–0.59, $p < 0.0001$). Allelic analysis further revealed that the T allele conferred a significantly reduced risk of SLE compared with the C allele (OR = 0.32, 95% CI: 0.22–0.46, $p < 0.0001$).

In contrast, rs6478106 exhibited a significant positive association with SLE susceptibility. The TT genotype was markedly more frequent among SLE patients than controls (53.85% vs. 19.33%), corresponding to an approximately fourfold increased risk of SLE (OR = 4.0, 95% CI: 2.05–7.79, $p < 0.0001$). Similarly, the T allele was significantly associated with increased disease risk (OR = 2.44, 95% CI: 1.67–3.53, $p < 0.0001$). However, neither the CT genotype nor the dominant model (TT+CT) showed statistically significant associations.

No statistically significant differences in genotype distribution, dominant genetic model, or allele frequencies were observed for rs7848647 between SLE patients and controls ($p>0.05$), indicating that this polymorphism may not contribute to SLE susceptibility in the studied population.

Correlation of TNFSF15 gene polymorphism with clinical features in patients with SLE

In the present study, three TNFSF15 polymorphisms (rs4979462 C/T, rs6478106 C/T, and rs7848647 T/C) were investigated for their association with clinical and immunological manifestations of systemic lupus erythematosus (SLE) under a dominant/recessive genetic model as represented in Table 4. The analysis revealed differential and phenotype-specific associations, suggesting a potential modulatory role of TNFSF15 variants in disease expression. The rs4979462 polymorphism demonstrated significant associations with key clinical manifestations of SLE, including malar rash ($p = 0.03$), serositis ($p = 0.02$), and arthritis ($p = 0.02$) Fig.2. These observations indicate a possible role of this variant in the regulation of inflammatory tissue involvement, particularly affecting cutaneous and serosal systems. Importantly, no consistent associations were observed with hematological or serological parameters, suggesting that its effect may be restricted to specific clinical phenotypes rather than global disease susceptibility or systemic immunological activity. Among the evaluated polymorphisms, rs6478106 showed the most consistent and biologically relevant associations with immunological markers of disease activity. Significant associations were observed with photosensitivity ($p = 0.02$) and anti-dsDNA positivity ($p = 0.02$) Fig.2, along with a borderline association with complement level abnormalities ($p \approx 0.05$).

These findings suggest that rs6478106 may contribute to immune dysregulation and enhanced autoantibody formation, potentially reflecting its involvement in pathways governing systemic immune activation and disease severity in SLE.

Table 3: Genotype and allele frequency between cases and controls.

SNP	Cases (n=130)	Controls (n=150)	p-value, Odds ratio (95% CI)
rs4979462			
CC	69 (53.08%)	42 (28%)	Reference
CT	11 (8.46%)	6(4%)	1, 1.12 (0.85-3.24)
TT	50 (38.46%)	102(68%)	<0.0001, 0.30 (0.18-0.49)
CT+TT	61(46.92%)	108(72%)	<0.0001, 0.34 (0.20-0.59)
C allele	149	90	Reference
T allele	111	210	<0.0001, 0.32 (022-0.46)
rs6478106			
CC	26 (20%)	43 (28.67%)	Reference
TT	70 (53.85%)	29 (19.33%)	<0.0001, 4 (2.05-7.79)
CT	34 (26.15%)	78 (52%)	0.39, 0.72 (0.37-1.40)
TT+CT	104	107	0.09, 0.62 (0.36-1.08)
C allele	86	164	Reference
T allele	174	136	<0.0001, 2.44 (1.67-3.53)
rs7848647			
TT	23 (17.69%)	19 (12.67%)	Reference
CC	64 (49.23%)	78 (52%)	0.32, 0.68 (0.34-1.35)
CT	43 (33.08%)	53 (35.33%)	0.36, 0.67 (0.32-1.42)
CC+CT	107	131	0.28,0.74 (0.42-1.28)
T allele	89	91	Reference
C allele	171	209	0.41, 0.84 (0.57-1.23)

* p-value calculated by using 2×2 contingency table

In contrast, rs7848647 exhibited predominantly non-significant associations across clinical and laboratory parameters. Only borderline associations were observed with lupus nephritis and complement levels ($p \approx 0.05$). Overall, this variant appears to have a limited or modest influence on SLE phenotypic expression in the present cohort, which may reflect weak functional relevance or population-specific genetic effects.

TNFSF15-62 C/T

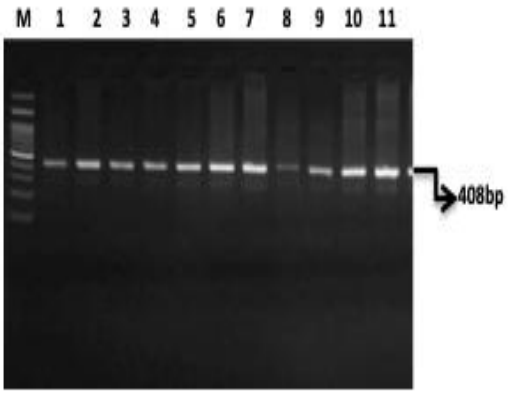


Fig. 1.1

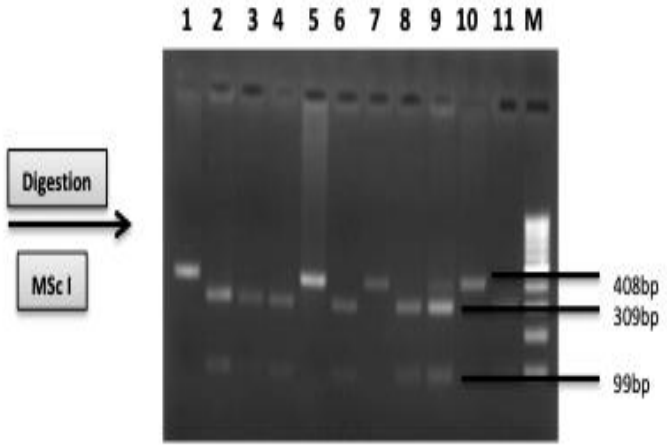
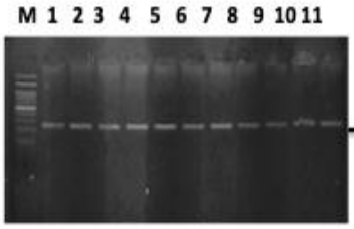


Fig 1.2

Table 4: Association of TNFSF15 polymorphism with Clinical characteristics												
Characteristics	**-rs4979462 C/T**				**-rs6478106 C/T**				**rs7848647 T/C**			
	CC n=26	**TT+CT n=104**	**p-value**	**χ2**	**CC n=26**	**TT+CT n=104**	**p-value**	**χ2**	**CC n=26**	**TT+CT n=104**	**p-value**	**χ2**
AGE (YEARS)												
<30 ≥30	10 16	49 55	0.42	0.56	34 35	28 33	0.70	0.05	35 29	36 30	0.99	0.0003
GENDER												
Female Male	24 2	102 2	0.26	0.69	6702	5803	0.47	0.52	631	633	0.32	0.97
ALOPECIA												
Present Absent	22 4	84 20	0.59	0.28	57 12	51 10	0.87	0.002	50 10	58 12	0.94	0.005
MALAR RASH												
Present Absent	14 12	78 26	0.03*	4.47	48 21	44 17	0.74	0.02	45 19	47 19	0.91	0.013
ORAL ULCER												
Present Absent	19 7	73 31	0.77	0.06	49 20	43 18	0.88	0.004	43 21	49 17	0.38	0.78
PHOTOSENSITIVITY												
Present Absent	16 10	79 25	0.13	2.26	49 20	32 29	0.02*	5.02	48 16	47 19	0.62	0.24
SEROSITIS												
Present Absent	4 22	3 101	0.02*	5.18	0465	0358	0.82	0.049	3 61	4 62	0.73	0.12
ARTHRITIS												
Present Absent	22 4	100 4	0.02*	4.79	6405	5704	0.87	0.021	62 2	59 7	0.09	2.83
DISCOID RASH												
Present Absent	20 6	83 21	0.74	0.02	55 14	48 13	0.88	0.022	47 17	56 10	0.10	2.58
PROTEINURIA												
Present Absent	18 8	57 47	0.18	1.76	43 26	32 29	0.29	1.29	33 31	42 24	0.16	1.94
THROMBOCYTOPENIA												
Present Absent	20 6	77 27	0.76	0.003	49 20	48 13	0.31	1.02	46 18	51 15	0.48	0.49
CRP												
Elevated Normal	10 16	35 69	0.64	0.22	19 50	26 43	0.20	1.61	17 47	28 38	0.05	3.61
ESR												
Elevated Normal	16 10	54 50	0.37	0.64	38 31	32 29	0.76	0.09	37 27	33 33	0.37	0.80
LUPUS NEPHRITIS												
Present Absent	10 16	43 61	0.78	0.07	28 41	26 35	0.81	0.055	19 41	34 36	0.05	3.82
COMPLEMENT LEVELS												
Low levels Normal levels	14 12	58 46	0.86	0.03	42 27	27 34	0.05	3.57	30 34	42 24	0.05	3.70
ANTI-dsDNA												
Positive Negative	21 5	80 24	0.67	0.10	59 10	42 19	0.02*	5.16	53 11	48 18	0.17	1.91
ANTI-SMITH												
Positive Negative	15 11	51 53	0.42	0.67	42 27	28 33	0.08	2.92	30 34	36 30	0.38	0.76

CRP= C-reactive protein, ESR= Erythrocyte sedimentation rate,

TNFSF15-06 C/T-



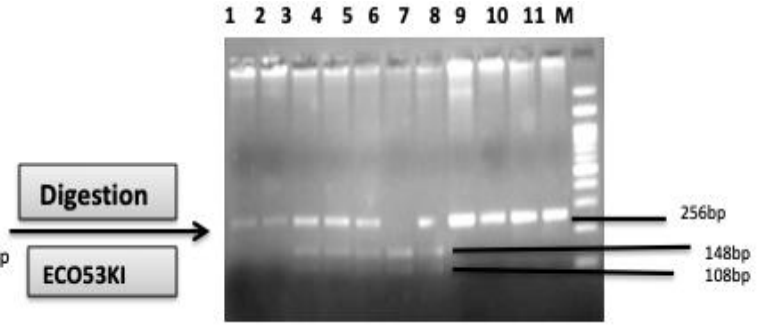
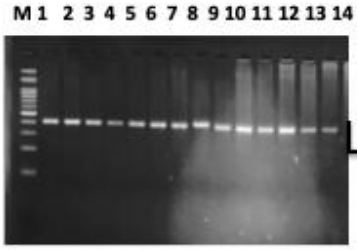


Fig. 1.3 Fig. 1.4

TNFSF15-47 T/C



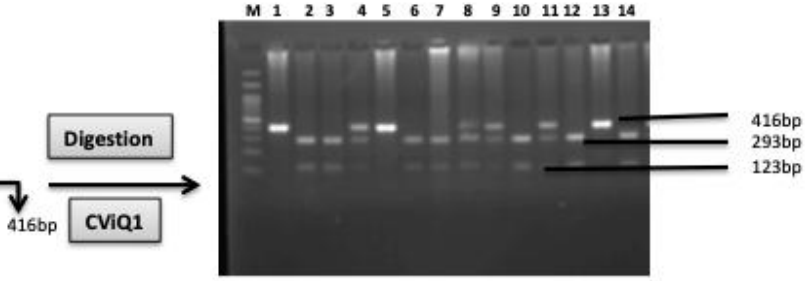
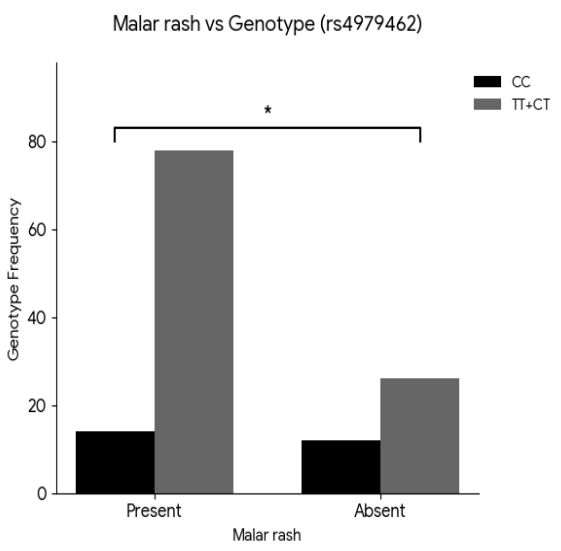
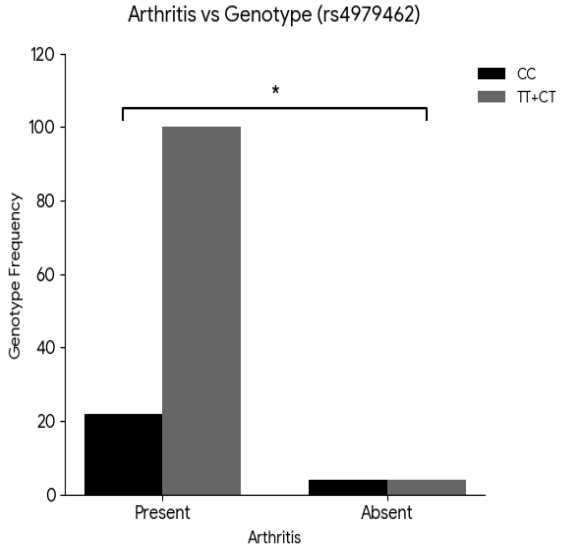


Fig. 1.5 Fig. 1.6

Fig. 1. Representative gel picture showing 1.1- TNFSF15-47 amplicon. 1.2- RFLP analysis of TNFSF15-47 PCR product. Lane M: 100bp marker & Lanes 1, 5 & 13: Shows wild TT genotype, Lane 2, 3, 6, 7, 10, 12 & 14: Shows homozygous CC genotype, & Lane 4, 8, 9 & 11: Shows heterozygous TC genotype. 1.3- TNFSF15-06 amplicon. 1.4- RFLP analysis of TNFSF15-06 PCR product. Lane M: 100bp marker, Lane 1, 2, 8, 9, 10 & 11: Shows wild CC genotype, Lane 6: Shows homozygous TT genotype & Lane 3, 4, 5 & 7: Shows heterozygous TC genotype. 1.5- TNFSF15-62 amplicon. Lane M: 100bp ladder. Lanes 1–5: 506bp amplicon of TNFSF15-62 gene in different DNA samples. 1.6- RFLP analysis of TNFSF15-62 PCR product. Lane M: 100bp ladder. Lane 1, 5, 7 & 10: Shows wild TT genotype, Lane 2, 3, 4, 6, 8 & 11: Shows homozygous CC genotype, & Lane 9: Shows heterozygous TC variant genotype.





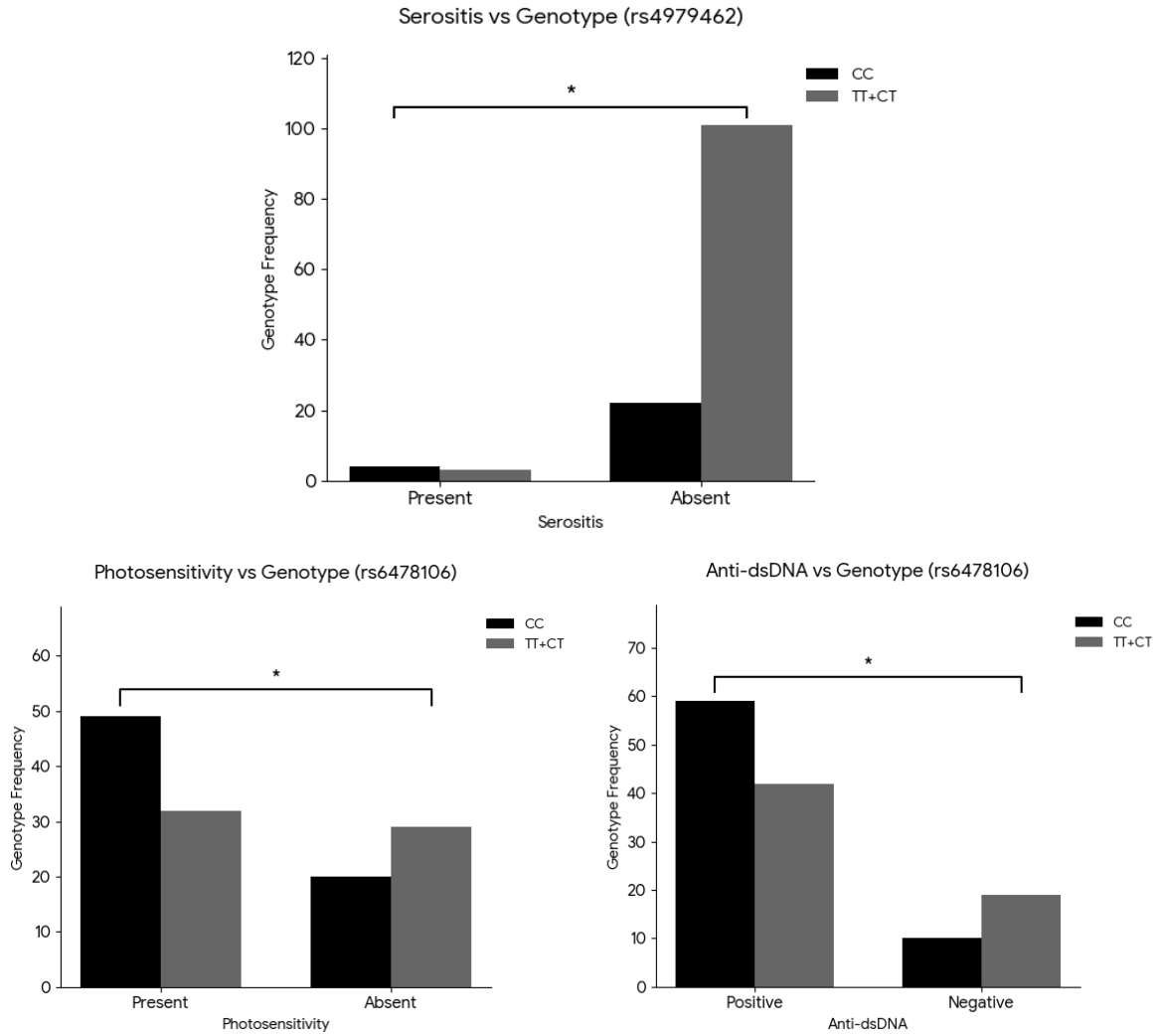


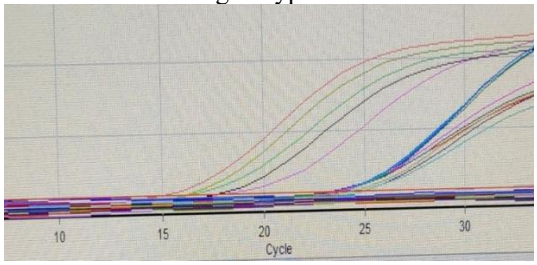
Fig. 2: Bar chart showing association of various characteristics with genotypic distribution.

Expression of TNFSF15 mRNA Levels in patients with systemic lupus erythematosus

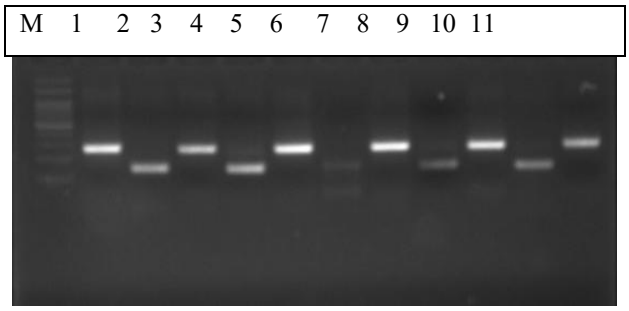
To evaluate transcriptional activity, TNFSF15 mRNA levels were measured in 40 SLE patients and 20 healthy subjects, with the latter serving as the control cohort for comparative analysis and it was found that expression levels of TNFSF15 mRNA was significantly elevated in SLE patients with mean ± standard deviation (SD) of 2.23±1.14 (p-value=0.0004; Fig 4) compared to controls. However, we could not find any significant association of TNFSF15 mRNA expression with various clinical characteristics of patients with SLE (P>0.05).

Correlation of TNFSF15 gene polymorphisms with TNFSF15 mRNA in patients with SLE

Furthermore, through analyzing the relationship between different SNPs and mRNA levels within TNFSF15, the patients who carried the TT genotype of rs6478106 had a higher TNFSF15 mRNA with mean ± standard deviation (SD) of 5.4±4.13 than that of the CC+CT genotype with mean ± standard deviation (SD) of 4.37±3.84, but the rs4979462 CC genotype carriers appeared to be associated with the decreased TNFSF15 mRNA with mean ± standard deviation (SD) of 3.00±2.36 on comparing with that of the CT+TT genotype with mean ± standard deviation (SD) of 5.32±4.87 (P=0.04) Fig 5. No significant associations were found between rs7848647 genotype and TNFSF15 mRNA (P>0.05).



a. Amplification plot of TNFSF15 and GAPDH genes.



→ → →

b. Gel picture

Figure 3: a. Representative picture of TNFSF15 qPCR analysis.
b.:
Lane M: Marker DNA ladder 100bp
Lane 1, 3, 5, 7, 9 & 11: Represents 260bp amplicon of GAPDH Gene
Lane 2 , 4, 8 and 10: Represents 139bp amplicon of TNFSF15 Gene
Lane 6: Blank

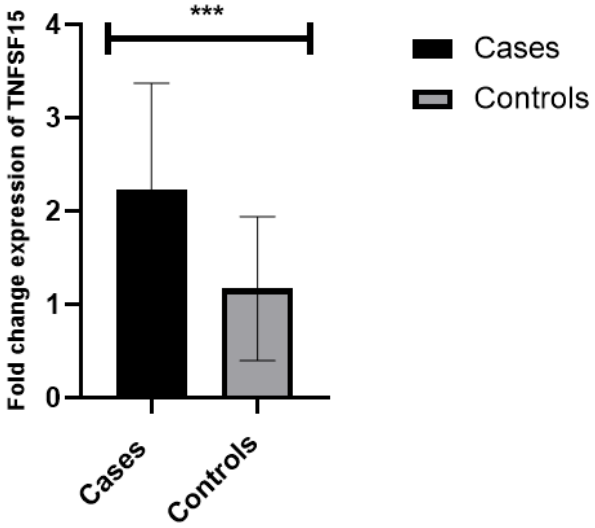


Fig. 4: Bar chart showing association between relative mRNA expression level of TNFSF15 and cases and controls.

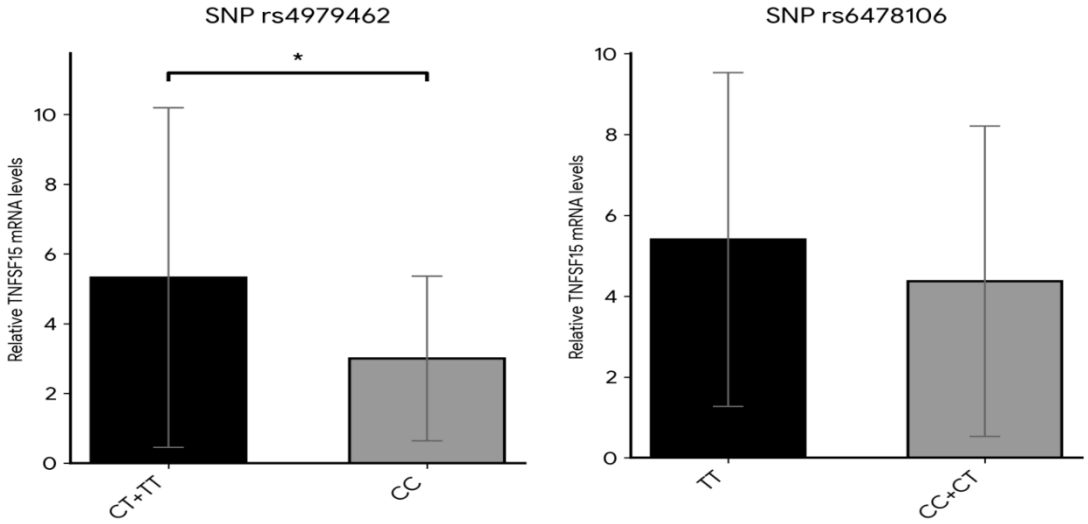


Fig. 5: Bar chart showing association between TNFSF15 SNPs and relative mRNA expression levels.

DISCUSSION

The genetic architecture of Systemic Lupus Erythematosus (SLE) is characterized by significant ethnic heterogeneity. This study investigated the association of TNFSF15 (TL1A) polymorphisms and mRNA expression with SLE in the Kashmiri population, providing evidence that genetic variations in the promoter and regulatory regions of this gene significantly modulate both disease susceptibility and clinical presentation.

Genetic Susceptibility and Allelic Heterogeneity

Our findings identified rs6478106 as a risk factor for SLE, with the TT genotype conferring a four-fold increase in disease risk (OR = 4.0). This aligns with the understanding that the upstream promoter regions of TNFSF15 are critical in determining transcriptional activity. Similar associations with TNFSF15 promoter variants have been observed in other autoimmune diseases, such as Crohn's disease, where regulatory SNPs are thought to disrupt binding sites for transcriptional repressors [14].

Conversely, the rs4979462 TT genotype demonstrated a strong protective effect in our cohort (OR = 0.30). While previous studies in East Asian populations have identified this locus as a susceptibility marker for various inflammatory conditions [15], the specific "protective" versus "risk" allele often shifts across different geographic populations. The association found here suggests that rs4979462 is a primary marker of immune homeostasis in the North Indian population. Notably, we found no significant association for rs7848647 ($p > 0.05$). This is a striking contrast to large-scale genome-wide association studies (GWAS) and meta-analyses in Caucasian and East Asian cohorts, where rs7848647 is frequently cited as a major risk variant for inflammatory bowel disease (IBD) and other systemic autoimmune conditions [16]. This discrepancy underscores the importance of population-specific studies; a variant that serves as a universal marker in one ethnic group may be non-contributory in another due to differences in linkage disequilibrium (LD) blocks.

Genotype and mRNA Expression

A significant contribution of this study is the correlation between genotype and functional output. We observed a significant elevation of TNFSF15 mRNA in SLE patients ($p = 0.0004$), supporting the hypothesis that TL1A acts as a pro-inflammatory driver in SLE pathogenesis.

Our data shows that the rs6478106 TT risk genotype correlates with higher mRNA expression, while the rs4979462 CC genotype is associated with significantly decreased expression ($p = 0.04$). In the context of SLE, overexpressed TL1A likely facilitates disease progression by enhancing the costimulation of T-cells and the production of autoantibodies by B-cells. This functional mechanism mirrors findings in Rheumatoid Arthritis (RA), where elevated TL1A levels in synovial fluid have been directly linked to the severity of joint inflammation [17].

Clinical Phenotypes and Disease Severity

The association of specific genotypes with clinical manifestations—such as rs6478106 with anti-dsDNA positivity and photosensitivity—suggests that TNFSF15 is not merely a marker of disease presence but a modulator of disease severity. The correlation between the rs6478106 risk allele and anti-dsDNA suggests that TNFSF15 signalling may specifically drive the loss of B-cell tolerance. Similar phenotype-specific associations have been noted in other cytokine genes, where genetic variants dictate the specific organ involvement (e.g., lupus nephritis or cutaneous manifestations) [18]. (Harley et al., 2008). A key finding of this study is the significant association between rs4979462 polymorphism and specific clinical manifestations of SLE, including malar rash ($p = 0.03$), serositis ($p = 0.02$), and arthritis ($p = 0.02$). These results suggest that TNFSF15 genetic variations do not merely influence disease susceptibility but also play a critical role in modulating the clinical heterogeneity of SLE. Our findings regarding these specific phenotypes are strongly supported by the work of Wang and Tu (2018) [19]. In their study of a Chinese cohort, they similarly identified that the rs4979462 polymorphism was significantly associated with butterfly (malar) rash, arthritis, and serositis ($p < 0.05$ for all). The consistency across different populations—Kashmiri and Chinese—underscores the robust nature of this SNP as a phenotypic marker.

CONCLUSION

In conclusion, our study demonstrates that TNFSF15 polymorphisms rs6478106 and rs4979462 are significant determinants of SLE risk in the Kashmiri population. The correlation between these risk genotypes and elevated mRNA expression provides a clear functional pathway for how these variants contribute to the chronic inflammatory state of SLE. These findings highlight TNFSF15 as a potential biomarker for disease susceptibility.

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