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Original Article

Tumor Necrosis Factor Superfamily Member 15 (*TNFSF15*) rs4979462 Variant and TNFSF15 Serum Levels Evaluation in Systemic Lupus Erythematosus

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Abstract

Background and objectives: Tumor necrosis factor (TNF) superfamily member 15 (TNFSF15) may have the potential to control vascular homeostasis and inflammation. Through binding to death receptor 3 (TNFRSF25), TNFSF15 promotes T-cell activation, proliferation, and the generation of multiple cytokines. TNFSF15-TNFRSF25 signaling is essential for effective T-cell immune responses in T-cell-mediated autoimmune diseases. Our goal is to study the role of the (*TNFSF15*) rs4979462 gene variant and TNFSF15 serum levels in systemic lupus erythematosus (SLE) in Egyptian patients.

Methods: A total of 118 patients with SLE and 102 age- and sex-matched healthy control volunteers were genotyped for the *TNFSF15* rs4979462 variant by polymerase chain reaction-restriction fragment length polymorphism and verified by direct sequencing. TNFSF15 serum levels were measured using an enzyme-linked immunosorbent assay.

Results: Regarding the *TNFSF15* rs4979462 gene variant, there was a significant increase in the frequencies of combined genotypes (CT + TT) and T-allele among female patients with SLE compared with the healthy female subjects (OR = 2.6, 95% CI = 1.1–6.3, p = 0.027; OR = 2.7, 95% CI = 1.2–6.3, p = 0.015, respectively). The T-variant was significantly associated with serositis and thrombotic manifestations (OR = 2.8, 95% CI = 1.1–7.1, p = 0.032; OR = 2.9, 95% CI = 1.1–7.8, p = 0.023, respectively). The median serum TNFSF15 concentration was significantly higher in patients with SLE compared to the healthy control group and was correlated with the disease activity (p = 0.023, 0.012, respectively).

Conclusions: The *TNFSF15* rs4979462 gene variant increases the risk of SLE in female subjects and modulates the clinical outcome of the disease. TNFSF15 serum level could be a biological marker of SLE disease activity.

Keywords: Systemic lupus erythematosus; *TNFSF15*; TNF-like ligand 1A; rs4979462; Single nucleotide variations.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence intervals; DR3, death receptor 3; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; HC, healthy control; HDLc, high-density lipoprotein cholesterol; IL, interleukin; LDLc, low-density lipoprotein cholesterol; NF-1, nuclear factor-1; OR, odds ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; TGs, triglycerides; Th, T helper; TL1A, TNF-like ligand 1A; TNF, tumor necrosis factor; TNFSF15, TNF superfamily member 15; uVTE, unprovoked venous thromboembolism; WBC, white blood cell.

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Introduction

Multiple organs and systems are affected by systemic lupus erythematosus (SLE), which is a chronic autoimmune inflammatory disease affecting primarily young adults, especially women of childbearing age.[1] Epidemiological data indicated that SLE prevalence ranges between 0 to 241 per 100,000 people worldwide, with regional and racial variations.[2] The exact pathogenesis that causes SLE development remains unclear.[3] Lupus develops because of abnormalities in the innate and adaptive immune system as well as environmental and genetic factors.[4] Recently, it was found that numerous loci are prone to SLE development.[5]

Tumor necrosis factor (TNF) superfamily member 15 (TNFSF15), a member of the TNF-α superfamily, is a proinflammatory cytokine also known as vascular endothelial growth inhibitor or TNF-like ligand 1A (TL1A).[6] It can serve as an essential

modulator of inflammation and vascular homeostasis.[7] Binding to its receptor, death receptor 3 (DR3, TNFRSF25), which is predominantly expressed in lymphocytes, TNFSF15 triggers the proliferation of T effector cells and cytokine production by these cells.[8] Effective T-cell immune responses in T-cell-mediated autoimmune and inflammatory illnesses, cell proliferation and death, angiogenesis, and tumor metastasis depend on TNFSF15-NFRSF25 signaling.[9] The *TNFSF15* gene, which has four exons and three introns and is found on chromosome 9 (9q32), encodes the TNFSF15 cytokine.[10,11]

The *TNFSF15* gene is polymorphic, and its variants induce the production of altered TNFSF15, aiding in the development of autoimmune and inflammatory disorders.[12] Many disorders, including tumors, Crohn's disease, ulcerative colitis, and other autoimmune diseases, have been linked to *TNFSF15* variations.[11,13] An earlier study in the Chinese population has found a strong association between the *TNFSF15* gene polymorphism and susceptibility to SLE.[14]

The substitution variant *TNFSF15* rs4979462 C>T has thymine instead of cytosine in the first intron of the *TNFSF15* gene. The rs4979462 variant lies in the transcription regulatory elements of the *TNFSF15* gene; consequently, the affinity for binding of the transcription factors could vary from the major to the minor sequence variants.[15]

We aimed to assess the role of the *TNFSF15* rs4979462 variant and the TNFSF15 cytokine in SLE in Egyptian patients.

Materials and methods

Patients

SLE patients were diagnosed according to the 2012 Systemic Lupus International Collaborating Clinics classification criteria and were collected from the Rheumatology and Rehabilitation Department outpatients clinic, Kasr Al-Ainy Hospitals, Faculty of Medicine, Cairo University.[16] Patients under 18 years of age and those suffering from other autoimmune or inflammatory illnesses were excluded from the study.

Complete clinical examination and history-taking were performed for each patient. The systemic lupus erythematosus disease activity index (SLEDAI) score was used to measure disease activity for all patients at the time of their enrollment in our study.[17]

Laboratory methods

Sample collection

A 5-mL sample of venous blood was obtained from all subjects under aseptic conditions: 2 mL were collected in an Ethylenediaminetetraacetic acid vacutainer and stored at −20°C until the genotyping time, and the other 3 mL were collected in a plain tube; serum was extracted and stored at −20°C till the time of measurement of TNFSF15 serum levels.

TNFSF15 rs4979462 genotyping

The GeneJet Whole Blood Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract the DNA from the Ethylenediaminetetraacetic acid whole blood. The extracted DNA was genotyped for the *TNFSF15* rs4979462 variant using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The total volume of the reaction mixture was 25.0 µL comprising 1.0 µL of each primer, 12.5 µL of the master mix, 3.0 µL of extracted DNA, and 7.5 µL of distilled water. The sequences of the primers were as follows: Forward primer: 5′-AAGGGCTCTCAGACATCATC-3′; Reverse primer: 5′-TCAAAGCATAGACACCACAAG-3′.

The thermal cycler was programmed as in the Han *et al.* protocol: denaturation at 94°C for 10 m, 35 cycles of melting for 30 s at 94°C, annealing for 35 s at 60°C, and extension for 60 s at 72°C, followed by final elongation at 72°C for 5 m.[18]

The 407-bp amplicon was subjected to digestion by *Msc*I restriction enzyme and analyzed at 2% agarose gel (Fig. 1).

Direct sequencing technique

Randomly selected samples from each genotype were repeated using the direct sequencing technique to confirm PCR-RFLP results (Fig. 2). The first step included the purification of the amplified PCR products through the PCR Purification Kit (Qiagen, Hilden, Germany). The second step was the cycle sequencing using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Waltham, MA, USA). Thereafter, cleaning of the reactions was conducted using the BigDye XTerminator Purification Kit. Finally, the cleaned-up products were injected into a genetic sequencer instrument (ABI 3500; Applied Biosystems Inc.).

Measurement of TNFSF15 serum levels

The serum levels of TNFSF15 were quantified by an enzyme-linked immunosorbent assay (ELISA) using the Bioassay Technology Laboratory human TNFSF15 ELISA kit (Bioassay Technology Laboratory, Shanghai, China).

Statistical analysis

Data about the patients were statistically analyzed using Statistical Package for the Social Sciences version 21 software (IBM Corp., Armonk, NY, USA). For parameters with normally distributed distribution, quantitative data were presented as a mean and standard deviation; for parameters with non-normally distributed distribution, median and percentiles were employed. The frequency and percentage were used to present qualitative data. The nonparametric Mann-Whitney *U* test and the student's test were employed to compare sets of quantitative variables. For qualitative variables, the chi-square test and Fisher's exact test were applied. The correlation analysis was evaluated using the Spearman coefficient. A *p*-value less than 0.05 was considered significant.

Results

Two hundred and twenty subjects (118 SLE patients and 102 healthy control volunteers with matching age and sex) participated in this study. The mean age of the SLE patients, which included 16 men (13.6%) and 102 women (86.4%), was 32.9 ± 9.6 years. The healthy control group included 94 females (92.2%) and eight males (7.8%), with an average age of 32.6 ± 8.6 years. The descriptive data of patients with SLE is shown in Table 1.

Distribution of TNFSF15 rs4979462 in the groups being studied

The *TNFSF15* rs4979462 variant was in Hardy–Weinberg equilibrium. The minor allele frequency was 0.10 in the patients' group and 0.05 in the control group.

There were no significant differences in genotypes and allele distribution between SLE patients and healthy control groups (Table 2). However, the frequencies of the combined (CT+TT) genotypes and T-allele were significantly higher in female SLE patients

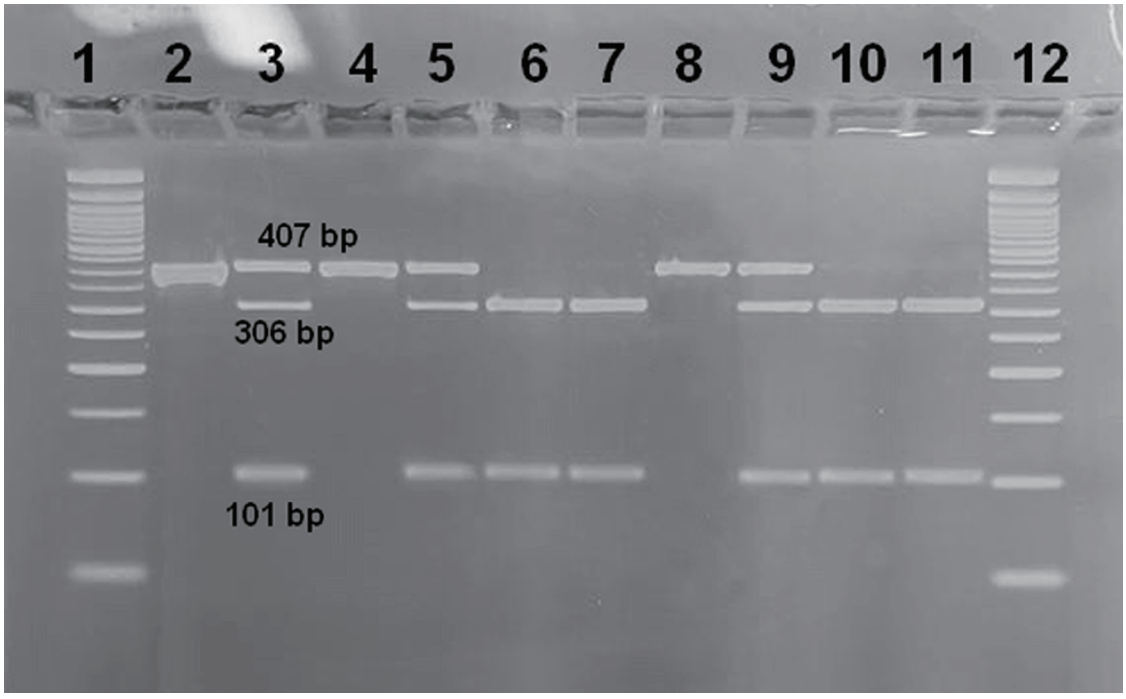

Fig. 1. Agarose gel showing RFLP analysis of the *TNFSF15 rs4979462* variant. Lanes 1 and 12 show 50 bp DNA ladder; Lane 2 shows the 407-bp amplicon; Lanes 4 and 8 show the homozygous TT genotype; Lanes 3, 5, and 9 show the heterozygous CT genotype; Lanes 6, 7, 10, and 11 show the homozygous CC genotype. *TNFSF15*, TNF superfamily member 15; RFLP, restriction fragment length polymorphism.

than in female control subjects [19.6% vs. 8.5%, odds ratio (OR) = 2.6, 95% confidence interval (CI) = 1.1–6.3, $p = 0.027$; 10.8% vs. 4.3%, OR = 2.7, 95% CI = 1.2–6.3, $p = 0.015$, respectively; Table 3]. In contrast, the distribution of the *TNFSF15* rs4979462 variant did not significantly differ in the male group.

Association of the TNFSF15 rs4979462 variant with the clinical and laboratory characteristics of SLE

The frequency of the combined genotypes (CT + TT) of *TNFSF15* rs4979462 was significantly higher in patients with SLE presenting with serositis and thrombotic manifestations (59.1% vs. 34.4%, OR = 2.8, 95% CI = 1.1–7.1, $p = 0.032$; 45.5% vs. 21.9%, OR = 2.9, 95% CI = 1.1–7.8, $p = 0.023$, respectively; Figs. 3 and 4). However, there was no significant association between *TNFSF15* rs4979462 and other clinical phenotypes of SLE.

Regarding the laboratory characteristics of patients with SLE, there was no significant association between the *TNFSF15* rs4979462 genotypes and any laboratory SLE finding.

TNFSF15 serum levels in the groups being studied

The median TNFSF15 serum levels were significantly higher in patients than in the healthy control subjects [11.4 (10.0–14.6) vs. 10.5 (9.6–12.2) ng/mL, $p = 0.023$] (Fig. 5).

The associations and correlation of TNFSF15 serum levels with the clinical phenotypes, disease activity, and laboratory features of SLE were further analyzed.

The TNFSF15 serum levels were significantly higher in patients with high disease activity who had SLEDAI scores of more than 20 than in those with lower activity [13.7 (10.3–15.2) vs. 10.8 (10.0–13.3) ng/mL, $p = 0.031$] (Fig. 6). In addition, there was a sig-

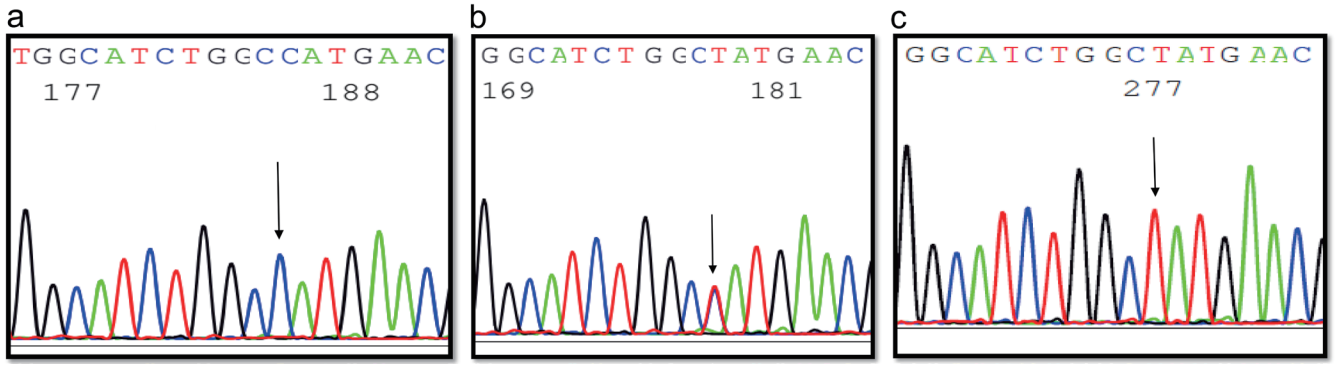

Fig. 2. Sequencing results of the *TNFSF15 rs4979462* variant. (a) The arrow indicates the presence of the major allele C. (b) The arrow indicates the presence of the heterozygous C/T genotype. (c) The arrow indicates the presence of the minor allele T instead of C at the polymorphic site. *TNFSF15*, TNF superfamily member 15.

Table 1. Descriptive data of patients with SLE

Feature	SLE patients, *n* = 118
Age in years	32.9 ± 9.6
Male sex, n (%)	16 (13.6)
Clinical manifestations, n (%)	
Malar rash	60 (50.8)
Vasculitic rash	25 (21.2)
Discoid rash	9 (7.6)
Alopecia	54 (45.8)
Oral ulcers	50 (42.4)
Photosensitivity	46 (39.0)
Arthritis	72 (61.0)
Serositis	46 (39.0)
Vasculitis	24 (20.3)
Thrombotic manifestations	31 (26.3)
Neurological manifestations	55 (46.6)
Nephritis	73 (61.9)
SLEDAI	18 (12–27)
High disease activity, SLEDAI >20	50 (42.4)
Renal SLEDAI	4 (0–8)
Laboratory findings	
Hb in g/dL	10.9 ± 2.1
WBC count in cell/mm^3	7.2 ± 3.1
Lymphocytes in cell/ mm^3	26.5 ± 11.9
Platelet count in platelets/mm^3	263.8 ± 108.2
ESR in mm/h	55.0 ± 32.7
Albumin in g/dL	3.4 ± 0.7
AST in IU/L	20 (15–29)
ALT in IU/L	19 (14–26)
Urea in mg/dL	38 (25–68)
Creatinine in mg/dL	0.9 (0.7–1.3)
Protein in urine in g/day	1.1 (0.4–2.6)
TGs in mg/dL	140 (100–247)
Cholesterol in mg/dL	204 ± 74
LDLc in mg/dL	132 ± 49
HDLc in mg/dL	48 (42–63)
Urinary findings, n (%)	
Hematuria	33 (28)
Proteinuria	67 (56.8)
Pyuria	43 (36.4)
Casts	12 (10.2)
Low complement, *n* = 105	86 (91.9)
Antinuclear antibodies, *n* = 113	108 (95.6)
Anti-ds-DNA antibodies, *n* = 84	65 (77.4)
Antiphospholipid antibodies, *n* = 71	23 (32.4)

Variables with normal distribution are presented as mean ± standard deviation; Skewed variables are presented as the median (interquartile range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; SLE, Systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; TG, triglyceride; WBC, white blood cell.

nificant positive correlation between TNFSF15 serum levels and the total SLEDAI score of patients with SLE ($r = 0.230$, $p = 0.012$; Table 4).

The patients with SLE who presented with hematuria and urinary casts had higher TNFSF15 serum levels than those without such urinary abnormalities [14.0 (10.5–17.2) vs. 10.8 (10.0–13.6) ng/mL, $p = 0.008$; 14.6 (12.8–15.5) vs. 10.95 (10.0–14.2) ng/mL, $p = 0.019$, respectively] (Figs. 7 and 8). In the same context, the TNFSF15 serum levels were significantly correlated with the renal SLEDAI score of patients with SLE ($r = 0.228$, $p = 0.013$; Table 4).

Furthermore, there were significant positive correlations between TNFSF15 serum levels and both the total and LDL cholesterol serum levels ($r = 0.326$, $p = 0.040$; $r = 0.348$, $p = 0.040$, respectively; Table 4).

Discussion

SLE is an autoimmune disease with an affection of multiple organs, its etiology and pathogenesis remain unknown.[19] The development of SLE was found to be influenced by multiple genetic factors.[20] Single nucleotide variations have become a vital tool to detect disease susceptibility genes, clarifying the pathogenic mechanisms of SLE and discovering new therapeutic approaches.[21]

We studied the role of the *TNFSF15* rs4979462 variant and the TNFSF15 cytokine in SLE in an Egyptian population.

There was no significant difference between SLE patients and healthy controls in the distribution of the *TNFSF15* rs4979462 variant. However, when our study subjects were accordingly stratified by sex, combined genotypes (CT + TT) genotypes and T-allele were significantly higher in female patients with SLE than in female healthy subjects (19.6% vs. 8.5%, OR = 2.6, 95% CI = 1.1–6.3, $p = 0.027$; 10.8% vs. 4.3%, OR = 2.7, 95% CI = 1.2–6.3, $p = 0.015$, respectively). In contrast, there was no such significant difference in the male subjects of our study population. This suggested that the *TNFSF15* rs4979462 variant is associated with a higher risk of SLE development in the female subjects of the Egyptian population.

In agreement with our results, Wang and Tu's study in 2018 has reported a significant association between the combined (CT + TT) genotypes and T-allele of *TNFSF15* rs4979462 variant and a higher risk of SLE in the Chinese population.[14]

In our study, the association between the *TNFSF15* rs4979462 variant and the clinical phenotypes of SLE was further analyzed. There was a significant association between the T-variant and the development of serositis and thrombotic manifestations in SLE patients' group (OR = 2.8, 95% CI = 1.1–7.1, $p = 0.032$; OR = 2.9, 95% CI = 1.1–7.8, $p = 0.023$, respectively). In Wang and Tu's study,[14] the authors found a significant association between the *TNFSF15* rs4979462 variant and butterfly rash, serositis, renal nephritis, and arthritis.

In this study, the median TNFSF15 serum levels were significantly higher in patients with SLE than in the healthy control subjects [11.4 (10.0–14.6) vs. 10.5 (9.6–12.2) ng/mL, $p = 0.023$]. The same results were found by Xu *et al.* in studies conducted in 2015 and 2019 on the Chinese population.[22,23] In the same context, Wang and Tu reported in their study a significantly higher level of *TNFSF15* mRNA in the SLE group than in the healthy group.[14]

The association and correlation of TNFSF15 serum levels with the clinical phenotypes, disease activity, and laboratory features of SLE were further analyzed. There was a significant association between high TNFSF15 serum levels and renal impairment in our patients with SLE, whereas patients with hematuria and

Table 2. Distribution of the *TNFSF15* rs4979462 variant in the studied groups

Genotype	SLE, *n* = 118	HC, *n* = 102	*p*-value	OR (95% CI)
CC	96 (81.4%)	91 (89.2%)	0.17	
CT	20 (16.9%)	11 (10.8%)		
TT	2 (1.7%)	0 (0.0%)		
CC	96 (81.4%)	91 (89.2%)		1.00
CT-TT	22 (18.6%)	11 (10.8%)	0.10	1.9 (0.9–4.1)
C (Major allele)	212 (90%)	193 (95%)		1
T (Minor allele)	24 (10%)	11 (5%)	0.06	1.9 (0.9–4.2)

CI, confidence interval; HC, healthy control; SLE, systemic lupus erythematosus; OR, odds ratio; *TNFSF15*, TNF superfamily member 15.

Table 3. *TNFSF15 rs4979462* variant distribution in the female subjects

Genotype	SLE, *n* = 102	HC, *n* = 94	*p*-value	OR (95% CI)
CC	82 (80.4%)	86 (91.5%)	0.060	
CT	18 (17.6%)	8 (8.5%)		
TT	2 (2.0%)	0 (0.0%)		
CC	82 (80.4%)	86 (91.5%)		1.00
CT-TT	20 (19.6%)	8 (8.5%)	0.027	2.6 (1.1–6.3)
C (Major allele)	182 (89.2%)	180 (95.7%)		1
T (Minor allele)	22 (10.8%)	8 (4.3%)	0.015	2.7 (1.2–6.3)

CI, confidence intervals; HC, healthy control; SLE, systemic lupus erythematosus; OR, odds ratio; *TNFSF15,* TNF superfamily member 15.

urinary casts had higher TNFSF15 serum levels [14.0 (10.5–17.2) vs. 10.8 (10.0–13.6) ng/mL, $p = 0.008$; 14.6 (12.8–15.5) vs. 10.9 (10.0–14.2) ng/mL, $p = 0.019$, respectively]. Moreover, there was a significantly positive correlation between TNFSF15 serum levels and the renal SLEDAI score ($r = 0.228$, $p = 0.013$).

In line with our findings, Al-Lamki *et al.* reported that TNFSF15 plays a significant role in renal tubular inflammation and injury.[24]

In this study, a significant association was found between TNFSF15 serum levels and SLE disease activity, where there was a significant positive correlation between TNFSF15 serum levels and the total SLEDAI scores of SLE patients ($r = 0.230$, $p = 0.012$). In addition, the TNFSF15 serum levels were significantly higher in patients with SLEDAI scores >20 than in those with lower SLEDAI scores [13.7 (10.3–15.2) vs. 10.8 (10.0–13.3) ng/mL, $p = 0.03$]. This comes hand in hand with the results of Xu *et al.* where the TNFSF15 serum levels were significantly higher in the newly diagnosed SLE patients with high disease activity and SLEDAI scores than in those with lower disease activity.[22] In addition, they reported a significant positive correlation between TNFSF15 serum levels and the SLEDAI score of their SLE patients.

The interesting finding in our study was that a significant positive correlation between TNFSF15 serum levels and both the to-

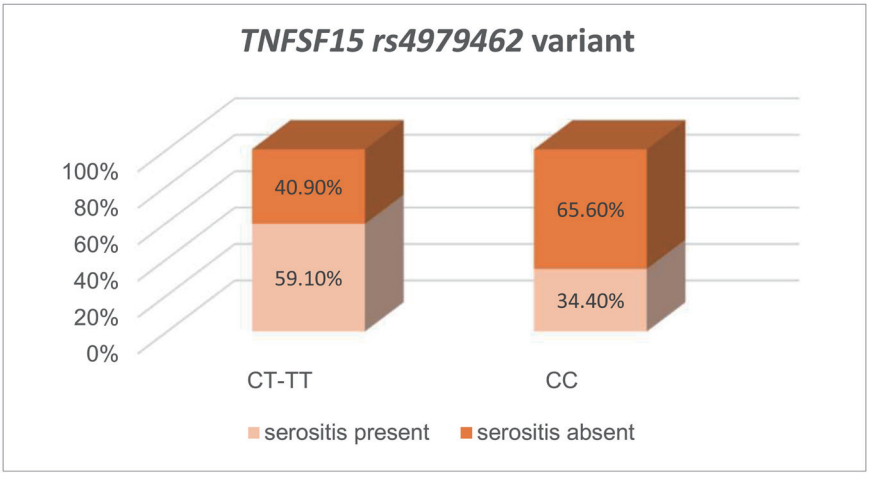

Fig. 3. Percentage of patients with serositis among the *TNFSF15* rs4979462 genotypes. *TNFSF15,* TNF superfamily member 15.

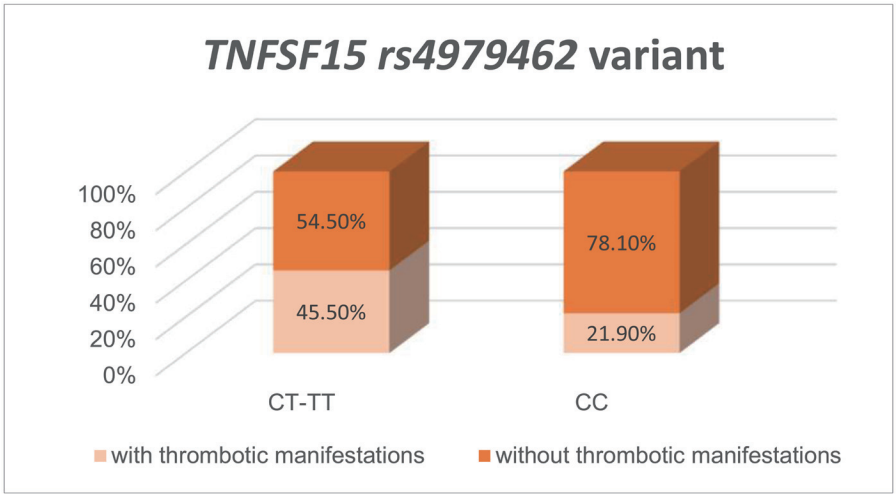

Fig. 4. Percentage of patients with thrombotic manifestations among the *TNFSF15* rs4979462 genotypes. *TNFSF15*, TNF superfamily member 15.

tal cholesterol and LDL cholesterol serum levels was found (r = 0.326, p = 0.040; r = 0.348, p = 0.040, respectively). As mentioned previously, the T-variant of *TNFSF15* rs4979462 was significantly associated with the thrombotic manifestations of our SLE patients (OR = 2.9, 95% CI = 1.1–7.8, p = 0.023). Recent studies proved that both arterial and venous thrombosis share the same risk factors, and dyslipidemia is presenting one of the most important.[25–27] Accordingly, our results suggest that both the TNFSF15 protein and *TNFSF15* rs4979462 variant play significant roles in developing dyslipidemia and thrombotic events. This could be through the effect of the rs4979462 variant on the *TNFSF15* gene function and transcription level.

In harmony with our results, in a recent study by Della Bella *et al.* investigated the underlying pathogenesis of unprovoked venous thromboembolism (uVTE),[28] the authors discovered the upregulation of TNFSF15 and its receptor TNFRSF25 (DR3) in the endothelial colony-forming cells isolated from the peripheral blood of patients with uVTE. In addition, the TNFSF15 levels were elevated in the sera of patients with uVTE. The authors further conducted functional analysis through blocking experiments; they proved that upregulation of the TNFSF15-TNFRSF25 axis impairs endothelial repair by minimizing the survival and proliferation of endothelial colony-forming cells and thus contributing to the pathogenesis of uVTE. According to the results of our study

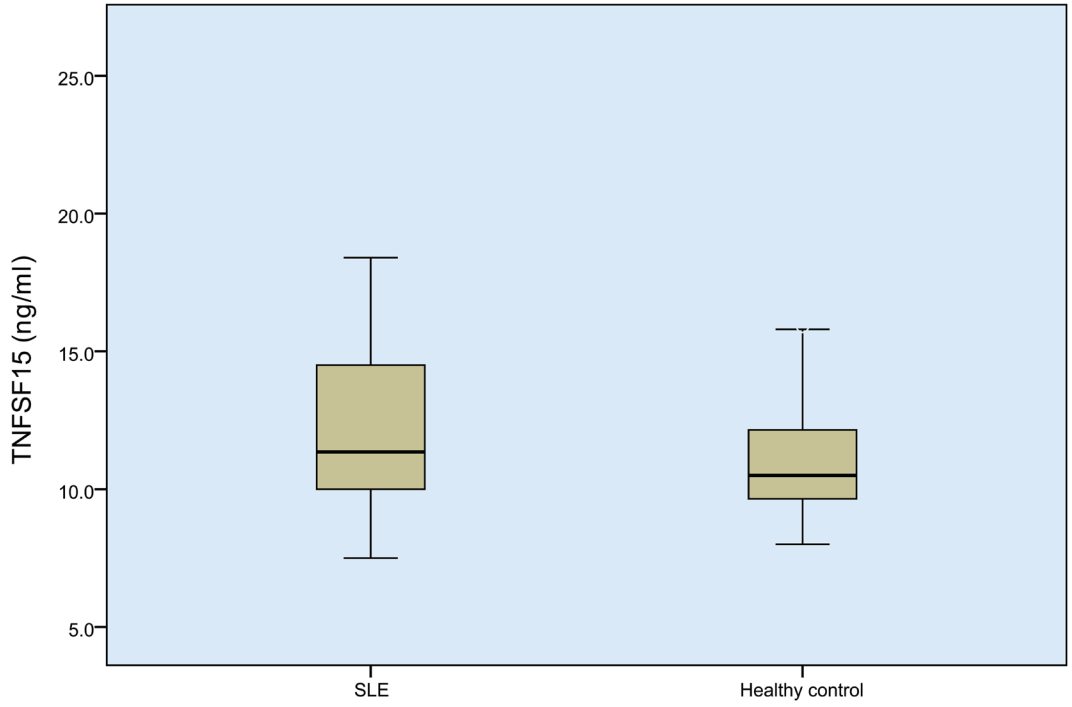

Fig. 5. Median TNFSF15 conc. in SLE patients and healthy control group. SLE, systemic lupus erythematosus; *TNFSF15,* TNF superfamily member 15.

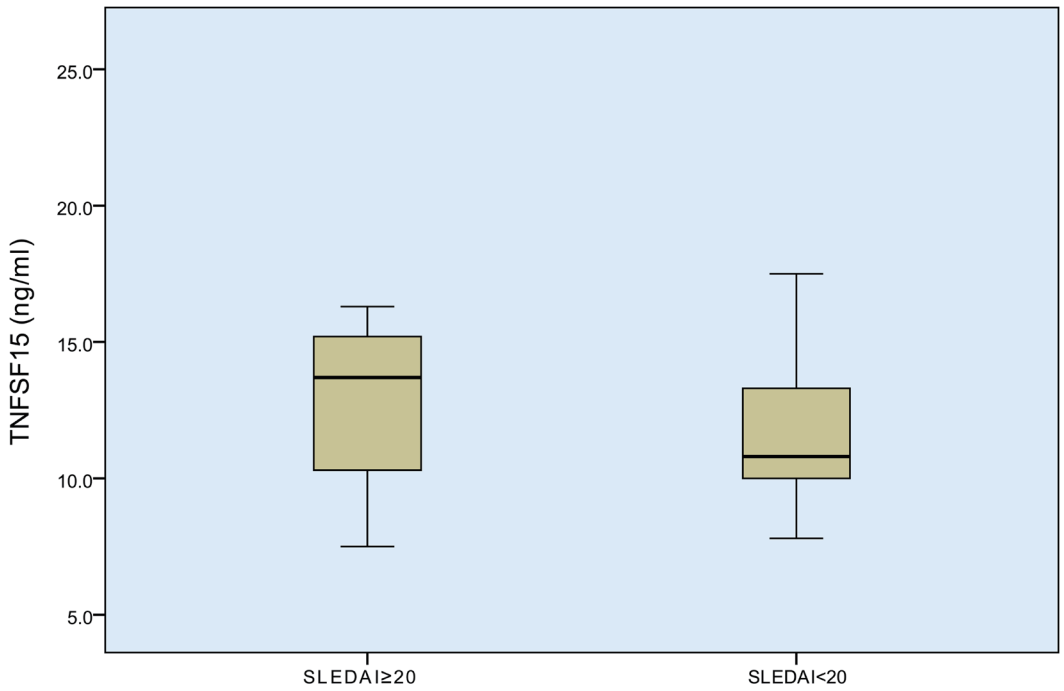

Fig. 6. Median TNFSF15 concentration in SLE patients with high disease activity (SLEDAI ≥ 20). SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; *TNFSF15*, TNF superfamily member 15.

Table 4. Correlation of TNFSF15 serum levels with clinical and laboratory parameters of SLE

Characteristic	TNFSF15 concentration	
	r	***p*-value**
Age of onset in years	−0.045	0.626
ESR in mm/h	0.014	0.888
WBC count in cell/mm^3	0.139	0.140
Lymphocytes in cell/mm^3	0.061	0.588
Hb in g/dL	−0.043	0.650
Platelet count as platelet count/mm^3	0.175	0.063
ALT in IU/L	−0.008	0.935
AST in IU/L	−0.015	0.879
Albumin in g/dL	−0.169	0.164
Creatinine in mg/dL	0.025	0.801
Urea in mg/dL	0.088	0.413
Protein in urine in g/day	0.066	0.544
TGs in mg/dL	0.077	0.630
Cholesterol in mg/dL	0.326	0.040
LDL in mg/dL	0.348	0. 040
HDL in mg/dL	0.095	0.618
TOTAL SLEDAI	0.230	0.012
Renal SLEDAI	0.228	0.013

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; *TNFSF15*, TNF superfamily member 15; TG, triglyceride; WBC, white blood cell.

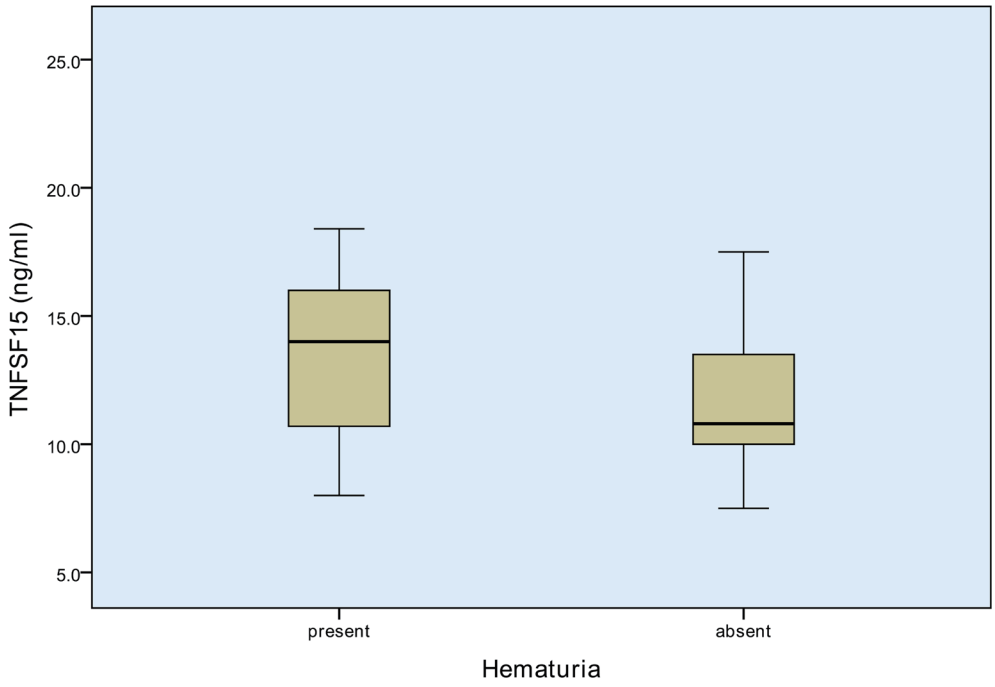

Fig. 7. Median TNFSF15 concentration in SLE patients with and without hematuria. SLE, systemic lupus erythematosus; *TNFSF15*, TNF superfamily member 15.

and that of Della Bella *et al.*,[28] it was postulated that TNFSF15 and its gene variant rs4979462 are involved in the development of dyslipidemia, atherosclerosis, and thromboembolism. However, further studies are needed to elucidate the exact role of TNFSF15 and its gene variants in the pathogenesis of thromboembolism and to confirm the efficacy of the therapeutic use of recombinant anti-TNFSF15 in treating thrombotic disorders.

In the present study, the TNFSF15 serum levels did not signifi-

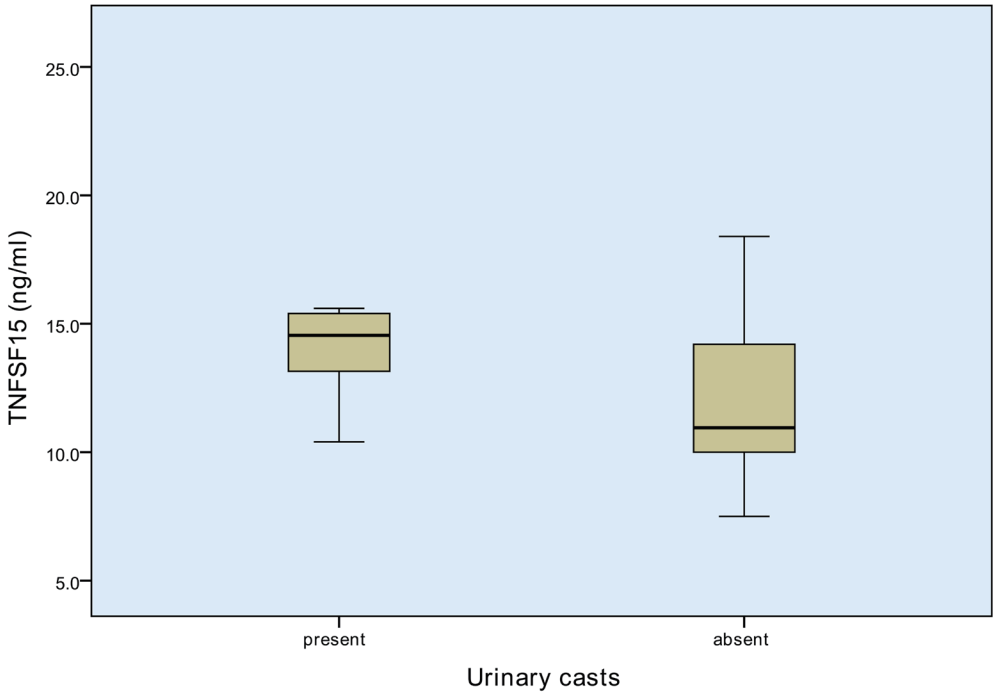

Fig. 8. Median TNFSF15 concentration in SLE patients with and without casts in urine. SLE, systemic lupus erythematosus; *TNFSF15*, TNF superfamily member 15.

cantly differ between the different *TNFSF15* rs4979462 genotypes. In Wang and Tu's study,[14] the T-variant of *TNFSF15* rs4979462 was associated with higher levels of *TNFSF15* mRNA expression. We suggest that *TNFSF15* rs4979462 increases the *TNFSF15* gene expression; however, it seems that there are multiple factors in addition to gene transcription that affect the TNFSF15 serum protein levels, like binding to various cell receptors, leakage in urine, and distribution through the body cells and fluids. In addition, the presence of *TNFSF15* gene variants other than rs4979462 could also affect the *TNFSF15* transcription and expression levels. Consistent with our results and hypothesis, Hitomi *et al.* have found no significant difference in TNFSF15 serum levels concerning different *TNFSF15* rs4979462 genotypes[15]; however, the authors reported that the T-variant of *TNFSF15* rs4979462 was associated with significantly higher *TNFSF15* mRNA expression levels.

TNFSF15, TL1A, is a transmembrane protein included in vascular and immune homeostasis. It is expressed by most immune cells as monocytes, dendritic cells, T cells, fibroblasts, macrophages, and endothelial cells.[7,29,30] TNFSF15 interacts with two types of receptors: TNFRSF25 (DR3) and TNFRSF6B (DcR3). DcR3 lacks the transmembrane and cytoplasmic domains, so it inhibits the immunological function of TNFSF15 by competing with DR3 for binding to it. The presence of DR3 and DcR3 is essential for maintaining the natural balance of the physiological functions of TNFSF15.[6,31–33]

The binding of TNFSF15 to TNFRSF25 (DR3) is essential to perform its immunological function as TNFSF15-TNFRSF25 (TL1A-DR3) enhances the production of stimulatory signals and the recruitment of adapter proteins and stimulates the production of inflammatory cytokines.[6,29] DR3 is extensively expressed by T helper (Th) 17 cells; TNFSF15-TNFRSF25 stimulates the activation and proliferation of Th17 cells and enhances the production of Th17 cytokines like interleukin (IL)-17 and IL-21.[34–37]

Xu *et al.* reported that TNFSF15 serum levels are significantly correlated with those of IL-17 and IL-21, providing proof that TNFSF15 controls the immune response and contributes significantly to the pathogenesis of autoimmune diseases through the regulation of Th17 cells and their cytokine production.[36] Th17/IL-17 dysregulation causes neutrophil accumulation and autoantibody production, thus increasing the risk of SLE development.[38]

TNFSF15 is one of the worthful cytokines that could be a therapeutic target for autoimmune disease. According to Xu *et al.*,[36] TNFSF15 serum levels were comparable to those of the healthy control participants and considerably lower in anti-TNF-treated patients than in anti-TNF-naive patients.

TNFSF15 gene variants have been involved in many autoimmune disorders such as primary biliary cirrhosis,[39] rheumatoid arthritis,[40] Crohn's disease,[11] and Graves' disease.[41] Hitomi *et al.* used in vitro functional analysis to detect the causal variants of the *TNFSF15* gene and the molecular mechanisms of TNFSF15 responsible for the development of primary biliary cirrhosis in the Japanese population.[15] They found that the *TNFSF15* rs4979462 risk allele variant generates a novel nuclear factor-1 (NF-1) binding site, which increased the expression levels of *TNFSF15* because of the binding of the transcription factor NF-1 to the novel binding site. Increased *TNFSF15* expression resulted in hyperactivation and proliferation of the Th17 cells with excessive production of the inflammatory cytokines and, finally, the development of autoimmune diseases.

The findings of our work and those of prior research studies suggest that *TNFSF15* rs4979462 changes the expression and the effector function of the *TNFSF15* gene and hence modulates the natural balance of Th17 effector cells and their related cytokines, IL-17 and IL-21, hence causing the dysregulation of the natural immune response and provide a key for developing SLE.

To our knowledge, only one study has investigated the role of the *TNFSF15* rs4979462 variant in SLE (in the Chinese population). Our study provides a step to improving the understanding of the pathogenesis and the underlying molecular mechanisms implicated in developing SLE in the Egyptian population. Larger-scale studies of various ethnicities are required to understand the exact role of TNFSF15 and its genetic variants in developing autoimmune diseases, especially SLE.

Conclusions

The *TNFSF15* rs4979462 variant and TNFSF15 protein are implicated in the development of SLE and modulate the clinical phenotypes and the severity of the disease, with sex being an essential modulator of the *TNFSF15* rs4979462 variant in the Egyptian population.

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There is nothing to declare.

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Conflict of interest

The authors declared no conflicts of interest.

Author contributions

Conception and design (AK and MMK), acquisition of the data (RAI), provision of study materials or patients (AK and NMAB), clinical evaluations (NMAB), laboratory analysis (RAI and AK), analysis and interpretation of the data (AK and MMK) collection of the scientific materials (RAI and AK), drafting of the article (AK, NMAB, and RAI), critical revision of the article for important intellectual content (AK and MMK).

Ethical statement

The study was approved by the Institutional Research of the Faculty of Medicine, Cairo University, Egypt. The study was conducted in accordance with the principles of the Declaration of Helsinki. An informed consent was obtained from each patient.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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