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Lupus disease activity state and *Foxp3* gene polymorphism

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Abstract

The autoimmune disease systemic lupus erythematosus (SLE) is presented with many clinical symptoms. The transcription factor fork head box protein 3 (Foxp3) is expressed on regulatory T (T-reg) cells and essential for its development and function. Functional single-nucleotide polymorphisms (SNPs) in the *Foxp3-3279* (rs3761548 C/A) gene influence SLE pathogenesis. We aimed to assess the relation between the functional polymorphism in *Foxp3-3279 (rs3761548 C/A)* gene and risk of SLE development and lupus disease activity state. This case-control study included SLE patients, diagnosed according to American College of Rheumatology/Systemic Lupus International Collaborating Clinics (ACR/SLICC) classification criteria. The degree of disease activity was assessed by Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS). *Foxp3-3279* (rs3761548 C/A) gene polymorphism was detected using the polymerase chain reaction restriction fragment length polymorphism-based analysis (PCR-RFLP). We found that AA and AC genotypes significantly increased the risk of SLE by 7.25 and 2.88 folds, respectively (p<0.001) and A allele significantly increased that risk by 3.12 folds (p<0.001). AA genotype significantly increased the risk of SLE moderate–severe disease activity and risk of lupus nephritis by 33.6 folds (p<0.001). In conclusion, *Foxp3 -3279* (rs3761548 C/A) gene polymorphism was associated with the risk of SLE and lupus nephritis. The relation of this SNP with SLE disease activity highlighted the role of *Foxp3* gene in SLE pathogenesis and manifestations that could potentially enhance the management of SLE patients by identifying each person's unique response to treatment.

Keywords: SLE, *Foxp3*, RFLP, LN.

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Introduction

The autoimmune disease systemic lupus erythematosus (SLE) presented with many clinical symptoms, ranging from limited cutaneous affection to potentially fatal affection of systemic organ.[1] Men and women of all ethnicities are affected by the condition, but women, particularly in their reproductive years, are more likely to contract it.[2]

Although the specific etiology of SLE is not known yet, development of the disease may involve multifactorial interactions between several susceptibility variables, such as genetic, hormonal, and environmental variables, rendering it even more complex and diverse.[3]

Abnormalities affecting B and T-cells are the hallmark of the disease.[4] The ability to regulate the proliferation and function of effector T-cells is a defining feature of regulatory T (T-reg) cells, which account for 1-2 percent of peripheral blood mononuclear cells.[5] Abnormalities in T-reg cells, either qualitative or quantitative, might compromise peripheral tolerance and cause autoimmune disorders.[6]

T-reg cells express the interleukin-2 (IL-2) receptor-chain (CD25) and the transcription factor fork head box protein 3 (Foxp3). It was found that Foxp3 is necessary for T-reg cell maturation and function. It is also regarded as a unique intracellular marker specific to T-reg cells.[7]

The fork head box P3 (Foxp3) gene belongs to the Fork head/Winged-helix family and is found on chromosome X (XP11.23). This gene's numerous single nucleotide polymorphisms (SNPs) have been identified in several types of immune disorders.[8] This gene's polymorphism has been associated with a number of autoimmune diseases by causing abnormal T-reg cell production and decreased T-reg cell quantity and function.[9]

A variant that appears to impact gene expression is the SNP rs3761548 C/A, located in the intron region of the *Foxp3* gene. Compared to the C allele, the A allele in this functional SNP is linked to decreased *Foxp3* gene expression. It has been observed that this SNP is linked to a number of autoimmune disorders.[10]

This finding attracted attention to research into the possible relation between the *Foxp3* gene polymorphism (rs3761548 C/A) and SLE development, as well as the impact of this SNP on the lupus disease activity. Our aim was to evaluate the relationship between the functional polymorphism in *Foxp3 -3279* (rs3761548 C/A) gene and risk of SLE development and lupus disease activity state.

Subjects and Methods

This case-control study, which included 120 participants (60 SLE cases and 60 controls), was conducted according to Strengthening the Reporting of Observational studies in Epidemiology. They were recruited by the Department of Rheumatology and Rehabilitation, Faculty of Medicine, Zagazig University Hospitals. The control group included apparently healthy volunteers, matched for age and gender. Laboratory work was carried out in the Department of Medical Microbiology and Immunology and the Scientific and Medical Research Center, Faculty of Medicine, Zagazig University.

The American College of Rheumatology/ Systemic Lupus International Collaborating Clinics (ACR/SLICC) classification criteria for SLE were used to diagnose SLE patients.[1] Patient who had severe comorbidities (e.g. cancer, and mental illness), those refused to give their written consent or refused to participate in the study were excluded from the study.

Assessment of disease activity

A complete examination and taking of the medical history were performed for each participant. The ACR/SLICC classification criteria for SLE were used to diagnose SLE.[11] Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS) was used to assess the degree of disease activity.[12] SLE patients were classified into three disease activity categories based on their SLE-DAS grades: low, mild, and moderate.[13] Laboratory data were gathered from the hospital patients' medical records. A venous blood sample (5 ml) was obtained from each participant and placed in EDTA tube for further detection of *Foxp3-3279* (rs3761548 C/A) gene polymorphism by the polymerase chain reaction restriction fragment length polymorphism-based analysis (PCR-RFLP).

Detection of Foxp3-3279 (rs3761548 C/A) gene polymorphism

Genomic DNA extraction: This was achieved by using commercial kits (gSYNCTM DNA Extraction Kit, Geneaid Biotech Ltd., Taiwan), according to the manufacturer's instructions. Gene amplification was done using commercial kits (2X TOPsimple™ DyeMIXn Taq Kit, Enzynomics Co., Ltd, Korea), according to manufacturer instructions. A reaction mixture (20 µl) was prepared for each sample by mixing 10 µl Dye MIX n Taq, 1 µl from each (template DNA, forward primer and reverse primer) then nuclease free water was used to complete the

volume to 20 µl. The sequence of used primers was (Forward-5'GCCCTTGTCTACTCCACGCCT CT3') and (Reverse-5'CAGCCTTCGCCAATACAG AGCC3').[14]

Thermal profile was as follows: initial denaturation for 5 minutes at 95°C followed by 35 cycles each of denaturation for 30 seconds at 95 °C, annealing for 45 seconds at 60° C and extension for 45 seconds at 72 °C, then a final extension for 5 minutes at 72 °C. PCR product was checked by agarose gel electrophoresis. The PCR product size for the *Foxp3-3279* (rs3761548) gene was 487 bp

RFLP genotyping: The PCR product was enzymatically digested by using the restriction enzyme PstI (Thermo Fisher, CA, USA). This was achieved by adding 10 µl of PCR product (~0.1-0.5 µg of DNA), 2 µl Buffer, 1 µl PstI and nuclease free water was used to complete the volume 25 µl. The mixture was incubated at 37°C for 16 hours. The fragments produced were separated by electrophoresis in 2% agarose gel then visualized under ultraviolet light. AA homozygous genotype, detected as non-digested single band was visualized at 487 bp, CC homozygous genotype, detected as 2 bands visualized at 329 and 158 bp and AC heterozygous genotype detected as 3 bands visualized at 487, 329 and 158 bp[14] (Figure 1).

Data were summarized using co-dominant, dominant and recessive genetic models, co-dominant model included CC, AC, and AA genotypes, dominant model compares (AA+ AC) versus CC, and a recessive model compares AA versus (CC+AC).[15]

Statistical Methods

The Statistical Package for the Social Sciences (SPSS) program version 26 was used to analyze the data. The percentages and absolute frequencies were used to describe the categorical variables. The percentages of categorical variables were compared using the chi square test, and the ordinal data between the two groups was compared using the chi square for trend test. Calculating the odds ratio (OR) was done at a 95% confidence interval (CI). Quantitative variables were characterized using either the median and interquartile range or the means and standard deviations, depending on the type of data. The independent samples Student's t-test was utilized to compare quantitative data between two groups. To compare quantitative data between more than two groups the one-way ANOVA test (for data that is normally distributed) and the Kruskal Wallis test (for data that is not normally distributed) were used. Pairwise comparison post hoc test was done to identify differences between each of the two individual groups when the difference was significant. A *p*-value of < 0.05 was considered statistically significant.

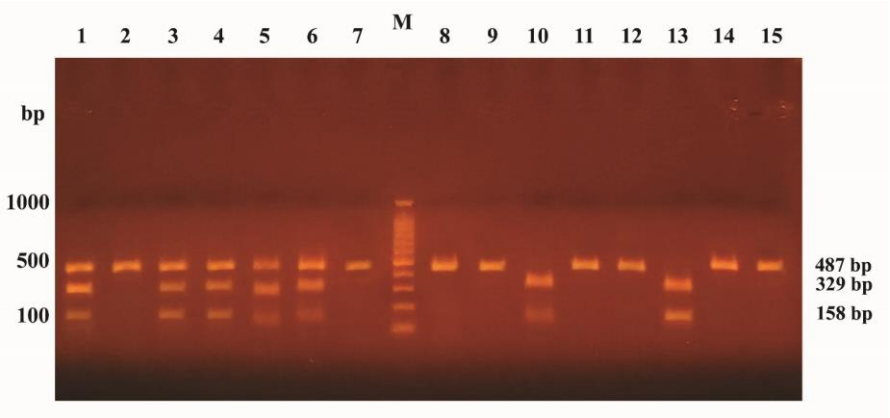

Figure 1. Agarose gel electrophoresis for *Foxp3-3279 (rs3761548 C/A)* gene polymorphism. Lane (M): presents100 bp DNA ladder. Lanes (2,7,8,9,11,12,14 and 15): present AA homozygous genotype (sigle band at 487 bp). Lanes (10 and 13): present CC homozyguse genotype (two bands at 158, 329 bp). Lanes (1,3,4,5 and 6): present A/C heterozygous genotype (three bands at 158, 329 and 487 bp).

Results

This case control study included 120 subjects, 60 SLE cases and 60 controls. There were 5 (8.3%) males and 55 (91.7%) females in the SLE patients' group, 6 (10%) males and 54 (90%) females in the control group. There was no significant difference in age and gender between the study groups (Table 1).

Table 1. Comparison of demographic data between the studied groups.

	SLE patients N= 60 No (%)	Control group N= 60 No (%)	*p* value
Gender			
Male	5 (8.3%)	6 (10%)	χ^2NS
Female	55 (91.7%)	54 (90%)	
Age (year)	Mean ± SD	Mean ± SD	*p* value
	34.53 ± 8.79	32.67 ± 4.54	^{t}NS

SLE: systemic lupus erythematosus, χ^2: Chi square test, t: independent samples, Student's t-test.
$p > 0.05$ is not significant (NS).

Regarding the *Foxp3-3279* (rs3761548 C/A) variant, there was a significant difference in the frequency of CC, CA, and AA genotypes between SLE patients and controls. Presence of AA and AC genotypes significantly increased the risk of SLE by 7.25 and 2.88 times, respectively (Figure 2, A). Upon evaluating the dominant and recessive genetic models in the SLE and control groups, it was observed that AA+AC and AA genotypes had considerably increased in the SLE group than controls, with an increased SLE risk of 4.33 and 3.74 folds, respectively. The A allele had significantly increased SLE risk by 3.12 folds (Table 2).

Table 2. Comparison of *Foxp3* genotypes and alleles between the studied groups.

	SLE patients N= 60 No. (%)	Control group N= 60 No. (%)	χ^2 *p* value	OR (95% CI)
Co-dominant				
AA	29 (48.3%)	12 (20%)		7.25(2.55 – 20.62)
AC	23 (38.3%)	24 (40%)	<0.001	2.88(1.08 – 7.69)
CC	8 (13.3%)	24 (40%)		1 (Reference)
Dominant				
AA+AC	52 (86.7%)	36 (60%)	0.001	4.33(1.75 – 10.72)
CC	8 (13.3%)	24 (40%)		1 (Reference)
Recessive				
AA	29 (48.3%)	12 (20%)	0.001	3.74(1.66 – 8.41)
CC+AC	31 (51.7%)	48 (80%)		1 (Reference)
Alleles				
A	81 (67.5%)	48 (40%)	<0.001	3.12(1.84 – 5.28)
C	39 (32.5%)	72 (60%)		1 (Reference)

SLE: systemic lupus erythematosus, χ^2: Chi square test, OR: Odds ratio, CI: Confidence interval §: Chi square for trend test.
$p \leq 0.05$ is significant.

According to SLE-DAS, 56.7% of SLE patients had moderate-severe disease activity. Regarding clinical and laboratory characteristics of SLE patients, 73.3% had mucocutaneous manifestations and 55% had nephritis. All cases had positive ANA, about 71.7% had high anti-dsDNA titer and 48.3% had hypocomplementemia (Table 3).

Table 3. Clinical and laboratory characteristics of SLE patients.

Variable	Number	%
Clinical manifestations		
Arthritis	13	21.7%
Mucocutaneous affection	44	73.3%
Neuropsychiatric affection	8	13.3%
Serositis	0	0%
Hematologic affection	17	28.3%
Vasculitis	0	0%
Nephritis	33	55%
Positive ANA	60	100%
Increased anti-dsDNA	43	71.7%
Hypocomplementemia	29	48.3%
SLE-DAS grade		
Remission	0	0%
Low activity	0	0%
Mild activity	26	43.3%
Moderate-severe activity	34	56.7%
	Median (IQR)	Range
Disease duration (year)	3(0.5 – 4)	0.16 – 10
WBCs count	7.7(5.6 – 11.1)	2.4 – 15.5
Platelet count	221(167 – 331)	45 – 409
BUN (mg/dl)	14.3(9.5 – 16.9)	6.4 – 27
Creatinine (mg/dl)	0.7(0.59 – 0.76)	0.49 – 28
24-hour protein in urine (gm)	0.52(0.22 – 1.4)	0.125 – 2.9
ESR (mm/hr)	26(20 – 35)	5 – 71
Anti-dsDNA (IU/ml)	37(21 – 56)	12 – 60
Complement C3 (mg/dl)	90(73 – 106)	58 – 185
Complement C4 (mg/dl)	18(9 – 33)	4 – 67
SLE-DAS	10.07(6.97 – 16.9)	4.11 – 32.88

ANA: Antinuclear antibodies, Anti-dsDNA: Anti double stranded DNA antibody, SLE-DAS: Systemic Lupus Erythematosus Disease Activity Score, IQR: inter quartile range, WBCs: White blood cells, BUN: Blood urea nitrogen, ESR: Erythrocyte Sedimentation Rate.

The *Foxp3* genotypes showed statistically significant relations with several laboratory parameters including the SLE-DAS, 24-hour urine protein, ESR, and anti-dsDNA. Post hoc test revealed significant difference between AA genotype and each other group (Table 4).

Table 4. Relation between *Foxp3* genotypes and laboratory related parameters among SLE patients.

Laboratory parameters	AA genotype	AC genotype	CC genotype	^{KW}p value
	Median (IQR)	Median (IQR)	Median (IQR)	
WBCs count	7.8(6.7 – 8.4)	5.5(3.8 – 1.4)	7.1(6.3 – 7.1)	NS
Platelet count	322(202 – 340)	178(167 – 230)	331(45 – 331)	NS
BUN (mg/dl)	13.7(9.5 – 15)	17.8(16.1 – 19.2)	10.85(7.6 – 14.1)	<0.001
Post hoc test [1]	p_1 0.001	p_2 0.001	p_3 0.274 (NS)	
Creatinine (mg/dl)	0.7(0.59 – 0.8)	0.59(0.51 – 0.7)	0.65(0.6 – 0.76)	NS
24-hour protein in urine (gm)	1.06(0.52 – 2.1)	0.36(0.25 – 0.66)	0.22(0.18 – 0.22)	<0.001
Pairwise test [1]	p_1 0.004	p_2 0.228 (NS)	p_3 0.001	
ESR (mm/hr)	35(20 – 67)	25(12 – 27)	22.5(20 – 25)	0.003
Pairwise test [1]	p_1 0.001	p_2 0.761(NS)	p_3 0.048	
Anti-dsDNA (IU/ml)	56(36 – 60)	30(15 – 38)	16.5(12 – 21)	<0.001
Pairwise test [1]	p_1 0.001	p_2 0.154(NS)	p_3 0.001	
Complement C3 (mg/dl)	96(73 – 106)	90(81 – 109)	73(60 – 86)	NS
Complement C4 (mg/dl)	26(9 – 35)	18(13 – 33)	30(8 – 30)	NS
SLE-DAS	11.4(9.83-26.34)	6.27(4.11 – 11.7)	7.47(6.97 – 7.47)	<0.001
Pairwise test [1]	p_1 <0.001	p_2 0.95(NS)	p_3 0.005	

IQR: inter quartile range, KW: Kruskal Wallis test, WBCs: White blood cells, BUN: Blood urea nitrogen, [1:] Post hoc test, *p*1: difference between AA and AC genotypes *p*2: difference between AC and CC genotypes, *p*3 difference between AA and CC genotypes, ESR: Erythrocyte Sedimentation Rate, Anti-dsDNA: Anti double stranded DNA antibody, SLE-DAS: Systemic Lupus Erythematosus Disease Activity Score. $p > 0.05$ is not significant (NS).

With regard to the relations between SLE disease activity state and *Foxp3-3279* (rs3761548 A/C) variant, our results indicated that the co-dominant genetic model (CC, CA, and AA) differed significantly between SLE patients with moderate-severe activity states and mild activity states. The AA genotype significantly increased the risk of moderate-severe disease activity by 33.6 folds (Figure 2, B). Patients with moderate-severe disease had a considerably higher frequency of the A allele than individuals with mild disease activity status, furthermore, it increased the risk of SLE moderate–severe activity by 6.05 folds. When the dominant and recessive genetic models were evaluated, the AA+AC and AA genotypes were significantly higher in moderate-severe than in mild disease activity state, and they increased the risk of SLE moderate–severe activity by 12.16 and 10.08 folds, respectively.

In addition, the risk of lupus nephritis (LN) was significantly elevated by the AA genotype and A allele, by 33.6 and 6.05 folds, respectively. The recessive and dominant genetic models showed that AA and AA+AC were significantly more prevalent in LN patients than in those without LN and they raised the incidence of LN by 11.2 and 11.73 folds, respectively (Table 5).

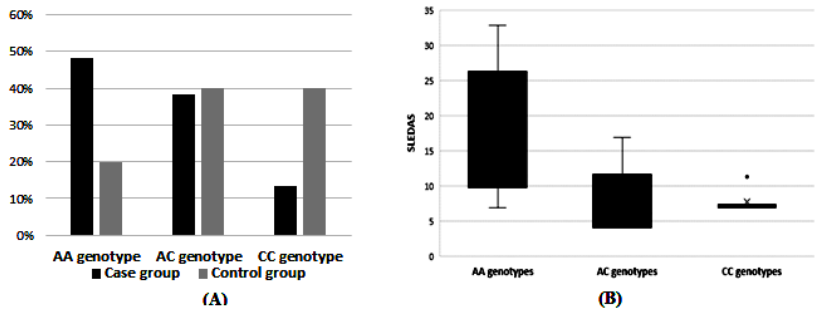

Figure 2. (A) Multiple bar chart showing *Foxp3* genotypes among studied groups. (B) Boxplot showing relation between *Foxp3* genotypes and SLE-DAS.

Table 5. Relation between *Foxp3* genotypes, alleles and SLE disease activity and lupus nephritis.

	Moderate-severe disease activity N= 34 No. (%)	Mild disease activity N= 26 No. (%)	*p* value	OR (95% CI)
Genotypes				
AA	24 (70.6%)	5 (19.2%)		33.6(3.35 – 337.23)
AC	9 (26.5%)	14 (53.8%)	χ^2<0.001	4.5(0.47 – 42.97)
CC	1 (2.9%)	7 (26.9%)		1 (Reference)
Dominant				
AA+AC	33 (97.1%)	19 (73.1%)	Fisher0.016	12.16(1.39 – 106.9)
CC	1 (2.9%)	7 (26.9%)		1 (Reference)
Recessive				
AA	24 (70.6%)	5 (19.2%)	Fisher<0.001	10.08(2.97 – 34.24)
CC+AC	10 (29.4%)	21 (80.8%)		1 (Reference)
Alleles				
A	57 (83.8%)	24 (46.2%)	Fisher<0.001	6.05(2.6 – 14.07)
C	11 (16.2%)	28 (53.8%)		1 (Reference)
	SLE with nephritis N=33 (%)	SLE without nephritis N=27 (%)	χ^2 *P*	OR (95% CI)
Genotypes				
AA	24 (72.7%)	5 (18.5%)		33.6(3.35 – 337.23)
AC	8 (24.2%)	15 (55.6%)	<0.001	3.73(0.39 – 35.93)
CC	1 (3%)	7 (25.9%)		1 (Reference)
Dominant				
AA+AC	32 (97%)	20 (74.1%)	0.009	11.2(1.28 – 97.95)
CC	1 (3%)	7 (25.9%)		1 (Reference)
Recessive				
AA	24 (72.7%)	5 (18.5%)	<0.001	11.73(3.41 – 40.42)
CC+AC	9 (27.3%)	22 (81.5%)		1 (Reference)
Alleles				
A	56 (84.9%)	25 (46.3%)	<0.001	6.5(2.75 – 15.34)
C	10 (15.1%)	29 (53.7%)		1 (Reference)

χ^2: Chi square test, OR: Odds ratio, CI: Confidence interval, §: Chi square for trend test. $p \leq 0.05$ is significant.

Discussion

SLE is an autoimmune illness which attacks several systems and causes organ damage over time. Autoantibodies and auto-reactive T cells are thought to be responsible for these pathological alterations. Recently, SNPs in the *Foxp3* gene have been implicated in impaired immune system function in SLE. These SNPs can alter the gene's expression level and impair the suppressive action of T-reg cells, one of these SNPs is the (rs3761548 C/A).[10, 16] This study evaluated the relationship between the functional polymorphism in *Foxp3-3279* (rs3761548) gene and risk of SLE development and lupus disease activity state. So, immunological profiling can be used to determine a patient's unique response to treatment and may be able to predict outcomes that enhance the care of people with SLE.[1]

There were 120 participants in this case-control study (60 SLE cases and 60 controls). There was no age and gender difference between the studied groups. Additionally, the results of Birjan et al., 2023 and Abbass et al., 2013 agreed our findings, demonstrating that both groups were well matched.[16, 17]

Our findings demonstrated a statistically significant relation between different *Foxp3-*

3279 (rs3761548) gene variants and the development of SLE, as AA and AC genotypes significantly increased risk of SLE by 7.25 and 2.88 folds, respectively. Also, A allele significantly increased SLE disease risk by 3.12 folds. In agreemnt with our results, the study by Stadtlober et al., 2021, found that A allele and the AA genotype were associated with SLE disease and increased risk of disease by 2.65, and 2.64, respectively.[18] Furthermore, a meta-analysis study was done to determine the relation between *Foxp3-3279 A/C* polymorphism and susceptibility for autoimmune diseases. They found that this gene polymorphism increased the risk of autoimmune diseases especially among carriers of A allele variant.[19]

On the other hand, two studies by Lin et al., 2011 and by Heydarinejad et al., 2022, examined the same variant in SLE patients, and reported no correlation with SLE susceptibility.[20, 21] Variations in the allelic/ genotypes frequencies across research studies may be attributed to the variety of disease features under investigation, ethnicity, the small sample size, genotyping technique and the features of the control group.[19]

The AA+AC and AA in dominant and recessive models were significantly higher in SLE patients and increased the risk of SLE disease by 4.33 and 3.74 folds, respectively. When Stadtlober et al., 2021, evaluated dominant and recessive genetic models, no significant association was detected in the frequency of AA+AC in dominant model. But in the recessive model, the presence of AA genotype was higher in SLE patients when compared to controls, it increased the risk of SLE by 2.644 folds.[18]

In the present study, we found a statistically significant relation between *Foxp3* genotypes and several laboratory parameters including, SLE-DAS, ESR, anti-dsDNA and proteinuria which were significantly higher in patients carrying the AA genotype than other genotypes. The study by Stadtlober et al., 2021, found that the recessive model (ACAC) haplotype, had significantly higher anti-dsDNA positivity and increased the risk of high anti-dsDNA by 3.026 folds.[18.]Despite the finding that anti-dsDNA antibodies have several specificities in SLE and are significantly linked to LN, T-cells were primarily thought to be involved in helping B cells to produce autoantibodies. The emphasis now is on T-cells as effectors causing tissue injury, glomerular sclerosis and interstitial inflammation even in the absence of immune complex deposition, rather than their role in lupus glomerulonephritis. When T-cells function influence lupus activity, these consequences should be considered, particularly when linked to functional polymorphism in Foxp3 transcription factor, that lead to reduction in T-reg cells' number and function, which is associated with higher T-cell activity.[7,22]

Different findings were also reported by other studies. The study by Lin et al., 2011, found that patients with *the Foxp3-3279* A allele had usually reduced anti-dsDNA levels. The study by Abbas et al., 2013, found no significant correlation between *Foxp3* gene expression and anti-ds DNA antibody titer, ESR, serum creatinine and proteinuria in the active SLE patients.[17, 20] Controversy in the relationship of laboratory parameters with genotypes in different studies could be attributed to variation of laboratory parameters levels along the SLE disease course in different patients and may be influenced by their treatment.

Furthermore, in our results, the state of SLE disease activity was significantly different among *Foxp3-3279* (rs3761548) genotypes. The A allele and the AA genotype greatly raised the risk of moderate-to-severe activity by 6.05 and 33.6 folds, respectively. In the dominant and recessive genetic models, AA+AC and AA can significantly increase the risk of SLE moderate–severe activity by 12.16 and 10.08 folds, respectively. Similar findings were reported by the study of Stadtlober et al., 2021, as patients with the dominant genetic model's A/C haplotype had a disease activity level that was significantly higher and increased risk by 1.119 folds.[18] Furthermore, the study by Shen et al., 2010, demonstrated that the (rs3761548) A allele is linked to lower *Foxp3* expression, which may result in a decrease in T-reg cell function or number that lead to high disease activity.[23]

In addition, the A allele and AA genotype significantly increased the risk of LN by 6.5 and 33.6 folds, respectively. The dominant and

recessive genetic models AA+AC and AA increased the risk of LN by 11.2 and 11.73 folds, respectively. However, the study by Stadtlober et al., 2021, found that in the dominant genetic model, patients with the AA+AC genotype had a decreased frequency of LN (p=0.038).[18] On the other hand, they reported that the risk of developing nephritis was 2.5 folds higher in patients with the ACAC haplotype.[18]

Some limitations of our study should be considered. All SLE patients included in this study had disease activity ranging from mild to moderate-severe activity and no one had disease remission or low activity. We studied one SNP of *Foxp3* gene, further research is required to determine the function of more SNPs related to SLE disease and other autoimmune diseases.

In conclusion, *Foxp3 -3279* (rs3761548 C/A) gene polymorphism was associated with the risk of SLE and LN. The relation of this SNP with SLE disease activity highlighted the role of *Foxp3* gene in SLE pathogenesis and manifestations that could potentially enhance the management of SLE patients by identifying each person's unique response to treatment.

Author Contributions

HIA, was responsible for the research conceptualization, proposal design, writing and editing the original manuscript; EIA, contributed to patient's data collection and interpretation; MAK, was responsible for PCR steps and data analysis. All authors shared in final revision and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University (approval date, September 2023).

Informed consent

All patients provided written informed consent prior to being included in the study.

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