

Association of *TNFAIP3* gene polymorphism (*rs5029939*) with susceptibility and clinical phenotype of systemic lupus erythematosus

Hala Gaballah¹ , Reham Abd-elkhalek¹ , Ola Hussein² , Shimaa Abd El-wahab¹ 

¹Department of Rheumatology and Rehabilitation, Faculty of Medicine, Zagazig University, Zagazig, Egypt

²Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

Objectives: This study aims to investigate the association of the tumor necrosis factor-alpha inducible protein 3 (*TNFAIP3*) (*rs5029939*) gene single nucleotide polymorphism (SNP) with the risk of systemic lupus erythematosus (SLE) and its clinical manifestations in a cohort of SLE patients.

Patients and methods: This study included a total of 180 participants (18 males, 72 females; mean age: 30.9±10.1 years; range 17 to 59 years) including 90 SLE patients and 90 healthy controls between March 2017 and February 2020. The *TNFAIP3 rs5029939* gene polymorphism was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in all participants.

Results: There was a significant difference in genotype distribution of the *TNFAIP3 rs5029939* SNP between SLE patients and healthy controls, where CG genotype was more common in SLE patients (53.3%) than controls (11.1%) ($p=0.001$). We found a significant difference in G allele frequency of *TNFAIP3 (rs5029939)* (37.8% with SLE vs. 5.6% with controls, $p=0.001$). Genotype CG was significantly associated with lupus nephritis and neuropsychiatric manifestations ($p<0.05$). Although the response to treatment was numerically higher with the genotype CC, it did not reach statistical significance ($p=0.4$).

Conclusion: Our study suggests that *TNFAIP3 rs5029939* gene polymorphism is associated with SLE susceptibility and may have an impact on its clinical phenotype. As such association differs among populations of diverse ethnic backgrounds, larger genome-wide association studies are warranted to further elucidate genetic associations.

Keywords: Gene polymorphism, lupus nephritis, systemic lupus erythematosus, *TNFAIP3*, tumor necrosis factor-alpha inducible protein 3.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease^{1,2} with an incidence and prevalence varying depending on age, sex, and race.³ The highest estimates of incidence and prevalence of SLE were in the North America (23.2/100,000 person-years and 241/100,000 individuals, respectively).⁴ Arab-Americans show a 2.1-fold higher incidence of SLE, compared to non-Arab Caucasians and African Americans,⁵ whereas the incidence and prevalence of SLE in the United Arab Emirates were estimated to

be 8.6 per 100,000/year and 103/100,000 individuals (84.5 to 124.4), respectively.⁶

Although the exact pathogenesis of SLE still remains to be elucidated, it has been proposed that the interaction of genetic, environmental, and hormonal factors, ultimately leading to the inappropriate function of the innate immune system, altered self-tolerance mechanisms, and apoptotic cell clearance are implicated.⁷ Several genetic polymorphisms have been suggested to predispose to such immune dysregulation,

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Correspondence: Reham Abd-elkhalek, MD. Department of Rheumatology and Rehabilitation, Faculty of Medicine, Zagazig University, 44519 Zagazig, Egypt.
Tel: 00201016073805 e-mail: ehamabdelkhalek@medicine.zu.edu.eg

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thus influencing SLE susceptibility and clinical features.^{8,9}

The tumor necrosis factor-alpha inducible protein 3 (*TNFAIP3*) gene encodes the ubiquitin-modifying enzyme A20 which inhibits nuclear factor-kappa B (NF-κB) activity in several signaling pathways, including those of TNF and Toll-like receptors.¹⁰ It is also required for the negative regulation of inflammatory responses.¹¹ Over the last decade, numerous studies have investigated the association between *TNFAIP3* gene polymorphisms and SLE.¹² Significant associations have been reported between different *TNFAIP3* gene polymorphisms, such as *rs2230926*, *rs5029939*, *rs5029937*, and *rs3757173* polymorphisms and SLE susceptibility and clinical manifestations.¹³⁻¹⁶ Such genetic predisposition varies with the ethnic background. In a meta-analysis, *rs5029939* and *rs3757173* polymorphisms were associated with SLE in Europeans and Asians, while *rs2230926* and *rs5029937* polymorphisms were associated with SLE in Europeans only.¹⁵

Currently, there is a limited number of data regarding the impact of *TNFAIP3 rs5029939* gene polymorphism among the SLE patients in Egypt. We, therefore, aimed to investigate the association of *TNFAIP3* gene polymorphism with SLE susceptibility and clinical manifestations in a cohort of patients with SLE.

PATIENTS AND METHODS

This case-control study was conducted at Faculty of Medicine, Zagazig University, Department of Rheumatology and Rehabilitation between March 2017 and February 2020. A total of 180 participants (18 males, 72 females; mean age: 30.9±10.1 years; range 17 to 59 years) including 90 SLE patients and 90 age- and sex-matched healthy controls recruited from other hospital units and clinics were included. The diagnosis of SLE was made according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE.¹⁷ Patients with juvenile SLE (<16 years) or other autoimmune diseases and patients with cardiac, respiratory, neuropsychiatric, and renal diseases due to causes other than SLE were excluded from the study. A written informed consent was obtained

from each participant. The study protocol was approved by the Institutional Review Board of the Zagazig University, Faculty of Medicine (No: 3845/17-6-2017, Date: 17.07.2017). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

Patients' data were collected from the medical records. Demographic data, clinical manifestations, laboratory and immunological parameters were noted. Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)¹⁸ and severity was assessed by the SLICC and American College of Rheumatology (ACR) Damage Index for SLE.¹⁹ The response to induction therapy was assessed using the SLE Responder Index (SRI).²⁰

Analysis for *TNFAIP3 rs5029939* gene polymorphism

The presence of the *TNFAIP3 rs5029939* variant was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).²¹

Deoxyribonucleic acid (DNA) extraction

Venous blood samples were collected in an ethylenediamine tetraacetic acid (EDTA) tube. The DNA was extracted from peripheral leukocytes using the GeneJET™ whole blood genomic DNA purification mini kit (Thermo Fisher Scientific® Inc., CA, USA) according to the manufacturer's instructions.

TNFAIP3 rs5029939 variant genotyping

Amplification was performed in a 50 µL reaction volume containing 25 µL of MyTaq™ Red Mix (Bioline Reagents Ltd., UK) genotyping master mix. The PCR test was carried out using the primer pair 5' GCC TTC ACC AGC AAA TCA AG 3' and 5' GAC ACC AAC TGC AAA GGA GCC AG 3'. The PCR conditions were initial denaturation at 95°C for 1 min followed by 35 cycles of 15 sec at 95°C, 15 sec at 50°C, 10 sec at 72°C, and a final extension at 72°C for 5 min. The PCR product (186 bp) was digested with AflIII enzyme (New England Biolabs® Inc., MA, USA). Two fragments were produced (142 and 44 bps) for the single nucleotide polymorphism (SNP) *rs5029939* CC genotype, and only one band of 186 bps for the GG genotype. The amplified

PCR products and digested fragments were visualized by ethidium bromide-stained agarose gel electrophoresis.

Statistical analysis

Power analysis and sample size calculation were performed using the G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).²² The study power was calculated as 95.9% with an effect (d) size=0.3 (depending on difference in minor allele frequency), $\alpha= 0.05$, and $df= 2$. A total of 180 participants (n=90 in each group) were needed to be recruited for the study.

Statistical analysis was performed using the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were expressed in mean \pm standard deviation (SD) or median (min-max), while categorical variables were expressed in number and frequency. Agreement with Hardy-Weinberg equilibrium

(HWE) was assessed by comparing the expected-to-observed genotype frequencies. The differences in allele and genotype frequencies between cases and controls were analyzed using the Pearson chi-square (χ^2) test and/or Fisher's exact test. The relative risk (odds ratio) for each genotype and allele was estimated with a 95% confidence interval (CI). The phenotypic comparisons were done by Pearson χ^2 test to compare the frequency of different clinical and laboratory characteristics, as well as the treatment response and SLICC/ACR-Damage Index scores among SLE patients with different genotypes. A p value of <0.05 was considered statistically significant.

RESULTS

Both groups were similar in demographic characteristics including age and sex ($p=0.9$ and

Table 1. Clinical and laboratory characteristics of patients with SLE (n=90)

Characteristics	n	%	Mean \pm SD	Median	Range
Age (year)			30.9 \pm 10.1		
Sex					
Male	18	20			
Female	72	80			
Disease duration (year)				5	1-17
Fever	19	21.1			
Malar rash	70	77.8			
Photosensitivity	60	66.7			
Oral/nasal ulcers	43	47.8			
Discoid lesion	6	6.7			
Lupus nephritis	49	54.4			
Cardiac	37	41.1			
Pulmonary	44	48.9			
Neuropsychiatric	46	51.1			
GIT	26	28.9			
Leucopenia	26	28.9			
Anemia	77	85.6			
Thrombocytopenia	7	7.8			
High ESR	87	96.7			
High CRP	42	46.7			
Positive ANA	88	97.8			
Positive anti-dsDNA	37	41.1			

SLE: Systemic lupus erythematosus; SD: Standard deviation; GIT: Gastrointestinal tract; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; ANA: Antinuclear antibody; Anti-dsDNA: Anti-double-stranded deoxyribonucleic acid.

Table 2. Genotype and allele frequency of *TNFAIP3* rs5029939 polymorphism among the study population

	SLE patients (n=90)		Controls (n=90)		p*	Odds (95% CI)
	n	%	n	%		
Genotypes						
CC	32	35.6	80	88.9	0.001	0.07 (0.03-0.15)
CG	48	53.3	10	11.1	0.001	9.1 (4.2-19.9)
GG	10	11.1	0.0	0.0	0.001†	2.1 (1.8-2.5)
Alleles						
C	112	62.2	170	94.4	0.001	0.09 (0.05-0.19)
G	68	37.8	10	5.6		

* Chi-square test (χ^2); † Fischer exact test; CI: Confidence interval.

p=0.4, respectively). Demographic and disease characteristics of patients with SLE are shown in Table 1.

We first compared the frequencies of *TNFAIP3* rs5029939 genotypes and alleles between the two groups (Table 2). Distribution of the three genotypes differed significantly where genotype CC was more frequent in controls (88.9%) than

SLE patients (35.6%), while genotypes CG and GG were more distributed among SLE patients than controls (53.3% vs. 11.1% and 11.1% vs. 0%, respectively; (p=0.001). Allele distribution also differed significantly between the two groups. The wild allele C was more frequent in controls (94.4%) than SLE patients (62.2%), while the mutant allele G was more frequent in SLE patients (37.8%) than controls (5.6%).

Table 3. Association between *TNFAIP3* rs5029939 polymorphism and the clinical and laboratory characteristics of SLE patients

Manifestations	n	Genotype CC (n=32)		Genotype CG (n=48)		Genotype GG (n=10)		p*
		n	%	n	%	n	%	
Fever	19	8	42.1	11	57.9	0	0.0	0.2
Malar rash	70	24	34.3	36	51.4	10	14.3	0.2
Photosensitivity	60	20	33.3	33	55.0	7	11.7	0.8
Oral/nasal ulcers	43	17	39.5	24	55.8	2	4.7	0.16
Discoid lesion	6	4	66.7	2	33.3	0	0.0	0.2
Lupus nephritis	49	19	38.9	24	48.9	5	12.2	0.001
Cardiac	37	9	24.3	21	56.8	7	18.9	0.06
Pulmonary	44	16	36.4	21	47.7	7	15.9	0.3
Neuropsychiatric	46	18	39.1	28	60.9	0	0.0	0.003
GIT	26	7	26.9	17	65.4	2	7.7	0.3
Leucopenia	26	8	30.8	16	61.5	2	7.7	0.6
Anemia	77	25	32.5	42	54.5	10	13.0	0.19
Thrombocytopenia	7	4	57.1	3	42.9	0	0.0	0.3
High ESR	87	32	36.8	45	51.7	10	11.5	0.2
High CRP	42	18	42.9	22	52.4	2	4.7	0.1
Positive ANA	88	30	34.1	48	54.5	10	11.4	0.1
Positive anti-dsDNA	37	9	24.3	24	64.9	4	10.8	0.1

SLE: Systemic lupus erythematosus; GIT: Gastrointestinal tract; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; ANA: Antinuclear antibody; Anti-dsDNA: Anti-double-stranded deoxyribonucleic acid; * Chi square test (χ^2).

Table 4. Association between *TNFAIP3* rs5029939 polymorphism and response to treatment and SLICC/ACR-Damage Index in SLE patients

	n	Genotype CC (n=32)		Genotype CG (n=48)		Genotype GG (n=10)		p*
		n	%	n	%	n	%	
Response to therapy								
Non-refractory	49	20	62.5	25	52.1	4	40.0	0.4
Refractory	41	12	37.5	23	47.9	6	60.0	
SLICC/ACR-Damage Index								
Less severe ≤ median (4)	50	19	38.0	26	52.0	5	10.0	0.8
More severe > median (4)	40	13	32.5	22	55.0	5	12.5	

SLICC/ACR: Systemic Lupus International Collaborating Clinics/American College of Rheumatology; SLE: Systemic lupus erythematosus; * Chi square test (χ^2).

Table 5. Comparative analysis between the current study and other studies regarding *TNFAIP3* rs5029939 association with lupus susceptibility and phenotype

Studies	Type of the study	Population	Risk allele	p	Phenotypic association*
Lodolce et al. ²⁷	Candidate gene study	African-Americans, Europeans	G	0.0453	Not studied
Kim et al. ²⁵	Candidate gene study	Koreans	G	0.01	Arthritis
Elghzaly et al. ²⁶	Candidate gene study	Egyptians	G	0.670	No significant association
Zhang et al. ¹⁵	Meta-analysis	Europeans, Asians, Africans	G	4.351×10 ⁻⁴	Not studied
Current study	Candidate gene study	Egyptians	G	0.001	Nephritis Neurological

* p<0.05 for all associations.

Subsequently, we examined the association between different genotypes and the clinical manifestations among SLE patients. There was a significant association between genotype CG with lupus nephritis and neuropsychiatric manifestations, while no significant association was evident between the genotypes and all other clinical, laboratory, and immunological parameters (Table 3).

Finally, we assessed the association between genotype frequencies and both SLE response to treatment and ACR-Damage Index. Although the genotype CC had a higher treatment response rate, it did not reach statistical significance (p=0.4). On the other hand, there was no significant association between the SLICC/ACR-Damage Index scores and the different genotypes (Table 4).

DISCUSSION

Multiple host genetic factors play an important role in both SLE pathogenesis and

the development of its clinical features.^{23,24} The *TNFAIP3* gene has been suggested to be implicated in B cell activation and autoantibody production related to SLE pathogenesis. Genetic variants in the *TNFAIP3* gene may, thus, be suggested as genetic risk factors for SLE susceptibility.²⁵ In our study, the SNP, rs5029939, in the *TNFAIP3* gene was found to have an association with the risk of SLE and some of its clinical patterns.

Our results also showed that SLE patients differed significantly from the control group in their genotype and allele frequency of *TNFAIP3* rs5029939 gene polymorphism. While allele C was dominant in healthy controls, the minor allele G occurred more frequently in the SLE patients. The genotype CC was the most frequent genotype in healthy controls, while the heterozygous genotype CG, followed by CC, was the most frequent in SLE patients. Results from previous studies assessing the *TNFAIP3* rs5029939 and SLE susceptibility and phenotype in different ethnic groups are inconsistent (Table 5). In contrast to our results, a previous study did not

show a significant association between *TNFAIP3 rs5029939* and lupus risk or clinical phenotype, yet the probability of type II statistical error could not be excluded.²⁶ However, this SNP (*rs5029939*) was previously demonstrated to be associated with SLE susceptibility both in the African Americans and Europeans.²⁷ A similar association between *TNFAIP3 rs5029939* gene polymorphism and SLE susceptibility was observed in a Korean study, where the minor allele of *TNFAIP3 rs5029939* SNP occurred more frequently in patients with SLE than in the controls.²⁵ A more recent meta-analysis showed a significant association between the minor *rs5029939* allele and SLE susceptibility in the overall population, while, based on the stratification according to ethnicity, *rs5029939* was significantly associated with SLE in Europeans, but not in Asians or Africans.¹⁵ These findings indicate that genetic predisposition is not uniform among different ethnic groups, but rather affected by the interplay of multiple host factors.

The clinical manifestations and disease course vary greatly among lupus patients. Such clinical heterogeneity can be correlated with specific polymorphisms in SLE-associated genes.²⁸ Our results suggest an impact of *TNFAIP3 rs5029939* gene polymorphism on disease phenotype among our lupus population. There was a significant association between genotype GC with lupus nephritis and neuropsychiatric manifestations. In the Korean SLE population, the arthritis phenotype was marginally associated with *TNFAIP3* SNPs; however, no association was found with the presence of nephritis.²⁵ Another study involving Caucasian SLE patients suggested that the risk of developing renal or hematological manifestations almost doubled in SLE patients with the heterozygous risk allele of *TNFAIP3 rs5029939*, compared to homozygous non-risk subjects.¹³ However, in a Chinese study, no significant association was identified between *TNFAIP3* gene polymorphisms and any of the studied clinical, hematological, or immunological parameters.¹²

Our study did not show a significant difference in SLICC/ACR-Damage Index scores among SLE patients of different genotypes. The treatment response rate was numerically higher with the genotype CC, but not statistically significant. Of note, SLE is an attractive candidate for a

personalized immunotherapy approach, since individual patients may have different molecular pathways driving their disease.²⁹ The *TNFAIP3* can be considered a central regulator of immunopathology in SLE, as it acts at multiple steps in the NF- κ B signaling pathway.³⁰ Based on our findings, we can recommend future studies to investigate therapies targeting the *TNFAIP3* pathway in patients with nephritis and neuropsychiatric SLE. This may represent an interesting potential therapeutic target in a subset of lupus patients.

The present study is limited by its single-center nature and relatively small sample size, thus hindering data generalization. However, it indicates the impact of genetic polymorphism not only on disease susceptibility, but also on its clinical phenotype among patients with SLE in our country.

In conclusion, our study suggests that *TNFAIP3 rs5029939* gene polymorphism is associated with the development of SLE and may have an impact on its clinical phenotype. Such an association, however, differs from previous studies involving population of other ethnic backgrounds. Therefore, further multi-center, genome-wide association studies are needed to further elucidate different genetic associations and their interactions in a larger population.

Declaration of conflicting interests

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