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Association between IL-35 Gene Single Nucleotide Polymorphisms and Systemic Lupus Erythematosus in Egypt

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease characterized by a reduced immune tolerance of the body's immune system and an enormous production of autoantibodies, which ultimately cause damage to most of body tissues and organs, including the skin, blood and its early diagnosis is important to reduce complications.
Aim of The Work: To investigate the possibility of a link between a single nucleotide polymorphism (SNP) in the IL-35 gene (rs4740) and susceptibility to SLE occurrence.
Patients and Methods: In this Observational cross sectional study, 30 patients already diagnosed with SLE (Group 1) were compared to 20 apparently healthy subjects (Group 2) who were matched for age and sex (all were females) and who had no history of autoimmune disease. Following informed consent for scientific research from the Al-Azhar Ethical Committee for Scientific Research, patients and a control group were enrolled in this study.
Results: The findings revealed that there was no statistically significant difference in age between SLE patients and healthy controls in this study. Butterfly rash (46.6 percent), arthritis (43.3 percent), haematological disease (70 percent), and immunological condition (73.3 percent) were the most prevalent clinical characteristics of SLE, according to the National Institute of Health. Furthermore, there was no statistically significant difference between SLE patients and healthy controls in the genotyped SNP rs4740 that was investigated in this study.
Conclusion: In conclusion, this study does not confirm an association between rs4740 SNP and the occurrence or susceptibility of SLE in Egypt.

Keywords: *Systemic Lupus Erythematosus; Il-35 gene; Single nucleotide polymorphism.*

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INTRODUCTION

SLE is defined as a chronic, relapsing, and intermittent autoimmune disorder which affects mainly women. It affects multiple organ systems and is distinguished by a wide range of clinical manifestations. Arthritis, cutaneous affection, nephritis, and vasculitis are the most common manifestations. [1]

It has been debated whether SLE is a single disease with numerous phenotypes or whether it is a common feature shared by many separate diseases with diverse pathogenic processes. This debate has arisen because of the high degree of heterogeneity in SLE. [2]

In Egypt, the prevalence of SLE was estimated in a national cross-sectional study in 2021 to be 6.1 for 100000 populations and it is more prevalent in females (11.3/100000 females) while in males (1.2/100000) [3]

In the United States, for example, documented cases ranging from 20 to 150 cases per 100,000 individuals, with the highest prevalence reported in

North America, and the frequency looks to be growing as the disease becomes more widely recognized and survival rates improve. When compared to those of other racial or ethnic groups, people of African, Hispanic, or Asian heritage had a higher incidence rate of SLE and more organs affection in the United States. [4]

Patients with severe and moderate SLE had significantly lower numbers of regulatory T cells (Treg) when compared to healthy controls, and the ratio of $CD4^+$ CD25 effector T cells to $CD4^+$ CD25 high CD127 Treg in severe and moderate SLE patients was significantly higher when compared to healthy controls, the researchers found. Patient's with moderate to severe SLE had significantly decreased gp130 expression on the $CD4^+$ Th cell surface and on the $CD14^+$ monocyte surface when compared to healthy controls, according to the findings. gp130 expression on $CD4^+$ T cells and the proportion of Tregs were shown to be positively related with one another, while being negatively associated with the SLEDAI, which stands for Systemic Lupus Erythematosus Disease Activity Index. [5]

Interleukin (IL)-35 is a member of the IL-12 family. It was found by Niedbala and Collison in 2007 and is composed of the interleukin-12 chain p35 and the interleukin-27 chain Epstein-Barr virus-induced gene 3

(Ebi3), which are joined by a disulfide bond. It is created by the immunological system of the human body.[6] When the EBV virus infects B lymphoblastoid cells, the expression of EBI3 is upregulated. [7] IL-35 may have a role in the development of SLE by directly inhibiting effector T cell growth, particularly that of Th17 cells, and so delaying the onset of inflammatory responses, as well as by indirectly decreasing effector T cell development. Moreover, serum levels of interleukin-35 (IL-35) were found to be significantly higher in lupus patients compared to healthy subjects [8], and IL-35 has been shown to significantly alleviate lupus flare-ups and renal affection, as well as increased proinflammatory cytokines (IFN- γ, TNF- α, and IL-6 and IL-17a) in plasma [8].

These findings confirm that the interleukin-35 (IL-35) gene plays a critical role in the development and progression of SLE, but patients from the previous studies were all Chinese which recommend to study the effect of this polymorphism in different ethnic groups, in this study, we examine the association between Il-35 gene polymorphism and occurance of SLE in Egypt.

PATIENTS AND METHODS

Thirty (30) female patients with SLE who had already been diagnosed (Group 1) according to the six diagnostic criteria from Systemic Lupus International Collaborating Clinics (SLICC)[9] for SLE and they were followed were included in this observational cross-sectional study, as well as twenty (20) healthy subjects who were age and sex-matched to the cases (Group 2) and had no history of autoimmune diseases. After informed consent was acquired, patients and a control group were enrolled in the study.

Inclusion Criteria for patients:

Already diagnosed with SLE.
Patients were selected from an outpatient clinic in their regular follow up.

All of the control idividuals are characterized by the following:

a. does not meet any of the criteria for being classified as having SLE;

b. neither the individual nor any near relatives have a history of autoimmune disorders.

The demographic profiles of the participants, as well as their clinical characteristics as mentioned in table 2, were acquired from the patients. The Medical Scientific Research Ethics Committee of Al-Azhar University gave its approval for this research project.

Specimen collection

Specimens from patients and control subjects were collected from Al-Azhar University hospitals; 2 mL was drawn from venous blood then added to EDTA tubes with stopper, then DNA extraction was done using a whole blood extraction kit manufactured by Hangzhou Bioer Technology Company Limited. Lot Number: 20201103.

Specimen storage

Specimens(DNA extract) were then capped and stored at -80° C till the time of the assay.
Genotyping for IL-35 gene

Studied SNP	EBI3 A/G (rs4740)
Sequence of primers	5′-GCTCCGTTGTGTGGTTCTGT-3′ 5′-AGTGACAGTTCAGTCAGCCC-3′
Product size	486 bp
Restriction enzyme used	HpyCH4IV
Enzyme digestion pattern	**A** : 388 + 98 bp **G** : 345 + 98 + 43 bp
Organism	Homo sapiens
Position	chr19:4236999 (GRCh38.p13)
Alleles	G > A
Variation Type	SNP (Single Nucleotide Polymorphism)

Table 1: SNP details, Rstriction enzyme and sequence of primers used in the study

Table 1 shows the primer sequences, restriction enzyme utilised, and restriction digestion pattern for each allele.

The polymorphism of EBI3 (rs4740) was investigated using the PCR-based (RFLP) technique.

The restriction enzyme used was manufactured by New England Biolabs incorporation Ipswich, MA, USA. cat no: R0619S, we also used a 50 bp ladder for the gel electrophoresis step.

Statistical Analysis

Continuous data, such as age, were displayed as mean ± SD to simplify the display. In the case of qualitative data, such as sex, it was supplied in the form of percentages. For numerical data Student t-test was used. For caategorical data. The Chi-square test was used. P-value at ≤ 0.05 was considered significant, which is the smallest possible value. The statistical analysis was done using IBM Statistical Package for Social Science (SPSS) software Version 21 for Windows.

RESULTS

Demographic data

Item	Patients (N=30)	Controls (N=20)	T test	p Value
Age (in years)	32.4 ± 9.82	30.35 ± 10.5	0.7	0.48
Arthritis	13 (43.3)			
Butterfly rash	16 (53.3%)			
Discoid rash	8 (26.6%)			
Hematological disorder	21 (70%)			
Immunological disorder	18 (60%)			
Oral Ulcers	7 (23.3%)			
Neurogenic disorder	2 (6%)			
Photosensitivity	11 (36%)			

Table 2: Age and clinical characteristics of two groups.

Regarding the age of SLE patients and healthy controls, there were no statistically significant differences between the two stdied groups (t = 0.7, p = 0.48). Table (2) also provides a list of the clinical characteristics of SLE patients.

Variable	SLE Patients	Healthy Controls	X^2	p Value	OR	95% CI
Genotype						
GG	11	6				
GA	14	10	0.258	0.878		
AA	5	4				
Allele						
G	36	22				
A	24	18	0.246	0.619	1.02	0.104–.118

Table 3: Genotypic and allelic frequency of various genotypes in controls or cases

Association between rs4740 SNP and SLE susceptibility

There were no statistically significant differences between SLE patients and healthy controls in the genotyped SNP rs4740 studied and also in allele frequency. (p > 0.05). (See Table 3)

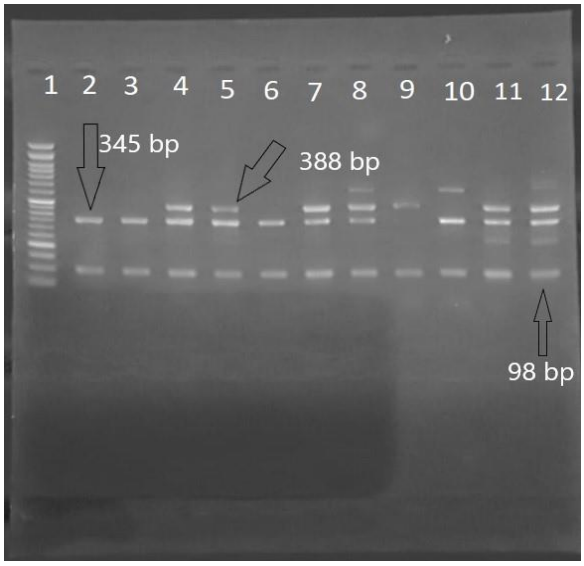

Fig. 1: The electrophoresis of EBI3 rs4740 PCR and restriction digestion products is depicted in Figure 1. Representative RFLP product samples were electrophoresed with ethidium bromide to determine their composition:
heterozygous A/G (lanes 4, 5, 7,8,11, and 12) 98 bp and 388 bp;
homozygous G/G (lanes 2, 3, 6, and 10) 98 bp and 345 bp;

homozygous A/A (lane 9) 98 bp and 388 bp.

DISCUSSION

SLE is an autoimmune illness with a significant degree of diversity in its development, making a proper and timely diagnosis challenging. Also, it is a severe disease and needs early diagnosis to prevent complications and organ damage.[10] Researchers investigated several biomarkers to early detect the disease one of these biomarkers is IL-35. [11] In our study we have tried to assess the genetic base of increased IL-35 in serum of SLE patients by investigating a common single nucleotide polymorphism that occurs in the IL-35 gene. IL-35 serum levels, as well as soluble serum gp130 levels, were found to be elevated in SLE patients compared to healthy control subjects in a study carried out by Cai et al . [11] IL-35 receptor (gp130) on CD4 + T cells was found to be low in SLE patients due to the high amounts of plasma IL-35 present in the patients' blood. Therefore, a high concentration of IL-35 may not be sufficient to induce an increase in (regulatory T) Tregs cells in SLE patients, showing that the cytokine may not respond adequately to the autoimmune disease in the absence of strong receptor expression. [11] Furthermore, increased levels of IL-35 may help to protect immune systems against pathogenic agents, hence reducing the risk of tissue and organ injury. Increased levels of IL-35 were protective responses to diseases, although the rise may have been countered by pro-inflammatory factors. [12]

Several researchers have investigated the relationship

between the IL-35 encoding genes (IL-12A and EBI3) and vulnerability to autoimmune disorders, Primary biliary cholangitis was shown to be highly related to genetic variations in the IL-12A locus (rs6441286, rs574808), according to the findings of a genome-wide association research conducted on white people from North America.[13] In this work, we investigated the relationships between IL-35 gene polymorphism and susceptibility to SLE in Egypt. No significant association between rs4740 polymorphisms and the occurrence of SLE was discovered in this study. Our results agree with Guan et al [14] findings who found than no statistically significant difference between Seven SNPs genotyped for the IL-35 gene in addition to allele frequencies for the mentioned gene including rs4740 polymorphism between SLE patients and healthy controls with the exception for occurrence of lupus nephritis and hematological disorder. [14]

The study carried out by Guan et al.[14] is the only published study on this topic. The similarity between our findings and those findings from Guan et al [14] regarding rs4740 SNP confirms that there is no association between this SNP and the occurrence of SLE not only in the Chinese population but also in the Egyptian race group. The difference between our study and Guan et al.'s study were that they studied Seven different SNPs for the IL-35 gene, while we studied one SNP (rs4740). They also compared gene polymorphism with IL-35 plasma levels. The only difference in our results was that there was an association between IL-35 SNP and the occurence of lupus nephritis and hematological disorders. It is recommended that several studies with a large Egyptian population with different manifestations of SLE to compare between them for determining the effect of IL-35 SNP on disease occurrence and complications development especially on those with lupus nephritis and hematological disorders.

CONCLUSION

In conclusion, there is no relationship between the rs4740 polymorphism and the occurrence of SLE in Egyptian patients.

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