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RESEARCH ARTICLE

ASSOCIATION OF STAT4 GENE SINGLE-NUCLEOTIDE POLYMORPHISM WITH SYSTEMIC LUPUS ERYTHEMATOSUS.

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Abstract

Background:- Systemic lupus erythematosus (SLE) is the prototypical systemic autoimmune disorder, with complex etiology and a strong genetic component. The signal transducer and activator of transcription-4 (STAT4) has been found to be a susceptible gene in the development of SLE in various populations.

Aim of the work:- To determine the association of STAT4 (rs7582694) gene polymorphism with thesusceptibility, clinical manifestation, and autoantibodies production in patients of SLE.

Patients and methods:- Ninety fourSLE patients andNinety four age and sex matchedhealthyvolunteers (control**)**were included in this study. Analysis ofSTAT4 genotyping was performed by using Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP).

Results:- The STAT 4 C/C genotype and G/C genotype frequencies were significantly higher in patients than controls (amounted to 17.0% and 4.3% for C/C genotype and amounted to 42.6% and 25.5% for G/C genotype respectively).The frequency of the STAT4 C allele was significantly higher in patients with SLE compared to control, with frequencies of 38.3% and 17.0% respectively**.**

Conclusion:- STAT4(rs7582694) gene polymorphism was significantly associated with development of SLE and occurrence of some clinical manifestation of the disease.

Introduction:-

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with strong genetic and environmental components. The disease is characterized by the presence of pathogenic auto-antibodies against a number of nuclear antigens, which results in severe immunologic abnormalities, subsequently leading to multiple tissue and organ damage [1]. Disease severity is wide ranging, with most suffering milder forms; however, it is potentially fatal depending on organ involvement.[2].The prevalence and incidence of SLE have been shown to vary across geographic regions around the world. It has been found more frequently in non-white populations compared to Caucasians, and the highest prevalence is reported among Afro-Caribbeans. Asian populations also have higher incidence and prevalence compared to Caucasians [3]. It is accepted that environmental factors together with genetic components are involved in the abnormal immune responses and pathogenesis of SLE. Flare-ups of SLE can be triggered by various environmental components, such as exposure to ultraviolet light, drugs, chemicals, and viral infections[4].

The STAT4 gene located on human chromosome 2q32.3, and consists of 24 exons spanning a 120 kb region. This gene encodes a transcription factor that can be activated by interleukin (IL)-12 and IL-23 and plays a key role in the signaling by type I IFN receptor.[5].Several genome-wide association studies have identified STAT4 as an SLE susceptible gene in Caucasian and Asian populations. Recently, many studies have demonstrated the contribution of intronic single nucleotide polymorphisms (SNPs) of STAT4 G/C (rs7582694) and G/T (rs7574865) to the incidence of SLE and its clinical manifestations. Both of these polymorphisms display complete linkage disequilibrium (LD) in Asian and Caucasian populations[6].

Signal transducer and activator of transcription 4 (STAT4) is a ranscription factor mainly activated by interleukin 12, which promotes the secretion of type 2 interferon (IFN) by T-helper 1 cells[7].The STAT4 gene encodes a transcription factor belonging to the STAT family expressed in lymphocytes, macrophages, and dendritic cells. STAT4 is essential for interleukin (IL)-12 signaling and induces interferon-gamma (IFNγ) production and Th1 differentiation. [8].

Signal transmission from the interferons involves STAT1 and STAT4, which are members of the signal transducer and activators of transcription (STAT) family of transcriptional factors. These proteins are involved in essential cellular events such as differentiation, proliferation, and apoptosis following cytokine and growth factor signaling . By binding to their receptors, interferons and other cytokines trigger Jak kinases to phosphorylate and activate STAT proteins. [9].The human STAT genes have been identified in three chromosomal clusters: STAT1 and STAT4 on human chromosome 2 (q12-33), STAT2 and STAT6 on chromosome 12 (q13-14) and STAT3, STAT5a, and 5b on chromosome 17 (q11.2-22) . The extensive involvement of type I and type II IFNs in the pathogenesis of SLE, made the cluster of STAT1 and STAT4 on chromosome 2q an obvious candidate region for genetic predisposition to this autoimmune disease **[10].**STAT4 is also activated by type I IFNs (IFNα/β) Moreover, the requirement of STAT4 in IL-23-induced IL-17 production has been suggested[11].

Aim of the work:-

To determine the association of STAT4 (rs7582694) gene polymorphism withthesusceptibility, clinical manifestation, and autoantibodies production in patients of SLE.

Patients and Methods:-

The current study was conducted on ninety-four SLE patientswith an average age 31.45 ± 8.33 years,attending the Rheumatology and Rehabilitation Department, Zagazig University Hospitals. They diagnosed according to Systemic Lupus International CollaboratingClinics classification criteria for SLE[12]. Ninety-fourhealthy controls with an average age 29.09 ± 8.67 years were recruited from individuals who took comprehensive examination in hospitals. All the precipitants were unrelated, gave their consent to participate in the study and to allow their biological samples to be analyzed.To investigate the association between STAT4(rs7582694)variation and clinical feature of SLE,the skin rash, photosensitivity,serositis,gastrointestinal symptoms, arthritis, renal manifestations of the patients the results were recordedin (Table 1). All the patients were subjected to the following:*Complete blood count ,routine urine examination.*Autoantibodies against double-stranded DNA (ds-DNA),and antinuclear antibodies were detected by immunofluorescence method (Diasorin,USA). Other autoantibodies ,including anti-sm, anti-SSA(Ro),anti-SSB(La)ani-RNP, anti-SCL 70,anti Jo1 were detected by Enzyme Linked Immunosorbent assay(Elisa).

Genotyping:-

Genomic DNA of each participants was extracted from peripheral Blood leukocytes using a QIA Amp DNA Minikit (Qiagen) according to manufactures instructions. Identification of the STAT4(rs7582694) polymorphic variant was performed by using Polymerase Chain Reaction- restriction fragment length polymorphism (PCR-RFLP). PCR was conducted employing primer pair.

Forward primer 5′ ATCCAACTCTTCTCAGCCCTT 3′.
Reverse primer 5′ TCATAATCAGGAGAGAGGAGT 3′.

The PCR amplified fragments of STAT4 that were 338bp in length were isolated and digested with endonuclease TAAl (Sigma). DNA fragments were separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistial analysis:-

The distribution of genotypes in patients and controls was examined using exact and log likelihood ratio χ^2tests The polymorphism was tested for association with SLE incidence using the χ^2 test for trend (ptrend). The χ^2 test was employed to examine differences in genotypic and allelic distribution between patients and controls, and a p value <0.05 was considered statistically significant. The Odds Ratio (OR) and 95 % Confidence Intervals (95 % CI) were calculated. Contribution of the STAT4 C/G polymorphism to clinical manifestations and the production of autoantibodies (Ab) was determined by χ^2test.

Table 1:- Characteristics of SLE patients.

Variable	Cases(n=94)	
	Positive	**Negative**
Malar rash:	47(50%)	47(50%)
Photosensitivity:	24(25.5%)	70(74.5%)
Arthritis:	60(63.8%)	34(36.2%)
Serositis:	18(19.1%)	76(80.9%)
Renal:	57(60.6)	37(39.4%)
Hematological:	63(67%)	31(33%)
GIT:	24 (25.5%)	70 (74.5%)
Hair fall:	54(57.%4)	40(42.6%)
Neurologic:	17(18.1%)	77 (81.9%)
Immunologic:	49(52.1%)	45 (47.9%)

Results:-

Demographic characteristics of SLE patients and healthy controls are presented in(Table2). there were no significant difference in the mean age between SLE patients and healthy control (p>0.05).

Table 2:- Demographic data of the two studied group.

Variable	Cases (n=94)		Control (n=94)		T	P
Age : (year) Mean ± SD Range	31.45 ± 8.33 20 – 56		29.09 ± 8.67 17 – 50		1.90	>0.05
Variable	No	%	No	%	χ^2	P
Sex: Female Male	86 8	91.5 8.5	82 12	87.5 12.5	0.90	>0.05

>0.05NS

The distribution of genotype and allele frequencies of rs7582694 was showed that, the STAT 4 C/C genotype and G/C genotype frequencieswere significantly higher in patients than controls (amounted to 17% and 4.3% for C/C genotype and for G/C genotype amounted to 42.6% and 25.5% respectively), (p value: 0.001),(table;3).

To evaluate the effect of minor allele as a risk factor in SLE incidence,assessment of minor allele's distribution in patients and controls was performed. The frequency of the STAT4 C allele was significantly higher in patients with SLE compared tocontrol, with frequencies of 38.3% and 17% respectively (p <0.001) , (Table3).

Table: 3:- genotype andallele distributionof rs7582694 between patients and controls

Variable	Cases (n=94)		Control (n=94)		OR	χ^2	P
	No	%	No	%			
Genotype:							
CC	16	17	4	4.3	6.95 (2.17-22.3)	12.89	<0.001
GC	40	42.6	24	25.5	2.9 (1.52-5.52)	10.74	0.001
GG	38	40.4	66	70.2	1	--	--
Allele frequency:							
C	38.3		17.02		3.03 (1.87 – 4.89)	21.27	<0.001
G	61.7		82.97				HS

P<0.001 HS

The relation between STAT4 genotypes and the presence of various autoantibodies in SLE patients were examined. As regard ANA ,and anti Scl -70, a higher percentage in C/C and G/C (100%) equally,and C/C (18.8%) were observed (p <0.05) , also for anti-SSB, anti RNP, and anti Jo-1 a higher percentage in G/G genotype (21.1%),C/C genotype (25%),and C/C genotype (2.5%) respectively(p <0.01,(Table 4).

Table 4:- Contribution of the STAT4 polymorphic variants to the presence of various autoantibodies in patients with SLE.

Variable	CC (n=16)		GC (n=40)		GG (n=38)		χ^2	P
	No	%	No	%	No	%		
Anti-DNA: (Positive)	8	50	21	52.5	19	50	0.06	>0.05
ANA: (Positive)	16	100	40	100	34	89.5	6.16	<0.05
Anti SS-A: (Positive)	4	25	12	30	16	42.1	1.97	>0.05
Anti SS-B: (Positive)	0	0	0	0	8	21.1	12.89	<0.01
Anti Sm:(Positive)	6	37.5	4	10	7	18.4	5.84	>0.05
Anti RNP: (Positive)	4	25	8	20	0	0	9.59	<0.01
Anti Scl 70:Positive)	3	18.8	4	10	0	0	6.4	<0.05
Anti Jo--1: (Positive)	2	12.5	0	0	0	0	9.97	<0.01

>0.05 Non-significant <0.05 Significant <0.01 Highly Significant

The association of STAT4 genotypes and clinical manifestation in patients, is shown in **Table (5)**.There was a significant association between the STAT4 genotypes and the presence of photosensitivity and GIT manifestation(p**<0.05)**, and a highly significant association between the STAT4 genotypes and the presence of malar rash.(p<0.01).

Table 5: Relation between clinical picture of the patients and genotype.

Variable	CC (n=16)		GC (n=40)		GG (n=38)		χ^2	P
	No	%	No	%	No	%		
Malar rash:								
No	12	75	12	30	23	60.5	12.08	<0.01***
Yes	4	25	28	70	15	39.5		
Photosensitivity:								
No	8	50	32	80	30	78.9	6.08	<0.05**
Yes	8	50	8	20	8	21.1		
Arthritis:								
No	7	43.8	12	30	15	39.5	1.24	>0.05*
Yes	9	56.2	28	70	23	60.5		
Serositis:								
No	10	62.5	36	90	30	78.9	5.73	>0.05*
Yes	6	37.5	4	10	8	21.1		
Renal:								
No	10	62.5	12	30	15	39.5	5.06	>0.05*
Yes	6	37.5	28	70	23	60.5		
Hematological:								
No	4	25	13	32.5	14	36.8	0.72	>0.05*
Yes	12	75	27	67.5	24	63.2		
GIT:								
No	16	100	28	70	26	68.4		
Yes	0	0	8	20	12	31.6	12.45	<0.05**
Oral ulcer	0	0	4	10	0	0		
Hair fall:								
No	6	37.5	18	45	16	42.1	0.27	>0.05*
Yes	10	62.5	22	55	22	57.9		
Immunologic:								
Negative	8	50	18	45	19	50	0.23	>0.05*
Positive	8	50	22	55	19	50		
Neurologic:								
Negative	12	75	31	77.5	34	89.5	2.51	>0.05*
Positive	4	25	9	22.5	4	10.5		

*** >0.05 Non-significant **<0.05 Significant***<0.01 Highly Significant**

Discussion:-

Theexpression of STATs has been observed in a vast range ofcell types, however the expression of STAT4 mainly takesplace in immune cells and the testis [13]. STAT4 isessential for signal transduction by interleukin-12 (IL-12),interleukin-23 (IL-23), and type 1 interferon (IFN) in Tcells and monocytes.STAT4 deficiency results in areduction of IFN biosynthesis in immune cells [5].Accordingly, an association between disease activity inSLE patients and activation of the type 1 IFN system hasbeen observed [14].

The scope of this study was to determine the association of STAT4 (rs7582694) gene polymorphism with the susceptibility, clinical manifestation, and autoantibodies production in patients of SLE.
In this study STAT4 rs7582694 intronic substitution was shown to be significantly increase the risk of SLE occurrence in the sample population.

studies carried out by Piotrowski et al. [4]. demonstrated a statistically significant contribution of STAT4 (rs7582694) to SLE incidence in polish.Luan et al. [15] demonstrated a statistically significant contribution of STAT4 (rs7582694) to SLE incidence in the Mainland Chinese female population.

A significantly higher level of theC allelefrequency (38.3%) was reported in SLE patients in relation to control(17.02%). Correspondingly, the G allele frequency showed a remarkable decrease. This suggests that this SNP was strongly associated with SLE patients and the C allele to be a risk allele . This was consisting withSigurdsson et al. who reported that 10 out of 53 analyzed SNPsin STAT4 were associated with SLE in Swedish patients with the strongest signal of association for two perfectly linked SNPs rs 10181656 and rs7582694 . [11].

The role of STAT4 (rs7582694) as a risk allele in development of renal and neurologic manifestation has not established in this study in concordance withLuan et al. [15] that reported no association between rs7582694 and sub phenotype of SLE in Chinese Han population.On the other hand, Piotrowski et al. [4] found that this SNP can be associated with renal and neurological symptoms of SLE. Since this autoimmune disease is vastly heterogeneous, further studies of this polymorphism's effects on clinical manifestationsof SLE in other populations would be valuable.

There was a significant association between the STAT4 genotypes and the presence of photosensitivity, GIT manifestation and a highly significant association with the presence of malar rash.The different effects of the STAT4 (rs7582694) or STAT4 SNPs on clinicalmanifestations in various ethnicities may result from different sizes of the studied groups, genetic heterogeneity or patient interaction with disparate environmental factors [16].

The frequencies of ANA positivity among SLE patients was nearly the same as reported in Hefny et al , [17].study, (95.7%) that was carried out in 2006 investigating ANA, anti ds-DNA and antibodies against extractable nuclear antigens in SLE Egyptian patients.

Anti ds-DNA, anti Sm, anti RNP, and anti La/SSB frequencies was slightly lower and amounted (51.1%), (18.1%), (12.8%), and (8.5%) respectively. However, anti Scl-70 was higher (7.4%) compared to (2.6%) in Hefny et al, [17]. 2006 study in which anti Jo-1 was not detected while amounted (2.4%) in our study. Anti Ro/ SSA was (34%) and was not included in the other study.

Conclusion:-

STAT4(rs7582694) gene polymorphism was significantly associated with development of SLE and occurrence of some clinical manifestation of the disease.

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