TNF-alpha promoter -308 and PTPN22 C1858T genes polymorphisms in Systemic lupus erythematosus

Thesis

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By

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بسم الله الرحمن الرحيم

(قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم)

صدق الله العظيم

Abstract

Objective: To assess the role of TNFα-308 G/A and PTPN22 C1858T SNP with respect to SLE susceptibility in Egyptian patients and whether these genetic polymorphisms are associated with the clinical and immunological features of the disease. Also determination of TNF alpha concenteration in relation to different genotypes and in relation to disease activity.

Methods: 40 SLE patients & 40 healthy subjects were tested for TNF alpha -308 and PTPN22 (C1858T) genotypes by PCR-RFLP and TNFα concenteration was measured in their serum using ELISA.

Results: No significant differences in TNFα-308 and PTPN22 (C1858T) genotypes or alleles frequencies could be identified between SLE cases and controls (P=0.108, 0.152 respectively). The level of serum TNFα was significantly higher in SLE patients when compared with the healthy control volunteers ($P < 0.001$). Furthermore, TNFα serum level was also statistically significantly higher in SLE patients with cardiac affection, with vasculitis and with low complement level (P=0.045, 0.016, 0.015 respectively). The serum level of TNF was statistically significantly higher in SLE group with high disease activity when compared with those low disease activity ($P = 0.001$). Also, there was a significant positive correlation between serum TNFα and SLEDAI ($r = 0.723$, $P < 0.001$).

Conclusion: The results of this study suggest that, TNFα -308 and PTPN22 (C1858T) polymorphisms, do not exhibit a significant influence on the susceptibility, disease course or laboratory characteristics in SLE in Egyptian patients. Nevertheless, serum TNFα level could be a sensitive marker of SLE disease activity.

Key words: Genetics - polymorphism - TNF - PTPN22 - SLE

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A	Adenine
aCL	anticardiolipines antibodies
ACR	American college of Rheumatology
ALT	Alanine aminotransferase
ANA	Antinuclear antibody
Anti-RNP	Anti ribonucleoprotein
Anti-Sm	Anti Smith
AP-4	activator protein-4
APCs	Antigen presenting cells
APLA	Antiphospholipid antibodies
APL	Antiphospholipid
APS	Antiphospholipid syndrome
ARMS	Amplification refractory mutation system
ASO	Allele specific oligonucleotide
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
B2GP1	Beta-2 glycoprotein 1
BCR	B-cell antigen receptor
BILAG	British Isles lupus assessment group
Bp	Base pair
BUN	Blood urea nitrogen
C	Cytosine
C3	Complement component 3
C4	Complement component 4
CBC	Complete blood picture
CL	Cardiolipin
CNS	Central nervous system

CPK	Creatine phosphokinase
CRP	C-reactive protein
Csk	C-terminal Src tyrosine kinase
CTH	C-terminal homology
Cu	Copper
CVA	cerebrovascular accident
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotides Triphosphate
dsDNA	Double stranded DNA
EBV	Epstein Barr virus
ECLAM	European Community Lupus Activity Measure
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme linked immunosorbant assay
ESR	Erythrocyte sedimentation rate
F	Forward
FAM	6-carboxyfluorescein
Fe	Iron
G	Guanine
GM-CSF	Granulocyte-monocyte colony stimulating factor
Grb2	growth factor receptor-bound protein 2
HLA	Human leukocytic antigen
HRP	Horseradish peroxidase
HRT	Hormonal replacement therapy
ICAM-1	Intercellular Adhesion Molecule 1
IF	Immunofluorescence
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ISN/RPS	International Society of Nephrology/Renal Pathology Society
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Kb	Kilo base

kD	Kilo Dalton
LAC	Lupus Anticoagulant
LDL	Low-density lipoprotein
Lt	Left
LYP	Lymphoid tyrosine phosphatase
M.W.	Molecular Weight
$MgCl_2.6H_2O$	Magnesium Chloride Hexahydrate
MHC	Major histocompatibility comlex
ml	Milliliter
mM	Millimole
n	Number
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
ng	Nanogram
NK	Natural killer
nm	Nanometer
NSAIDs	Nonsteroidal anti-inflammatory drugs
oxLDL	Oxidized LDL
P Value	Probability Value
PCR	Polymerase chain reaction
PEP	Proline-enriched protein tyrosine phosphatase
pg	Picogram
PPi	Pyrophosphate
PTPN22	Protein tyrosine phosphatase non-receptor 22
PTT	Partial thromboplastin time
R	Reverse
R	Arginine
r	Correlation coefficient
RA	Rheumatoid arthritis
RBCs	Red Blood Cells
RFLP	Restriction fragment length polymorphism

RHD	Rheumatic heart disease
RIA	Radioimmunoassay
RNA	Ribonucleic acid
Rt	Right
S35	serine 35
SD	Standard deviation
SH3	Src homology 3
SLAM	Systemic lupus activity measure
SLE	Systemic lupus erythematosus
SLEDAI	Systemic lupus erythematosus disease activity index
SNP	Single nucleotide polymorphism
sTNFRs	soluble TNF receptors
T	Thymine
TAMRA	Tetramethylrhodamine
TBE	Tris Borate EDTA
TCR	T-cell receptor
TET	Tetrachlorofluorescin
TGFß	Transforming growth factor beta
Th	T helper
TMB	Tetramethylbenzidine
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFα	Tumor necrosis factor alpha
TNFβ	Tumor necrosis factor beta
Tris-HCl	Tris – Hydrochloric Acid
ul	Microlitre
UV	Ultraviolet light
W	Tryptophan
W.H.O.	World health organization
Zn	Zink

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease, characterized by the production of multiple autoantibodies, complement activation, and immune-complex deposition, resulting in tissue and organ damage ***(Pan et al., 2011).***

Investigators have studied several cytokines involved in SLE pathogenesis. The association between SLE and inflammation emphasize the importance of cytokine network genes ***(Lin et al., 2009).***

Tumour necrosis factor alpha (TNFα), an important proinflammatory cytokine, exerts a variety of physiological and pathogenic effects, including the activation of a cascade of inflammatory events, which lead to tissue destruction in autoimmune diseases ***(Serrano et al., 2006).***

The presumptive pathophysiological role of TNFα in SLE suggests that genetic polymorphisms affecting the TNFα production capacity may influence the susceptibility to SLE. The single-nucleotide polymorphism TNFα –308 G/A is located in the promoter region of TNFα gene. The TNFα –308A allele has been reported to be a stronger transcriptional activator in vitro than the common TNFα –308G allele ***(Zou et al., 2010).***

Multiple abnormalities of T and B lymphocytes are frequently found in patients with SLE and are central to pathogenesis of the disease ***(Mustelin et al., 2004).***

The gene protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) encodes the lymphoid protein tyrosine phosphatase (Lyp) that is known to be involved in the control of T-cell activation. Under normal conditions, this enzyme (Lyp) works as a 'negative regulator' and keeps immune cells from becoming overactive ***(Reddy et al., 2005).***

The functional PTPN22 C1858T (R620W) polymorphism resides in a motif involved in C-terminal Src tyrosine kinase (Csk) binding. When a tryptophan (W) residue replaces an arginine (R) at this site, it disrupts the interaction of Lyp with Csk, thereby disturbing the regulation of the T cell receptor (TCR) signaling kinases ***(Akosy et al., 2011).***

It seems that the R620W polymorphism, by suppressing TCR and BCR (B cell receptor) signaling, globally alters maturation, selection, and function of both T- and B-lymphocytes that predisposes to inducing autoimmunity ***(Stanford et al., 2010).***

AIM OF THE WORK

The aim of the present study was to assess the role of TNFα-308 G/A and PTPN22 C1858T SNPs with respect to SLE susceptibility in Egyptian patients and whether these genetic polymorphisms are associated with the clinical and laboratory features of the disease. Also determination of serum TNF alpha concenteration in relation to different genotypes and in relation to disease activity.