



# **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 العظيم الحكيم)

صدق الله العظيم

# Abstract

**Objective:** To assess the role of TNF $\alpha$ -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 concentration 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 TNF $\alpha$  concentration was measured in their serum using ELISA.

**Results:** No significant differences in TNF $\alpha$ -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 TNF $\alpha$  was significantly higher in SLE patients when compared with the healthy control volunteers ( $P < 0.001$ ). Furthermore, TNF $\alpha$  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 TNF $\alpha$  and SLEDAI ( $r = 0.723$ ,  $P < 0.001$ ).

**Conclusion:** The results of this study suggest that, TNF $\alpha$  -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 TNF $\alpha$  level could be a sensitive marker of SLE disease activity.

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

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## **LIST OF ABBREVIATIONS**

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

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

<b>kD</b>	Kilo Dalton
<b>LAC</b>	Lupus Anticoagulant
<b>LDL</b>	Low-density lipoprotein
<b>Lt</b>	Left
<b>LYP</b>	Lymphoid tyrosine phosphatase
<b>M.W.</b>	Molecular Weight
<b>MgCl<sub>2</sub>.6H<sub>2</sub>O</b>	Magnesium Chloride Hexahydrate
<b>MHC</b>	Major histocompatibility complex
<b>ml</b>	Milliliter
<b>mM</b>	Millimole
<b>n</b>	Number
<b>NaCl</b>	Sodium Chloride
<b>NaOH</b>	Sodium hydroxide
<b>ng</b>	Nanogram
<b>NK</b>	Natural killer
<b>nm</b>	Nanometer
<b>NSAIDs</b>	Nonsteroidal anti-inflammatory drugs
<b>oxLDL</b>	Oxidized LDL
<b>P Value</b>	Probability Value
<b>PCR</b>	Polymerase chain reaction
<b>PEP</b>	Proline-enriched protein tyrosine phosphatase
<b>pg</b>	Picogram
<b>PPi</b>	Pyrophosphate
<b>PTPN22</b>	Protein tyrosine phosphatase non-receptor 22
<b>PTT</b>	Partial thromboplastin time
<b>R</b>	Reverse
<b>R</b>	Arginine
<b>r</b>	Correlation coefficient
<b>RA</b>	Rheumatoid arthritis
<b>RBCs</b>	Red Blood Cells
<b>RFLP</b>	Restriction fragment length polymorphism

<b>RHD</b>	Rheumatic heart disease
<b>RIA</b>	Radioimmunoassay
<b>RNA</b>	Ribonucleic acid
<b>Rt</b>	Right
<b>S35</b>	serine 35
<b>SD</b>	Standard deviation
<b>SH3</b>	Src homology 3
<b>SLAM</b>	Systemic lupus activity measure
<b>SLE</b>	Systemic lupus erythematosus
<b>SLEDAI</b>	Systemic lupus erythematosus disease activity index
<b>SNP</b>	Single nucleotide polymorphism
<b>sTNFRs</b>	soluble TNF receptors
<b>T</b>	Thymine
<b>TAMRA</b>	Tetramethylrhodamine
<b>TBE</b>	Tris Borate EDTA
<b>TCR</b>	T-cell receptor
<b>TET</b>	Tetrachlorofluorescein
<b>TGFβ</b>	Transforming growth factor beta
<b>Th</b>	T helper
<b>TMB</b>	Tetramethylbenzidine
<b>TNF</b>	Tumor necrosis factor
<b>TNFR</b>	Tumor necrosis factor receptor
<b>TNFα</b>	Tumor necrosis factor alpha
<b>TNFβ</b>	Tumor necrosis factor beta
<b>Tris-HCl</b>	Tris – Hydrochloric Acid
<b>ul</b>	Microlitre
<b>UV</b>	Ultraviolet light
<b>W</b>	Tryptophan
<b>W.H.O.</b>	World health organization
<b>Zn</b>	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 (TNF $\alpha$ ), 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 TNF $\alpha$  in SLE suggests that genetic polymorphisms affecting the TNF $\alpha$  production capacity may influence the susceptibility to SLE. The single-nucleotide polymorphism TNF $\alpha$  –308 G/A is located in the promoter region of TNF $\alpha$  gene. The TNF $\alpha$  –308A allele has been reported to be a stronger transcriptional activator in vitro than the common TNF $\alpha$  –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 TNF $\alpha$ -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 concentration in relation to different genotypes and in relation to disease activity.