The Influence of the MDR1 C3435T Polymorphism on Methotrexate Responsiveness in Rheumatoid Arthritis Patients

Thesis

Submitted for Partial Fulfillment of M.D. Degree in Clinical Pathology

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2019

Acknowledgement

No words can express my deepest appreciation and profound respect for ***Prof. Dr. Shahira Fathy El Fedawy,*** *Professor of Clinical Pathology, Ain Shams University, for her continuous guidance and support. She has generously devoted much of her time and her effort for planning and supervision of this study.*

Also, my profound gratitude to ***Prof. Dr. Abeer Al Sayed Ali Shehab,*** *Professor of Clinical Pathology, Ain Shams University, for her kind supervision and support. It was great honor to work under her supervision.*

I would like also to thank ***Prof. Dr. Rania Ahmed Abo-Shady,*** *Professor of Clinical Pathology, Ain Shams University Hospitals, for her support, help and constructive criticism during this work.*

I would like also to thank ***Prof. Dr. Samah Abdel Rahman ElBakry,*** *Professor of Internal Medicine and Rheumatology Ain Shams University Hospitals, for her support and help during this work.*

Also, my profound gratitude to ***Ass. Prof. Dr. Dina Aly Mohamed Aly Ragab,*** *Assistant Professor of Clinical Pathology, Ain Shams University, for her great care and support.*

Last but not least, I dedicate this work to my family, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.

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List of Abbreviations

- **ABC** : ATP binding cassette proteins
- **ABCB1** : ATP binding cassette subfamily B member 1 gene
- **ACPA** : Anti-citrullinated protein antibodies
- **ACR** : American College of Rheumatology
- **ADP** : Adenosine diphosphate
- **AICAR** : 5-aminoimidazole-4-carboxamide ribonucleotide
- **ALT** : Alanine aminotransferase
- **AMP** : Adenosine monophosphate
- **ANA** : Antinuclear antibodies
- **anti-CarP** : Anti-carbamylated protein
- **anti-CCP** : Anti-cyclic citrullinated peptide antibodies
- **anti-MCV** : Anti-mutated citrullinated vimentin antibodies
- **APC** : Antigen-presenting cell
- **AST** : Aspartate aminotransferase
- **ATIC** : 5-aminoimidazole-4-carboxamide ribonucleotide
- **ATP** : Adenosine triphosphate
- **bDMARDs** : Biologic disease modifying antirheumatic drugs
- **CBC** : Complete blood count
- **CCP** : Cyclic citrullinated peptide
- **CCP1** : First generation ACCP assay
- **CCP2** : Second generation ACCP assay
- **CCP3** : Third generation ACCP assay
- **CD** : Cluster of differentiation
- **cDMARDs** : Conventional disease-modifying antirheumatic
- **CH2-FH4** : 5,10-methylenetetrahydrofolate
- **CI** : Confidence intervals
- **CRP** : C-reactive protein
- **CTLA4** : Cytotoxic T-lymphocyte antigen 4
- **DAS 28** : Disease Activity Score 28 deiminases
- **DHFR** : Dihydrofolate reductase
- **DIP** : Distal interphalangeal joints
- **DMARDs** : Disease-modifying antirheumatic drugs
- **DNA** : Deoxyribonucleic acid
- **dNTP** : Deoxyribonucleotide triphosphate drugs
- **dTMP** : Deoxythymidine monophosphate

- **dTTP** : Deoxythymidine triphosphate
- **dUMP** : Deoxyuridine monophosphate
- **EDTA** : Ethylenediaminetetraacetic acid
- **ELISA** : Enzyme-linked immunosorbent assay
- **ESR** : Erythrocyte sedimentation rate
- **EULAR** : European League Against Rheumatism
- **Fab** : Fragment antigen-binding
- **Fc** : Fragment crystallizable
- **FDA** : Food and Drug Administration
- **FH_2** : Dihydrofolate
- **FH_4** : Tetrahydrofolate
- **FOLT** : Folate transporter
- **FPGS** : Folylpolyglutamate synthetase
- **GGH** : γ-glutamyl hydrolase
- **GH** : The patient global health assessment
- **GIT** : Gastrointestinal tract
- **Hb** : Hemoglobin
- **HCQ** : Hydroxychloroquine
- **HCV** : Hepatitis C virus
- **HLA** : Human leucocyte antigen
- **HS** : Highly significant
- **IFN-γ** : Interferon γ
- **Ig** : Immunoglobulin
- **IL** : Interleukin
- **IL-1ra** : Interleukin -1 receptor antagonist
- **IMP** : Inosine monophosphate
- **IQR** : Interquartile range
- **ITP** : Inosine triphosphate
- **ITPA** : Inosine triphosphate-pyrophosphatase
- **IU** : International unit
- **JAK** : Janus kinase
- **ln ESR** : The natural logarithm of the ESR
- **MCP** : Metacarpophalangeal joint
- **MDR1** : Multidrug resistance 1 gene
- **MHC** : Major histocompatibility complex

- **ml** : Milliliter
- **MMPs** : Matrix metalloproteinases
- **mRNA** : Messenger RNA
- **MRP** : MDR-associated protein
- **MTP** : Metatarsophalangeal joint
- **MTX** : Methotrexate
- **MTX-Glu** : Polyglutamated methotrexate
- **NF-κB** : Nuclear factor kappa light chain enhancer of activated B-cells
- **NS** : Non-significant
- **OPGL** : Osteoprotegerin ligand
- **OR** : Odds ratio
- **P. ging** : Porphyromonas gingivalis
- **PADs** : Peptidyl-arginine deiminase
- **PCR** : Polymerase chain reaction
- **Pgp** : Permeability glycoprotein
- **PIP** : Proximal interphalangeal joint
- **PLT** : Platelets
- **PPAD** : Porphyromonas gingivalis peptidyl-arginine
- **PTPN22** : Protein tyrosine phosphatase non-receptor 22
- **RA** : Rheumatoid arthritis
- **RF** : Rheumatoid factor
- **RFC1** : Reduced folate carrier protein
- **RNA** : ribonucleic acid
- **rpm** : Revolution per minute
- **rs** : Reference SNP cluster
- **S** : Significant
- **SE** : Shared epitope
- **SJC** : Swollen joint counts
- **SLC19A1** : Solute carrier family 19 member 1
- **SNP** : Single nucleotide polymorphism
- **SPSS** : Statistical package for the social sciences
- **SSZ** : Sulfasalazine
- **TCR** : T cell receptor
- **TGF-β** : Transforming growth factor β
- **Th17** : Type 17 helper T

- **TJC** : Tender joint counts
- **TLC** : Total leukocyte count
- **TLR** : Toll-like receptor
- **TNF** : Tumor necrosis factor
- **TS** : Thymidylate synthetase
- **tsDMARDs** : Targeted synthetic disease modifying antirheumatic drugs
- **TYMS** : Thymidylate synthetase
- **ULN** : Upper limit of normal

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease primarily targeting the synovial joints causing joint damage and significant functional impairment ***(Choy, 2012).***

The management of RA rests primarily on the use of disease-modifying anti-rheumatic drugs (DMARDs). These agents are commonly characterised by their capacity to reduce signs, symptoms and progression of joint damage ***(Smolen et al., 2007)***. Methotrexate (MTX), one of the DMARDs, remains the mainstay for treatment in the majority of patients with RA ***(Smolen et al., 2014).***

However, methotrexate is associated with immunosuppression, which may lead to bone marrow depression and increased susceptibility to infection ***(van Ede et al., 1998).***

The human multidrug resistance gene 1 (MDR1) encodes a plasma membrane, P-glycoprotein (P-gp), which functions as a transmembrane efflux pump for various structurally unrelated anticancer agents and toxins. Polymorphisms in the MDR1 gene may have an impact on

the expression and function of P-gp, thereby responsiveness to drugs ***(Chen et al., 2012)***.

Lately, a silent C3435T polymorphism in exon 26 of MDR1 has been reported to be importantly associated with the expression and function of P-gp, and thus, the MDR1 polymorphism may have an impact on the expression and influence the response to methotrexate in RA patients ***(Chen et al., 2012).***

Aim of the Work

The aim of this study is to determine the influence of the MDR1 C3435T polymorphism on Methotrexate responsiveness in rheumatoid arthritis patients.

Rheumatoid Arthritis

Rheumatoid arthritis is a chronic inflammatory autoimmune and systemic disease characterized by chronic synovial inflammation, hyperplasia and joint damage leading to functional decline and disability. Systemic features of RA include cardiovascular complications, pulmonary complications and skeletal disorders. Although systemic manifestations may be present at the onset, they develop more usually as the disease progresses ***(Giasuddin et al., 2014).***

In socio-economic terms, RA is the most common and most important of the inflammatory rheumatic diseases, with a prevalence of ~1% of the population worldwide and 0.3% in Egypt ***(Usenbo et al., 2015).***

The relatively high prevalence, irreversible joint damage and widespread occurrence of co-morbidities determine the huge social impact of this disease. A therapeutic window of opportunity exists early in the course of the disease during which the introduction of aggressive antirheumatic therapy can result in a change in the course of disease, leading to protection against progressive joint destruction, prevention of disability and potential lowering of the risk of cardiovascular co-morbidity. Advances in understanding the pathogenesis of the disease have

fostered the development of new therapeutics, with improved outcomes ***(Myasoedova et al., 2010).***

I- Predisposing Factors for RA:

A. Genetic factors:

Several factors have strongly suggested that genetics are a major influence on the development of RA. These factors include the general increased prevalence of RA within families, leading to estimations of familial risk contribution of ~40-50% of seropositive RA, with strongest risks seen in first-degree relatives ***(Nordang et al., 2013).***

The largest genetic risk factor for RA lies within the human leucocyte antigen (HLA) class II region that encodes the HLA-DRB1 molecule. Specific HLA-DRB1 alleles as DRB1*04 (*04:01, *04:04) and *01 (*01:01) alleles, and DRB1*10 and *14 alleles (*10:01, *10:02, *14:02 and *14:17) were reported to be associated with anti-citrullinated protein antibodies (ACPA)-positive RA which has been related to a more aggressive disease with more frequent erosions RA ***(Nordang et al., 2013).***

A characteristic for these alleles is that they encode a conserved sequence of amino acids, the so-called "shared epitope" (SE), comprising residues 70–74 in the third hyper variable region of the DR1 chain. These residues constitute a helical domain forming one side of the antigen binding site, a site likely to affect antigen presentation ***(Newton et al., 2004).***