Anti-Citrullinated Protein Antibodies and Rheumatoid Factor In First-Degree Relatives Of Rheumatoid Arthritis Patients

Thesis

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By

Jillan Reyad Abdel Aziz

M.B.B.Ch

Supervised By

Prof. Dahlia Abdel Mohsen Hussein

Prof. of Internal Medicine and Rheumatology
Ain Shams University-Faculty of medicine

Dr. Amina Badr Eldin Abd Elaziz

Assist. Prof. of Internal Medicine and Rheumatology
Ain Shams University- Faculty of medicine

Dr. Sameh Abdel Moteleb Hassan

Assist. Prof. of Internal Medicine and Rheumatology
Ain Shams University- Faculty of medicine

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Jillan Reyad Abdel Aziz

List of Contents

List of Abbreviations

ACPA	Anti-citrullinated proteins antibodies
anti-CarP	Anti–carbamylated protein antibodies
AP-2α	Activator protein 2α
APRIL	proliferation-inducing ligand
BANK1	B-Cell Scaffold Protein With Ankyrin Repeats 1
BLyS	B-lymphocyte stimulator
C5	complement 5
CC	β-chemokines
Csk	C-terminal Src kinase
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CXC	α-chemokines
ELISA	enzyme-linked immunosorbent assay
FCGR2A	Low affinity immunoglobulin gamma Fc region receptor II-a
FDRS	First degree relatives
FLS	fibroblast-like synoviocytes
Flt3L	Fms-like tyrosine kinase 3 ligand
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
HLA	Human leukocyte antigen
ICAM-1	Intercellular Adhesion Molecule 1
IDDM	Insulin dependent diabetes mellitus
IL-11	Interleukin 11
IRAK1	Interleukin-1 receptor-associated kinase 1
IRF7	Interferon regulatory factor 7
ITGAM	integrin subunit alpha M
JIA	Juvenile idiopathic arthritis
KLF	Kruppel-like transcriptional regulatory factors
LIF	leukemia inhibitory factor
MEIA	Microparticle Enzyme Immunoassay
MHC	major histocompatibility complex
MMP	matrix metalloproteinases
MS	Multiple sclerosis

List of Abbreviations (Cont.)

MTP	Metatarsophalangeal
NF-κB	nuclear factor κB
OX40L	ligand for CD134
PADI	peptidyl arginase deiminas
PBMC	peripheral blood mononuclear cells
PDCD1	Programmed Cell Death 1
PMN	polymorphonuclear leukocytes
PTPN22	Protein tyrosine phosphatase non-receptor 22
RA	Rheumatoid arthritis
RF	Rheumatoid Factor
SE	shared epitope
SNP	single-nucleotide polymorphism
ST2	suppression of tumorigenicity 2
STAT4	Signal transducer and activator of transcription 4
Th1	type 1 helper
TIMP	tissue inhibitors of metalloproteinases
TLR	Toll-like receptors
TLR7/9	Toll-like receptor 7 and 9
TNF	Tumor necrosis factor
TNFAIP3	Tumor necrosis factor-α-induced protein 3
TNFSF4	Tumor Necrosis Factor Ligand Superfamily, Member 4
TRAF1	TNF receptor associated factor 1
TREX1	Three prime repair exonuclease 1
TYK2	tyrosine-protein kinase
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor

List of Figures

List of Tables

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown etiology that leads to joint damage, significant disability, and reduced life expectancy ***(Gabriel et al., 2003).***

Nearly 70% of the cases of established RA are characterized by the presence of autoantibodies, either rheumatoid factor (RF) or antibodies to citrullinated protein antibodies (ACPAs) ***(Nishimura et al., 2007).***

Citrullination is a posttranslational modification of endogenous proteins in which arginine is converted to citrulline in the presence of calcium and is catalyzed by peptidylarginine deiminase. This process occurs completely in the presence of inflammation and apoptosis ***(Vossenaar et al., 2003).*** However, antibodies targeting citrullinated proteins and peptides (ACPAs) are >95% specific for rheumatoid arthritis (RA) ***(Taylor et al., 2011).***The concomitant evaluation of RF and anti-CCP represents the most powerful prognostic marker for RA. ***(Van Venrooij., 2008).***

ACPAs have been detected up to 15 years before the onset of RA and have been associated with an increased risk of RA in patients were diagnosed as undifferentiated inflammatory arthritis ***(Kokkonen et al., 2011).***

Because First-degree relatives of patients share some of the genetic and environmental risk factors for RA so they may represent a pre-RA state ***(Kolfenbach et al., 2009).*** As First-Degree relatives do not have clinically apparent disease but are at increased risk of future RA, they are an informative population in which to study relationships between RA-related autoantibodies, epidemiologic exposures, and potential etiologies of RA ***(Arlestig et al., 2012).*** Familial RA has been reported as a key risk factor for severe joint damage in various populations ***(Rojas-Villarraga., 2009).***

Aim of The Work

The purpose of this study is to determine the prevalence of ACPA and RF in the First-Degree Relatives of RA patients and correlate their levels with the prevalence of joint symptoms

Pathogenesis of Rheumatoid Arthritis

RA is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular and systemic effects. It has been reported that RA affects ~0.5–1% of the adult population of developed regions. ***(Carbonell et al., 2008).*** Mortality rates are more than twice as high in patients with RA as in the general population ***(Gonzalez et al., 2007).***

Etiology:

1-Genetic and Environmental Factors:

A) Genes:

Rheumatoid arthritis involves a complex interplay among genotype, environmental triggers, and chance. Twin studies implicate genetic factors in rheumatoid arthritis, with concordance rates of 15 to 30% among monozygotic twins and 5% among dizygotic twins ***(MacGregor et al., 2000)***. The long-established association with the human leukocyte antigen (HLA)–DRB1 locus has been confirmed in patients who are positive for rheumatoid factor or ACPA; alleles that contain a common amino acid motif (QKRAA) in the HLA-DRB1 region, termed the shared epitope, confer particular susceptibility ***(Gregersen et al., 1987)***. Other possible explanations for the link between rheumatoid arthritis and the shared epitope include molecular

mimicry of the shared epitope by microbial proteins, increased T-cell senescence induced by shared epitope–containing HLA molecules, and a potential proinflammatory signaling function that is unrelated to the role of the shared epitope in antigen recognition ***(De Almeida et al., 2010).***

Many other identified risk alleles in ACPA-positive rheumatoid arthritis consistently aggregate functionally with immune regulation, implicating nuclear factor κB (NF-κB)–dependent signaling (e.g., *TRAF1–C5* and *c-REL*) and T-cell stimulation, activation, and functional differentiation (e.g., *PTPN22* and*CTLA4*) ***(Plenge et al., 2007).*** Genetic risk factors for ACPA-negative disease appear to be no less important than those for ACPA-positive disease. However, they are less well established and involve different HLA alleles (e.g., *HLA-DRB1*03*), interferon regulatory factors (e.g., interferon response factor 5), and lectin-binding proteins (e.g., C-type lectin domain family 4 member A). Patients with ACPA-positive disease have a less favorable prognosis than those with ACPA-negative disease, which suggests that such molecular subsets are clinically useful ***(Klareskog et al., 2008)***.

B) Smoking:

Smoking is the best defined environmental risk factor for seropositive RA. The reason for its influence on the development

of synovitis is not fully defined but could involve the activation of innate immunity and Peptidylarginine Deiminase (*PADI)* in the airway. Citrullinated proteins have been detected in bronchoalveolar lavage samples of smokers, and this could provide a stimulus for generation of ACPAs in susceptible individuals ***(Linn-Rasker et al., 2006).*** Although smoking and the shared epitope (SE) alone modestly increase the likelihood of developing RA, the combination is synergistic ***(Lundström et al., 2009).***

C) Gender:

RA is one of many chronic autoimmune diseases that predominate in women. Molecular explanations for such phenomena are emerging from animal models of inflammation, which show a link between the hypothalamic–pituitary–adrenal axis and cytokine production ***(Capellino et al., 2010).***

Pregnancy is often associated with remission of the disease in the last trimester. More than three quarters of pregnant patients with RA improve in the first or second trimester, but 90% of these experience a flare of disease associated with a rise in RF titers in the weeks or months after delivery. The mechanism of protection is not defined but might be due to the expression of suppressive cytokines such as IL-10 during pregnancy, production of α-fetoprotein, or alterations in cell-mediated immunity ***(Firestein et al., 2013)***

Fetal DNA levels in the maternal peripheral blood correlate with the propensity for improved symptoms in pregnant RA patients. It is not certain whether the DNA itself contributes or whether it is a marker for increased leakage of fetal cells into the maternal circulation ***(Yan et al., 2006).***

D) Infectious agents:

Antibodies to certain organisms such as *Proteus* are reportedly elevated in the blood of patients with RA, but this could represent an epiphenomenon or a nonspecific B cell activation. Most RA and reactive arthritis patients contain bacterial DNA sequences in their synovium.

The bacteria identified are not unique and generally represent a cross-section of skin and mucosal bacteria including *Acinetobacter* and *Bacillus* spp ***(Firestein et al., 2013).***

It is possible that the synovium functions as an adjunct to the reticuloendothelial system in arthritis, allowing local macrophages to accumulate circulating bacterial products. In addition to prokaryotic DNA, bacterial peptidoglycans have been detected in RA synovial tissue. Antigen-presenting cells containing these products express Toll-like receptors (TLRs) and produce proinflammatory cytokines such as TNF. Several animal models of arthritis are dependent on TLR2, TLR3, TLR4, or TLR9 ***(Choe et al., 2003).***