The influence of HLA genotyping on the clinical expression and predicting response to treatment in juvenile rheumatoid arthritis

Thesis

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Taher Ismail Osman Ayoub

CONTENTS

LIST OF ABBREVIATIONS

AA protein	:	Amyloid A Protein
ACCP	:	Anti-cyclic citrullinated peptide
ACR	:	American College of Rheumatology
AGEs	:	Advanced Glycation End Products
AIDS	:	Acquired Immunodeficiency Syndrome
AKA	:	Antikeratin antibody
ANA	:	Antinuclear antibodies
Ang	:	Angiopoietin
APF	:	Antiperinuclear factor
APR	:	Acute-phase reactants
BMC	:	Bone mineral content
BMD	:	Bone mineral density
CRP	:	C-Reactive Protein
CVD	:	Collagen Vascular Disease
DPA	:	Dual photon absorptiometry
DXA	:	Dual energy X-ray absorptiometry
EBV	:	Epstein-Barr Virus
EGF	:	Epidermal growth factor
EIA	:	Enzyme Immuno-assay
ESR	:	Erythrocyte sedimentation rate
FDA	:	Food Drug Administration
FGF	:	Fibroblast growth factor
GAGs	:	Glycosaminoglycans
G-CSF	:	Granulocyte colony-stimulating
GM-CSF	:	Granulocyte-macrophage colony-stimulating factor
gp	:	Glycoprotein
HGF	:	Hepatocyte growth factor
HIF-1	:	Hypoxia-inducible factor-1
HIV	:	Human Immunodeficiency Virus
HLA	:	Human Leucocyte Antigen
Hp	:	Haptoglobin
HSP	:	Heat shock proteins
IGF-1	:	Insulin-like Growth Factor-1

Igs	:	Immunoglobulins
IL	:	Interleukin
IP-10	:	Interferon-inducible protein-10
JRA	:	Juvenile rheumatoid arthritis
KDa	:	Kilo Dalton
MHC	:	Major Histocompatibility Complex
MIG	:	Monokine induced by interferon-γ
MMPs	:	Matrix metalloproteinases
MRI	:	Magnetic Resonance Imaging
NK	:	Natural killer
NSAIDs	:	Non-steroidal antiinflammatory drugs
PD-ECGF	:	Platelet-derived endothelial cell growth factor
PDGF	:	Platelet-derived growth factor
PF-4	:	Platelet factor-4
PGs	:	Proteoglycans
QCT	:	Quantitative computed tomography
QUS	:	Quantitative ultrasound
RA	:	Rheumatoid arthritis
RF	:	Rheumatoid factor
RFLP	:	Restriction Fragment Length Polymorphism
TGF	:	Transforming growth factor
TIMP	:	tissue inhibitors of metalloproteinases
TNF-α	:	Tumor necrosis factor-α
VEGF	:	Vascular endothelial growth factor

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

Aim of the Work

The aim of the present study is to detect the frequency prevalence of HLA-DRB1 genes, to study the influence of such alleles on JRA susceptibility/resistance among a group of Egyptian children having polyarticular onset JRA. Also, the contribution and association of the shared epitope positive HLA-DRB1 alleles (alleles containing QKRAA, QRRAA, and RRRAA) with disease severity variables, (clinical and radiological destructive arthritis). Also, to determine the predictive value of shared epitope alleles for response to therapy.

INTRODUCTION

HLA class II antigens, HLA-DR, -DQ, and -DP, are heterodimeric glycoproteins consisting of an α and a β chain. They are cell-surface molecules, mainly expressed on B cells, activated T cells, antigen-presenting cells, and thymic epithelium. They play a role in the presentation of foreign antigens to T cells to start an adequate immune response. They are also involved in the development of specific immune recognition of self. Additionally, they play a crucial role in transplantation immunology and in the susceptibility of autoimmune diseases ***(Kinikli et al., 2003).***

The HLA class II genes are located on the short arm of chromosome 6. Both the HLA-DQ and -DP regions contain one functional gene for each of their α- and β-chains (DQA1 and DQB1; DPA1 and DPB1). For HLA-DR, one gene coding for the DR α-chain, DRA, and one gene coding for the β-chain, DRB1, is always present. Depending on the DRBl-type, a DRB3, DRB4, or DRB5 may be present, and may be accompanied by pseudogenes ***(Brodsky, 2001***).

Among the α-chain loci, the DQA1 and DPA1 locus show some polymorphism with respectively 19 and 13 alleles. The β-chain loci however, are highly polymorphic. Population surveys in a variety of human populations have identified over 80 alleles at the DPBl locus, over 35 alleles at the DQB1 locus, and over 195 at the DRB1 locus. Virtually all of the sequence diversity is localized on the second exon, encoding the amino-terminal extracellular domain which functions as the peptide-

binding groove formed by the α-β heterodimer ***(Weyand and Goronzy, 2002).***

With respect to the multiple functions of the HLA class II antigens, adequate typing protocols are extremely important. Matching for solid organ and for bone marrow transplantation, typing for diseases susceptibility, for population studies, as well as for paternity or other forensic investigations are all applications demanding an accurate but simple typing technique ***(Thiel and Radbruch, 1999).***

For many of rheumatic diseases, genetic susceptibility is an important member of a triad of triggering factors implicated in pathogenesis. This genetic contribution is an important determinate of disease susceptibility but does not act alone. Environmental contributions and developmental factors act in contact to trigger disease. Thus disease susceptibility genes are best reviewed as permissive for disease.

The association between rheumatoid arthritis (RA) and class II major histocompatibility complex markers was first recognized in 1978. Juvenile RA (JRA) is a polygenic disorder to which the HLA region contributes about 30% of the genetic component. It is strongly associated in most populations and ethnic groups with a certain series of HLA-DRB1 alleles encoding a conserved sequence of amino acids between positions 70 and 74 in the third hypervariable region of DRB1 chain, (^{70}Q/R K/R R A A^{74}). The binding site of these antigen-presenting molecules is often referred to as the shared epitope (SE). The strongest associations are with certain HLA-DRB1*04 alleles (*0401, *0404, *0405 and *0408) but other SE-positive DRB1 alleles (*0101, *0102 and *1001) are also associated with RA ***(Wollheim et al., 2003).***

HLA-DR4 is associated with RA in populations of Northern European ancestry, and the *0401 allele is the most frequent DR4 subtype. This shared epitope has also been found to be associated with disease severity, especially when present in a double dose. However, because about 30% of patients are negative for DRB1*04 and 15% are SE negative, a unifying pathological mechanism has proven elusive. On the contrary, in Chilean patients the disease is only weakly associated with HLA-DR4. Among Greeks, Spaniards, Israeli Jews, and African blacks, RA is associated with DR1 and DR10, while among North American Natives; the RA-linked marker is DR14. HLA-DR9, which lacks the shared epitope, represented a novel disease marker. However, it was in only 20% of patients and apparently makes no contribution to severity ***(Harney et al., 2003).***

In addition to their role in JRA susceptibility, DRB1 alleles that contain the shared sequences are associated with more severe erosions. These insights into the genetics of JRA were made possible by observations in subjects from a variety of ethnic backgrounds, underscoring the importance of studying JRA susceptibility in diverse populations ***(Brintnell et al., 2004).***

JUVENILE RHEUMATOID ARTHRITIS

Epidemiology

Rheumatoid arthritis (RA) is the most common chronic inflammatory and destructive arthropathy that cannot be cured and that has substantial personal, social, and economic costs. The long-term prognosis is poor: 80 percent of affected patients are disabled after 20 years, and life expectancy is reduced by an average of 3 to 18 years ***(Heiberg et al., 2005).***

RA affects about 1 percent of the general population worldwide. Juvenile RA (JRA) is the most common rheumatic disease in children. It is one of the most frequent chronic illnesses of childhood ***(Choy and Panayi, 2001).*** The incidence of JRA is approximately 13.9/100,000 children (16 years or less) per year with a prevalence of approximately 113/100,000 children ***(Schaller, 1997).*** In Egypt, ***El-Gamal, (1998),*** reported a frequency prevalence of 9.8 JRA patients per year per 100,000 children attending the outpatients' clinics, Children's Hospital, Ain Shams University.

Although RA is properly considered a disease of the joints, it is important to recognize that it can exhibit a variety of extra-articular manifestations. These manifestations clearly show that RA has features of a systemic disease that is capable of involving a variety of major organ systems. Current slow-acting antirheumatic drugs have limited efficacy and many side effects. Moreover, they do not improve the long-term prognosis of rheumatoid arthritis ***(Loetscher and Moser, 2002).***