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Research Article

The Presence of HLA-DRB1Alleles in Children with Juvenile Rheumatoid Arthritis in Tabriz Year 2016

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Abstract

Babies idiopathic arthritis, the most common rheumatic disease in newborns and in different geographical areas based on the criteria, the prevalence is different. HLA-DRB1 alleles in this project in Tabriz-Iran childrens with juvenile rheumatoid arthritis oligularticular 57 were evaluated. Peripheral blood samples from 57 children with rheumatoid arthritis and 100 healthy people as a control group oligularticular young people, the preparation and the presence of HLA-DRB1 alleles were compared.

The evaluation of the HLA with SSP-PCR techniques were used for this study, as well as in those patients with the HLA-DRB1 subtypes were determined nucleotide sequence of exon two. Most symptoms of JRA disease in HLA-DRB1* 08 allele was found. In terms of frequency, allele HLA-DRB1* 11, the most frequent allele in patients who showed a significant difference with the control group.

Keywords: Allele HLA-DRB1, Juvenile Rheumatoid Arthritis, Oligoarthritis, Rheumatoid Factor.

Introduction

HLA-DR (Human Leukocyte Antigen - antigen D Related) is a class II MHC cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6 region 6p21.31. This complex and its ligand is a peptide of 9 amino acids in length with a ligand for the T-Cell Receptor (TCR). HLA (Human Leukocyte Antigens) were the antigen responsible for the rejection of tissue transplants in cases of HLA-mismatched donors. HLA were originally defined as cell surface antigens that mediated graft-versus-host disease. With the recent identification of the mismatch property has led to a greater success and longevity rate in organ transplants. The antigen described responsible for graft loss in organ transplant are HLA-DR (first six months), HLA-B (first two years) and HLA-A (long-term survival) [1]. A perfect match between the antigens of the host and the donor is the critical step in achieving a good graft survival rate. HLA-DR is also the reason for several autoimmune conditions, disease susceptibility and disease resistance. Its close relativeness to HLA-DQ has made it difficult in resolve the most causative factor in the disease [1].

HLA-DR molecules often get up-regulated in response to signaling. During an infection the peptide molecule e.g. staphylococcal enterotoxin I peptide bounds into a DR molecule are presented to the T-cell receptor on TH cell. On binding to these antigens on the B-cells stimulates B-cell proliferation. The primary function of HLA-DR circles around to present peptide antigen, basically of foreign origin to the immune system to elicitate or suppress the response of TH cell that leads to the production of antibodies against the same peptide. The DR 'antigen' is found abundantly on the cell surface of Antigen presenting cells (macrophages, B-cells and dendritic cells) often in response to stimulation thus being a key marker for immune stimulation [2].

The HLA-DR αβ heterodimers each subunit contains two extracellular domains, a membrane-spanning domain and a cytoplasmic tail. The α and β chains both are anchored in the membrane where the N-terminal domain forms an alpha-helix constituting the exposed portion of the binding groove and the C-terminal cytoplasmic region interacts with other chains forming a beta-sheet

under the binding groove spanning throughout the cell membrane. In each chain the first 80 residues form the contact positions for majority of the peptide chain. Genetics of HLA-DR is complex because it is encoded by several loci and the 'genes' at each locus perform different function. The DR α-chain encode by HLA-DRA locus lacks functional variation in the mature gene region. (Note: The table shows the number of Variant Alleles within the HLA-DR loci which reduces its potential for functional combination in the regions from ~1400 to ~400 [Table details are not exact because new allele functions are continually added, among which all not specifically classified as functional variants]). [2] The DR β-chain[3] is encoded by 4 loci out of which not more than 3 functional loci are present in a single individual in a ratio of not more than two on a single chromosome. The HLA-DRB1 is responsible for encoding a very large number of functionally variable gene products i.e. from HLA-DR1 to HLA-DR17. Inspit of being second to the HLA-B locus in terms of number of allelic variants HLA DRB1 has a high level of allelic variants. This directly implies that HLA-DRB1 rapidly evolves much more than the other protein encoding loci. High percentage of the variation occurs mainly at the peptide contact position within the binding groove resulting in a change in the way the DR binds peptide ligands and changes the repertoire each receptor can bind. This clearly states that the variations are functional in nature [4](Table: 1-3).

Positive control	Products (bps)	Forward	Reverse
Beta Actin	354	CACTCTTCGAGCCTTCCTTC	AGTCCGCCTAGAAGCATTTG

Table 1: sequences of primers used for the positive control group and reaction products PCR.

Group	HLA-DRB1 Alleles	Products (bps)	Forward	Reverse
1	HLA-DRB1*04	251	GTTTCTTGGAGCAGGTTAAAC	CCGCTGCACTGTGAAGCTCT
2	HLA-DRB1*08	278	AGTACTCTACGGGTGAGTGTT	CCGCTGCACTGTGAAGCTCT
3	HLA-DRB1*11	185	ACGTTTCTTGGAGTACTCTACG	CTGGCTGTTCCAGTACTCCT

Table 2: Sequences of primers used for the PCR reaction products allele HLA-DRB1.

Alleles	Controls (100) N	Controls (100) Ferquency	Patients (57) N	Patients (57) Ferquency	P value	OR(%95Cl)
DRB1*04	7	13.4	9	14.7	NS	2.134
DRB1*08	5	11.2	6	31.4	0.007	5.312
DRB1*11	19	41.7	21	67.2	0.009	2.161

Table 3: frequency of HLA-DRB1 alleles in patients with OA-JIA in children Tabriz and the control group.

The evolution of HLA is generally through the process of gene conversion, a form of short distance or 'abortive' genetics recombination method. In this the functional motifs within the gene are exchanged to form new form of alleles, forming functionally different DR isoforms and HLA-DR is the best example explaining this. In a survey conducted on the X-linked loci revealed that most of the human loci has undergone fixation in the last 600,000 years and similar is the case with the diploid loci. In nearly 100,000 to 150,000 years ago it was considered that due to the deep branching at the X-linked loci these were closed to fixation or restricted to the end of the human population bottleneck and HLA-DR locus was an exception to this observation. Most of the HLA alleles currently present in the human population can be explained by gene conversion between these ancient ancestral types, [6] some that persist into the extant population [5].

Juvenile Rheumatoid Arthritis is a chronic inflammatory disease of unknown etiology [1]. This is the most common rheumatic disease in children 2. HLA-DRB1 genes on the short arm of chromosome 6 for 6p 21.3 and has exons 2 and 3.

JRA diagnosis based on diagnostic criteria of the International Union of Rheumatology (ILAR) that in accordance with arthritis (joint inflammation) is detected for a period of 6 weeks, 3. The outbreak is 8-150 per 100000 births 4. Youth oligoarticular rheumatoid arthritis (OA-JRA) are classified according to the International Union of rheumatology, pediatric idiopathic arthritis is one of the subgroups seen in girls and boys.

DRB1 beta chain an HLA class II histocompatibility antigen, is a protein encoded by the HLA-DRB1 gene in humans[2]. DRB1 is responsible for encoding the most prevalent beta subunit of HLA-DR. Research report that the increased incidence of rheumatoid arthritis are assumed to be associated with several alleles of DRB1 [3]. Class II, an heterodimer consist of an alpha (DRA) and a beta chain (DRB) anchored on the membrane playing a central role in the immune system by presenting extracellular protein derived peptides to TH cells (T helper cells).

They are constitutively expressed in professional antigen presenting cells and also non-professional APCs. The beta chain is around 26-28 kDa encoded by 6 exons, from which the first exon encodes the leader peptide, the 2nd and the 3rd exon encodes the two extracellular domains, the 4th encodes the trans-mem-

brane domain and the 5th exon encodes for the cytoplasmic tail. The beta chain within the DR molecule contains all the polymorphisms which specify the peptide binding specificities and these polymorphism typing is usually done for bone marrow and kidney transplantation [2]. There are hundreds of DRB alleles discovered among which DRB1 is expressed five times higher than its paralogs DRB3, DBR4 and DRB5 and present in all individuals. DRB2, DRB6, DRB7, DRB8 and DRB9 are the related pseudo-genes described [2].

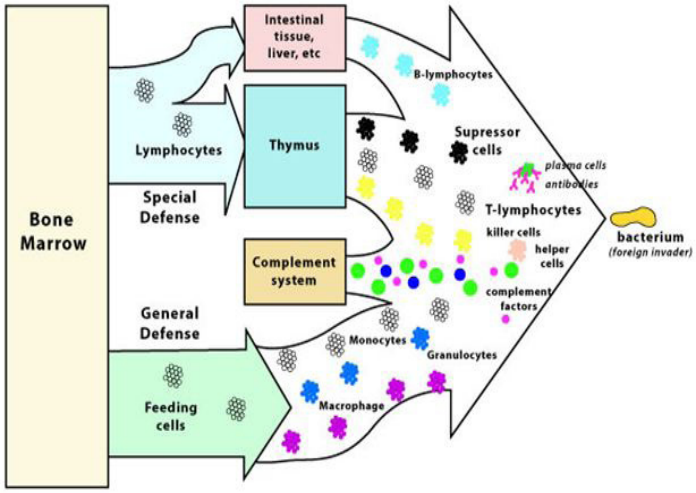


Figure 1: Schematic view of how HLA immune system by bacterial pathogens.

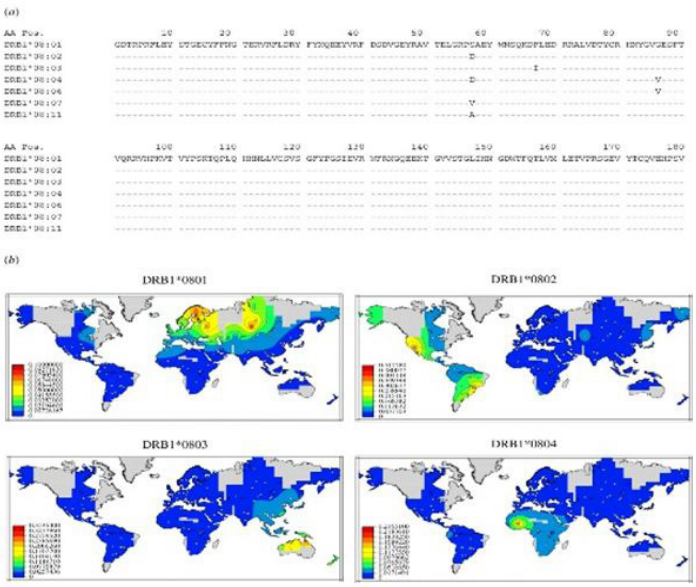


Figure 2: HLA-DRB1 allele frequencies between populations in different countries.

Materials and Methods

In this study, 57 children with OA-JIA and 100 healthy people as a control group were evaluated. The patient group included 41 girls and 16 boys, ages 3 to 15 years. Diagnosis is made by physical examination and diagnostic criteria of the International Union of Rheumatology (ILAR) was performed.

After consent from patients and healthy individuals, DNA genomic samples of blood from patients and control individuals using extraction kit product Qiagene South Korean company were extracted. HLA-DRB1 alleles were determined and with Olerup SSP-PCR technique was performed and Zetterquist. 50-100 ng of genomic DNA PCR reactions on the final volume of 25 ml for 35 cycles in 1 minute 94C0, 60C0 for 1 minute, and 72C0 for two minutes in order to reproduce exon 2 HLA-DRB1 was performed using specific primers. (Figure: 3-5)

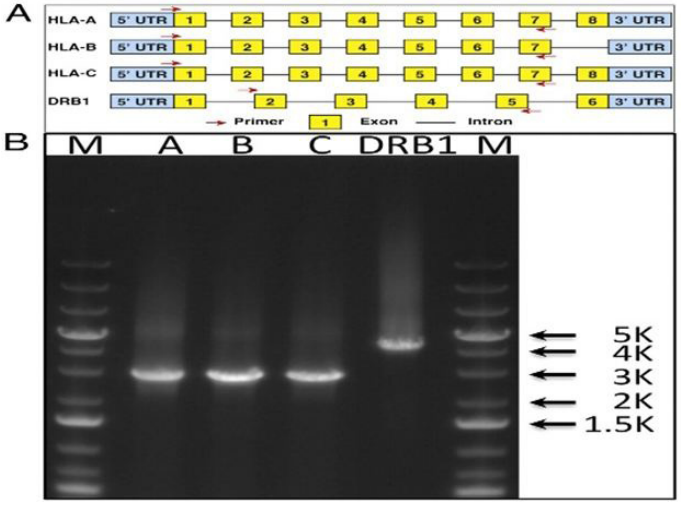
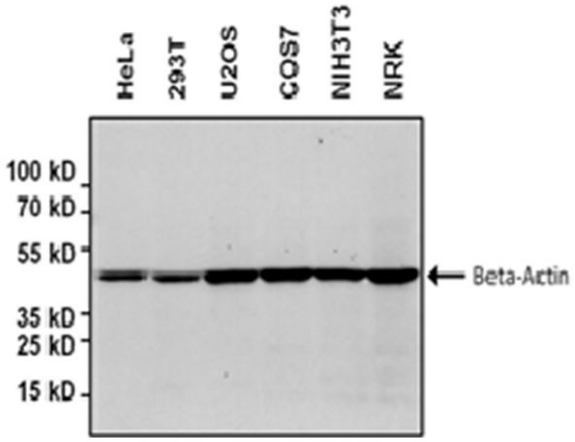


Figure 3: HLA-DRB1 alleles band formed in response PCR.



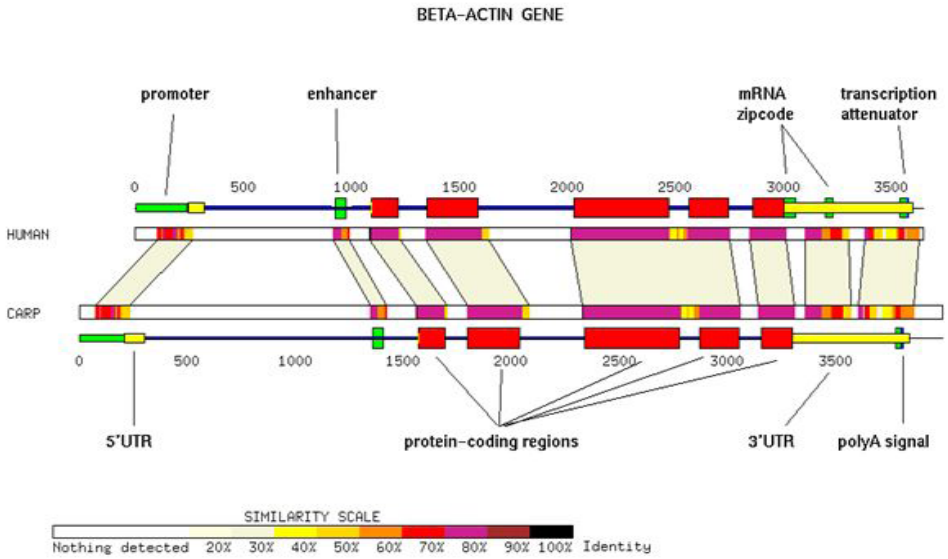
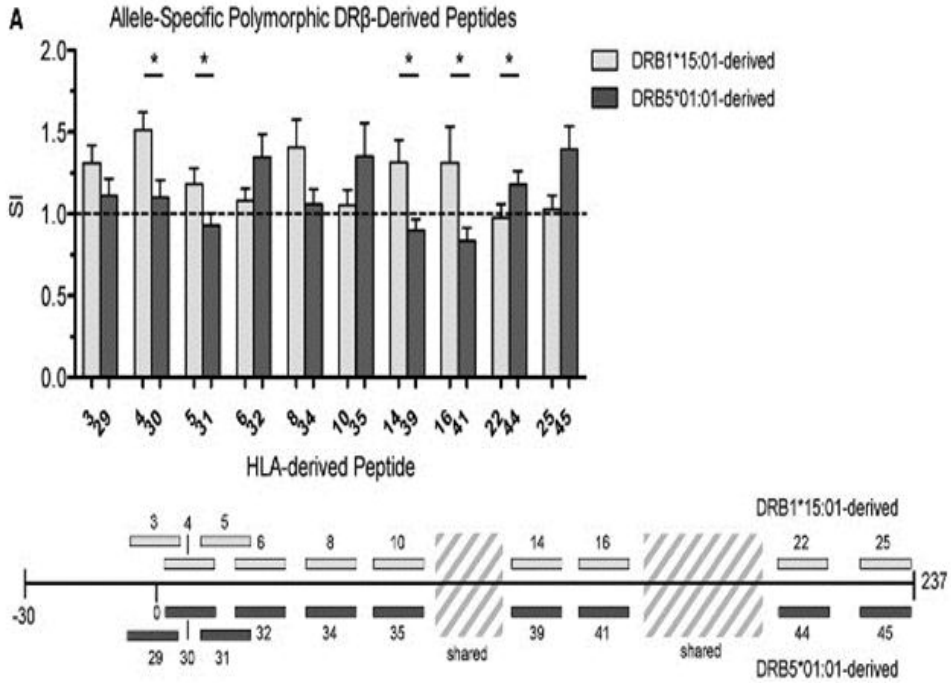


Figure 4: The formation of beta-actin gene bank in PCR reactions and location of the gene locus in a molecule of DNA.



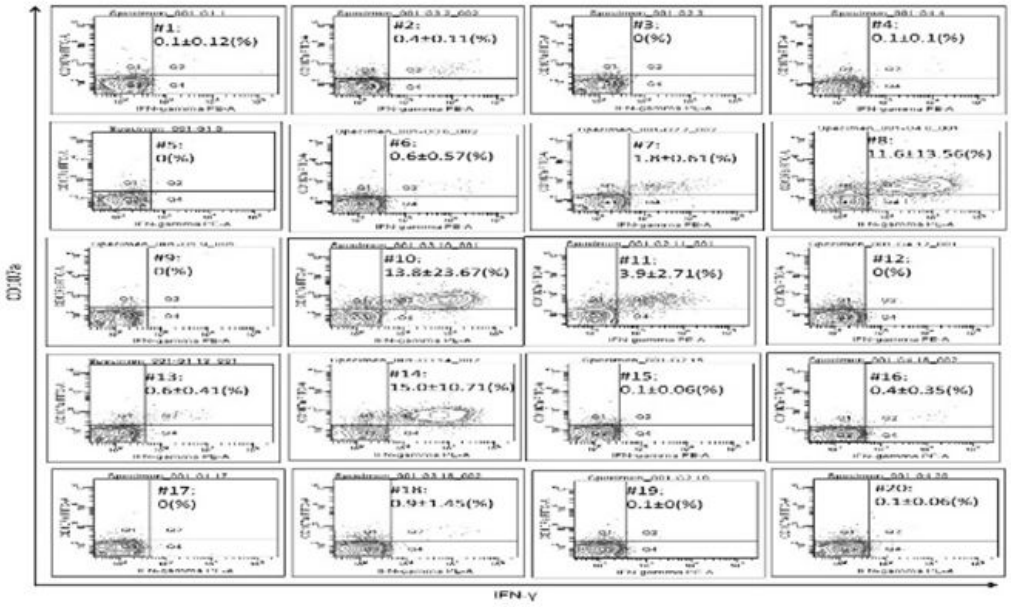


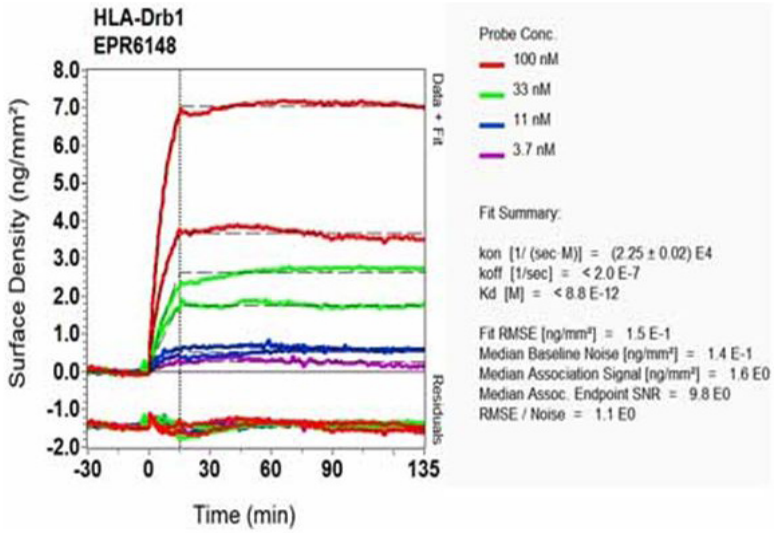
Figure 5: schematic diagram of the DRB1 gene polymorphisms and DRB5 in control and patient samples.

A pair of primers for amplification of beta-actin gene as a positive control reaction limited PCR cycles were used at all.

2.5% agarose gel electrophoresis of PCR reaction on the product's specific bands have been observed using UV light.

Conclusion

The project was performed for the first time in Tabriz, Iran, HLA-DRB1* 08 allele in patients with rheumatoid arthritis, type oligularticular greatest impact in Tabriz showed children as well as frequent allele HLA-DRB1* 11 allele in the patient group and the control group were determined. According to the results obtained in this study, we found that patients with rheumatoid youth are associated with antigen HLA-DRB1. These antigens for the population and the various human races are equally plays a role. The findings of this study can be concluded that environmental factors and life stresses important role in the physiological antigen is HLA-DRB1. This is the first study that has been done in the city of Tabriz, Iran. The main purpose of this study was to investigate the pattern of genetic inheritance and the people with rheumatoid arthritis. We hope also inspired researchers of this study could have significant advances in the treatment of juvenile rheumatoid arthritis (figure 6).



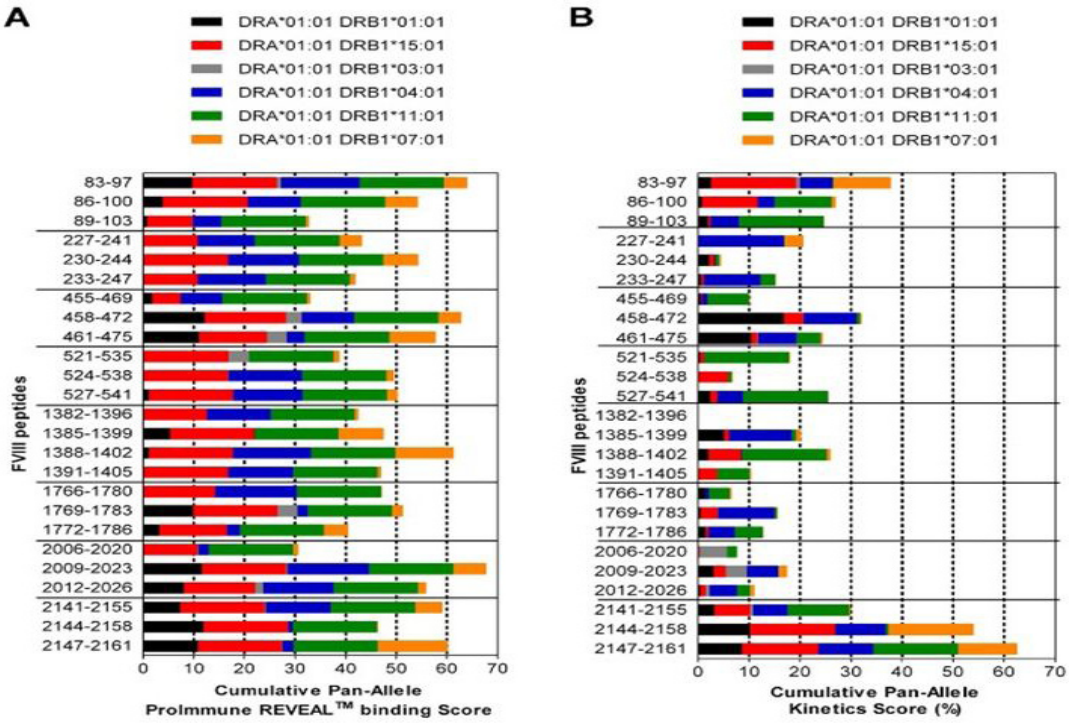


Figure 6: Schematic view of the frequency of the DRB1 gene polymorphisms and DRB5 in control and patient samples.

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