Available online at www.sciencedirect.com
ScienceDirect
Joint Bone Spine 75 (2008) 554–558
JOINT BONE SPINE
http://france.elsevier.com/direct/BONSOI/

Original article

HLA polymorphism and early rheumatoid arthritis in the Moroccan population

Ouafa Atouf [a,*], Karima Benbouazza [b], Chehrazade Brick [a], Fatiha Bzami [b], Naima Bennani [a], Bouchra Amine [b], Najia Hajja-Hassouni [b], Malika Essakali [a]

[a] *Service de transfusion sanguine et d'hémovigilance, Unité d'Immunologie, CHU Ibn Sina, Rabat, Moroco.*
[b] *Service de Rhumatologie, Hôpital El Ayachi, CHU Ibn Sina, Rabat, Morocco*

Accepted 15 January 2008
Available online 18 September 2008

Abstract

Objectives: Rheumatoid arthritis (RA) is an autoimmune multifactorial disease which has a great socio-economic impact in Morocco. The association of HLA genes with RA was studied in various ethnic groups but not in the Moroccan population. Our study focused on evaluating the distribution of class I and class II HLA genes among Moroccan patients presenting early signs of RA.
Methods: Forty nine patients diagnosed with early RA were compared to a group of healthy controls matched by age, sex, and ethnic origin. Among the patient group, 34 were seropositive (presence of the rheumatoid factor). HLA typing of the patients and the controls was performed using microlymphocytotoxicity for class I (A and B) and PCR-SSP for class II (DR and DQ).
Results: We found a significant increase of the frequency of the HLA-A24 antigen ($p = 0.03$), the DRB1*04 ($p = 0.004$) and DQB1*03 ($p = 0.03$) alleles and a significant decrease of the DRB1*07 allele ($p = 0.03$) in seropositive patients. The analysis of the frequency of the DRB1*01, DRB1*10, and DRB1*14 alleles did not show any difference between the RA patients and the controls. The frequency of DR4-DQ2 and DR4-DQ4 haplotypes was increased in the patients compared to the controls while that of DR7-DQ2 and DR13-DQ6 was decreased.
Conclusions: Our study suggests that DRB1*04 predisposes to RA while DRB1*07 seems protective for the Moroccan patients population. In addition we show the influence of some haplotypes DR-DQ in the susceptibility and protection against the disease.

Keywords: Early rheumatoid arthritis; Rheumatoid factor; HLA; Moroccan population

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease, characterized by chronic inflammation and synovial hyperplasia leading to the progressive destruction of both the articular cartilage and bone, with a functional incapacity in the long term. The rheumatoid factor (RF) is a useful marker for diagnosis, but it is found only in 80% of the patients diagnosed with RA [1]. Rheumatoid arthritis affects between 0.5 and 1% of the world's population and can occur at any age, more particularly between 40 and 70 years, with a higher prevalence in females [1].

Genetic, environmental and hormonal factors have been involved in the predisposition to the disease. Among the genetic factors, the HLA genes have been incriminated in 1/3 to 1/2 of the cases of RA [2]. A high frequency of the HLA-DRB1*04 allele has been shown to be associated with RA [3–5]. The discovery of other alleles which can be associated with RA, such as -DRB1*01 and -DRB1*10, led to the shared epitope hypothesis (SE) [6]. Based on this hypothesis, the alleles associated with the susceptibility to RA (DRB1*0101, *0102, *0401, *0404, *0405, *0408, *1001 and *1402) encode a common sequence of amino acids, located in position 70–74 (QKRAA or QRRAA) of the third

* Corresponding author. 11, lotissement raha, Nahda IV, Rabat, Moroco. Tel.: +212 61 23 38 93; fax: +212 37 67 36 67.
E-mail address: atoufoufa@gmail.com (O. Atouf).

1297-319X/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.jbspin.2008.01.027

hypervariable region of the β chain. The biological mechanism that could explain the role of SE in increasing the susceptibility to RA still has to be elucidated [7,8].

In Morocco, the incidence of RA has not been clearly established yet. However, the disease has an important impact on the social and occupational activity and on the financial standing of the patients [9]. The association between early RA and the HLA genes has never been studied on a Moroccan population. The purpose of this study was to evaluate the distribution of the HLA molecules (-A, -B, -DR and -DQ) in patients with early RA to analyse the impact of the genetic factor in RA.

2. Methods

2.1. Patients and controls

We recruited 49 Moroccan patients diagnosed with early RA (less than a year) from the Department of Rheumatology of the Ibn Sina University Hospital of Rabat. Diagnosis was established on the basis of criteria established by the American College of Rheumatology (ACR). The sample consisted of 39 women and 10 men whose age varied between 25 and 60 years. The presence of the RF, defining seropositivity, was observed in 34 patients (69%). The patients were compared with healthy controls without family ties (155 for class I and 183 for class II) and matched by age, sex and ethnic origin. These controls were either volunteers as bone marrow- or kidney-donors or just volunteers taking part in the study.

2.2. HLA typing

HLA typing of class I (-A and -B) was performed according to a standard complement-dependent microlymphocytotoxicity assay using lymphocytes isolated from peripheral blood with the aid of immuno-magnetic beads (One Lambda). Plates used for typing possessed 25 specificities for the allele A and 49 for B (One Lambda). HLA class II alleles (DRB1 and DQB1) was tested by "Polymerase chain reaction sequence specific primers" (PCR-SSP). DNA was first extracted from the buffy coat fraction of blood samples using a commercial kit (Qiagen) and then tested on plates for generic typing (One Lambda) according to the manufacturer's instructions.

All analyses were performed in the laboratory of immunology of the Ibn Sina University Hospital of Rabat.

2.3. Statistical analysis

Calculations were performed using the commercial SPSS software package for Windows, version 10.0 (frequencies, p values, odds ratio (OR) and a 95% confidence interval (CI)). The frequencies in patients and the controls were compared with Fisher's exact test. p values below 0.05 were considered statistically significant.

3. Results

Tables 1 and 2 show HLA-A and -B antigen frequencies observed in the Moroccan patients with early rheumatoid arthritis and in healthy controls. HLA-A24 antigen frequency increased significantly from 5.8% to 12.5% ($p = 0.03$) with a relative risk of 2.5 and confidence interval (CI) between 1.1 and 5.7. Concerning HLA-B antigens, no significant difference between the patients and controls was appreciable.

DNA based HLA class II typing for DRB1 and DQB1 studies did not reveal any statistically significant difference between the patients and the controls (Table 3). The slight increase observed with HLA-DRB1*04, -DQB1*03 and -DQB1*04 alleles in the patients, did not turn out be significant after statistical analysis. On the other hand, the generic typing of DRB1* and DQB1* in patients with a positive RF showed a significant increase of HLA-DRB1*04 and -DQB1*03 allele frequency reaching respectively 30.9% ($p = 0.004$, OR = 3.07, $1.4 < CI < 6.5$) and 36.5% ($p = 0.04$, OR = 2.5, $1.1 < CI < 5.5$) compared with 17.2% and 26.5% respectively found in the controls (Table 4).

Interestingly, the frequency of the HLA-DRB1*07 allele decreased in a significant manner by 11.2% in the controls compared to 2.9% in the RA patients ($p = 0.03$). DRB1*13 allele frequency is also less frequent in RA; however the value is not significant ($p = 0.1$) (Table 4).

The frequency of HLA DR4-HLA DR7 genotype was about 6.6% in the controls and 5.9% in the seropositive patients.

HLA DRB1-DQB1 haplotype frequencies observed in the 49 patients with early rheumatoid arthritis were comparable to the group of healthy controls (Table 5). However, examination of the patient group with positive RF (Table 6) revealed a highly significant increase of DRB1*04-DQB1*02 (from 4.2%

Table 1
HLA-A antigen frequencies in RA patients and healthy controls

Antigens	Controls		Patients		p	OR	CI
	$n = 155$	AF (%)	$n = 49$	AF (%)			
A1	31	10	7	7.3	0.5	0.6	0.2–1.6
A2	55	17.7	13	13.6	0.3	0.6	0.3–1.3
A3	34	10.9	10	10.8	1	0.9	0.4–2.1
A10	22	7.1	8	8.35	0.6	1.2	0.5–2.9
A11	13	4.2	3	3.2	0.8	0.7	1.9–2.6
A23	15	4.9	7	7.3	0.4	1.5	0.6–4.1
A24	18	5.8	12	12.5	0.03	2.5	1.1–5.7
A29	15	4.9	7	7.8	0.4	1.5	0.6–4.1
A30	30	9.7	6	6.2	0.3	0.5	0.2–1.5
A31	8	2.6	1	1.6	0.6	0.3	0.04–3.2
A32	10	3.7	3	3.2	1	0.9	0.2–3.6
A33	17	5.5	4	4.2	0.8	0.7	0.2–2.3
A34	4	1.3	3	3.2	0.4	2.5	0.5–11.9
A68	20	6.5	7	7.3	0.9	1.5	0.4–2.9
A69	1	0.3	0	–	1	–	–
A74	2	0.65	1	1.5	1	1.6	0.1–18.3
A80	1	0.3	0	–	1	–	–
A-	16	5.65	7	7.3	0.5	1.4	0.5–3.8

AF: antigen frequency; CI: confidence interval; OR: odds ratio.