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Immunological markers of rheumatoid arthritis in the Moroccan population

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Abstract

Aim: few studies exist on HLA-DRB1 alleles and their association with RA in North Africa. We aimed to provide an evaluation of the distribution of the HLA-DRB1* and -DQB1* genes in Moroccan patients with early RA. Additionally, we sought to analyze the relationship between HLA molecules and the production of rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP) in these patients.

Methods: 65 patients with rheumatoid arthritis benefited from HLA class II typing (DRB1* and DQB1*) and testing for anti-CCP and RF antibodies. The frequency of HLA alleles was compared to that of 180 healthy controls. Logistic regression analysis was used to investigate the association of HLA-DR and -DQ molecules in the different subgroups according to anti-CCP and RF status.

Results: The frequency of HLA-DRB1*04 and *11 alleles is increased in patients compared to controls (OR = 1.9; 95% CI (1.06-3.30), P=0.029; OR=2, 95% CI (1-3.83, P=0.047, respectively). 56 patients (86.1%) were RF positive, and 67.7% were anti-CCP positive. A significant increase in the frequency of DRB1*04 alleles was also noted in seropositive patients.

Conclusion: Our results suggest the predisposition of the HLA-DRB1*04 allele and the seropositive status of patients with RA. This could be useful in predicting the evolution of RA and establishing a diagnosis in some patients at an early stage of disease use.

Keywords: Rheumatoid Arthritis; Rheumatoid Factor; Anti-Cyclic Citrullinated Peptides; HLA-DRB1; Moroccan Population

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune rheumatic disease that involves chronic inflammation, specifically the synovial joints.[1] The pathogenesis of RA involves two autoantibodies called rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP).[2–4] These antibodies play a role in diagnosing RA based on the criteria set by EULAR/ACR.[5]

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These antibodies can be present for many years before joint symptoms develop and predict progression to RA. [6] Additionally, their presence is closely associated with radiological progression and severe disease prognosis.[7]

Various studies have shown that certain HLA-DRB1 alleles encoding for the "shared epitope" (SE), such as DRB1*04, *01, *14, and *10, [3] are significantly linked to a higher risk of developing arthritis (RA) accompanied by anti-CCP antibodies. [4,8,9]

The first Moroccan study examining the distribution of the HLA-DRB1 and -DQB1 alleles in patients with early RA did not show a contribution from HLA-DRB1 alleles to cases of RA in general. However, a significant frequency of the HLA-DRB1*04 allele was observed among patients who tested positive for RF antibodies.[10]

This paper aims to analyze and assess the distribution and frequencies of HLA-DRB1 and -DQB1 alleles in a larger group of patients who recently developed RA and compare them with those found in healthy individuals within the Moroccan population.

Additionally, we were interested in understanding the occurrence of antibodies (anti-CCP and RF) among these patients and examining the relationship between HLA-DR1 and -DQB1 molecules in various subgroups based on their anti-CCP and RF status.

2. Patients and methods

This study is a case-control analysis involving 65 patients from the ESPRIM database (evolution and monitoring of recent undifferentiated polyarthritis in Morocco). [11] The database consists of a cohort of 200 recruited patients between December 2008 and March 2012. These patients had arthritis that had been developing for more than a year. All the patients met the criteria set by the American College of Rheumatology (ACR) in 2010. [5]

A biobank containing buffy coat and serum samples was collected from the patients at the time of inclusion. Ethical approval for the ESPRIM cohort was obtained from the Biomedical Research Ethics Committee in Rabat.

To compare the patient group, we selected 180 healthy controls who shared characteristics regarding age, gender, and ethnicity. These controls were randomly selected from a pool of Moroccans who have volunteered as bone marrow or kidney transplantation donors.

For HLA typing (HLA-DRB1* and -DQB1*), we adopted the specific Primer Polymerase Chain Reaction (PCR-SSP) technique using generic HLA genotyping plates provided by One Lambda.

All experimental procedures followed the manufacturer's recommendations. Subsequent data analysis and interpretation of PCR SSP results were performed using HLA Fusion software developed by One Lambda.

The analyses were conducted at the histocompatibility laboratory of CHU Ibn Sina in Rabat, Morocco.

We used the Enzyme Linked Immuno Sorbent Assay (ELISA) technique with the ZEUS ELISA Rheumatoid Factor (RF) IgM Test System to search for RF. A positive result was defined as a cut-off value ≥ 6 IU/ml following the manufacturer's instructions. To analyze anti-CCP antibodies, we used the second-generation anti-CCP ELISA kit from Euroimmun (Anti-CCP ELISA). We considered a threshold value of 5 IU/ml positive for detecting anti-CCP antibodies.

We performed all calculations using SPSS software package version 20 for Windows. Through regression models, we derived odds ratios and 95% confidence intervals to measure the strength of the association between HLA alleles and arthritis (RA) risk and serological status. A p-value of .05 determined statistical significance.

3. Results

Our study mainly included women (87.7%) with an average age of 47 (11) years. Tables 1 and 2 display the frequencies of HLA-DRB1 and DQB1 alleles in both RA patients and healthy individuals.

In RA patients, we observed that the frequencies of the HLA-DRB1*04, *11, and HLA-DQB1*03 alleles were significantly higher compared to the control group (OR=1.9, 95% CI (1.07-3.30), P=0.029; OR=2, 95% CI (1.01-3.83), P=0.047; and OR=1.9, 95% CI (1.25-3.02), P=0.003), respectively. Although the frequency of DRB1*10 alleles was higher in RA

patients (6.2%) than in healthy subjects (3.3%), this difference did not reach significance (OR=1.9, 95% CI (0.76-4.76), P=0.17) (table1).

Table 1 The distribution of HLA-DRB1 in patients with rheumatoid arthritis (RA) (n=65) and healthy controls (n=180)

HLA-DRB1* alleles	healthy controls (2n=360)		RA patients (2n=130)		OR	95% CI	P value
DRB1*01	28	7.8	5	3.8	0.5	(0.18-1.26)	0.133
DRB1*03	42	11.7	20	15.4	1.4	(0.77-2.45)	0.276
DRB1*04	37	10.3	23	17.7	1.9	(1.07-3.30)	0.029
DRB1*07	68	18.9	15	11.5	0.6	(0.31-1.02)	0.058
DRB1*08	20	5.6	5	3.8	0.7	(0.25-1.85)	0.450
DRB1*09	1	0.3	2	1.5	5.6	(0.5-62.39)	0.161
DRB1*10	12	3.3	8	6.2	1.9	(0.76-4.76)	0.170
DRB1*11	24	6.7	16	12.3	2.0	(1.01-3.83)	0.047
DRB1*12	1	0.3	2	1.5	5.6	(0.5-62.39)	0.161
DRB1*13	51	14.2	15	11.5	0.8	(0.43-1.46)	0.453
DRB1*14	11	3.1	2	1.5	0.5	(0.10-2.27)	0.366
DRB1*15	62	17.2	13	10	0.5	(0.28-1.01)	0.053
DRB1*16	3	0.8	2	1.5	1.8	(0.31-11.3)	0.5

OR: odds ratio. CI: confidence interval at 95%. Differences were considered significant at $P < 0.05$.

Furthermore, we found that the frequency of HLA-DQB1*06 in RA patients (28.3%) was significantly lower than that in healthy subjects (16.9%) (OR= 0.5, 95% CI (0.31-0.86), P=0.011). The control group showed an occurrence of DRB1*01, *07, *13, *14, and *15 alleles compared to the RA patients. However, this difference did not reach significance.

Table 2 The distribution of HLA-DQB1 in patients with rheumatoid arthritis (RA) (n=65) and healthy controls (n=180)

HLA-DRB1* alleles	Healthy controls (2n=360)		RA patients (2n=130)		OR	95% CI	P value
DQB1*01	4	1.1	2	1.5	1.4	(6.25-7.68)	0.705
DQB1*02	109	30.3	31	23.8	0.7	(0.45-1.14)	0.165
DQB1*03	77	21.4	45	34.6	1.9	(1.25-3.02)	0.003
DQB1*04	17	4.7	11	8.5	1.9	(0.85-4.10)	0.12
DQB1*05	49	13.6	19	14.6	1.1	(0.61-1.93)	0.77
DQB1*06	102	28.3	22	16.9	0.5	(0.31-0.86)	0.011

OR: odds ratio. CI: confidence interval at 95%. Differences were considered significant at $P < 0.05$.

When analyzing RA patients precisely the frequencies of DRB1*11/DRB1*13 genotypes (OR= 14.91, 95% CI (1.71-130.23), P=0.01) and DRB1*03/DRB1*11 genotypes (OR= 7.12; 95% CI (1.78-28.43), P=0.005) were found to be significantly higher compared to the control group (Table 3).

Table 3 Comparative analysis of the frequency of HLA-DRB1*/-DRB1* genotypes between patients with RA and healthy controls

Genotype	Healthy controls (n=180)		RA patients (n=65)		OR	95% CI	P value
DRB1*01/DRB1*01	3	1.7	1	1.5	0.92	(0.09-9.02)	0.94
DRB1*01/DRB1*11	1	0.6	0	-	0.34	(0.001–146.88)	0.73
DRB1*01/DRB1*13	6	3.3	1	1.5	6.45	(0.05-3.84)	0.46
DRB1*01/DRB1*14	1	0.6	0	-	0.34	(0.001–146.88)	0.73
DRB1*01/DRB1*15	6	3.3	1	1.5	0.45	(0.05-3.84)	0.46
DRB1*01/DRB1*03	0	-	1	1.5	19.12	(0.04-8152.29)	0.34
DRB1*01/DRB1*04	2	1.1	2	3.1	2.82	(0.39-20.48)	0.30
DRB1*01/DRB1*07	5	2.8	0	-	0.34	(0.22-5.13)	0.43
DRB1*01/DRB1*08	1	0.6	0	-	0.34	(0.001-146.88)	0.73
DRB1*10/DRB1*11	1	0.6	0	-	0.34	(0.001-146.88)	0.73
DRB1*10/DRB1*13	1	0.6	1	1.5	2.79	(0.17-45.37)	0.46
DRB1*10/DRB1*15	5	2.8	0	-	0.34	(0.02-5.13)	0.43
DRB1*11/DRB1*11	0	-	1	1.5	19.12	(0.04-8152.29)	0.34
DRB1*11/DRB1*13	1	0.6	5	7.7	14.92	(1.71-130.23)	0.01
DRB1*11/DRB1*15	8	4.4	0	-	0.33	(0.04-2.88)	0.31
DRB1*12/DRB1*13	1	0.6	2	3.1	5.68	(0.51-63.75)	0.15
DRB1*13/DRB1*13	1	0.6	0	-	0.34	(0.001-146.88)	0.73
DRB1*13/DRB1*14	2	1.1	1	1.5	1.39	(0.12-15.598)	0.78
DRB1*13/DRB1*15	9	5.6	1	1.5	0.3	(0.04-2.39)	0.25
DRB1*13/DRB1*16	1	0.6	1	1.5	2.8	(0.17-45.37)	0.46
DRB1*15/DRB1*15	6	3.3	2	3.1	0.92	(0.18-4.68)	0.92
DRB1*03/DRB1*10	2	1.1	1	1.5	1.39	(0.12-15.6)	0.78
DRB1*03/DRB1*11	3	1.7	7	10.8	7.12	(1.78-28.43)	0.005
DRB1*03/DRB1*13	8	4.4	2	3.1	0.68	(0.14-3.30)	0.63
DRB1*03/DRB1*14	2	1.1	1	1.5	1.39	(0.12-15.6)	0.78
DRB1*03/DRB1*15	9	5.0	2	3.1	0.6	(0.13-2.87)	0.52
DRB1*03/DRB1*04	0	-	4	6.2	-	-	1
DRB1*03/DRB1*07	15	8.3	0	-	-	-	1
DRB1*03/DRB1*08	3	1.7	2	3.1	1.87	(0.31-11.47)	0.49
DRB1*04/DRB1*10	2	1.1	3	4.6	4.3	(0.70-26.38)	0.11
DRB1*04/DRB1*11	6	3.3	1	1.5	0.45	(0.05-3.84)	0.46
DRB1*04/DRB1*13	7	3.9	0	-	-		1
DRB1*04/DRB1*15	4	2.2	4	6.2	2.88	(0.70-11.89)	0.14
DRB1*04/DRB1*04	3	1.7	3	4.6	2.85	(0.56-14.52)	0.20
DRB1*04/DRB1*07	7	3.9	1	1.5	0.39	(0.05-3.20)	0.37

DRB1*04/DRB1*08	2	1.1	2	3.1	2.82	(0.39-20.48)	0.30
DRB1*04/DRB1*09	1	0.6	0	-	-	-	1
DRB1*07/DRB1*10	0	-	3	4.6	-	-	1
DRB1*07/DRB1*11	3	1.7	1	1.5	0.32	(0.09-9.02)	0.94
DRB1*07/DRB1*13	8	4.4	1	1.5	0.33	(0.04-2.73)	0.30
DRB1*07/DRB1*14	5	2.8	0	-	-	-	1
DRB1*07/DRB1*15	8	4.4	1	1.5	0.34	(0.04-2.74)	0.30
DRB1*07/DRB1*16	1	0.6	1	1.5	2.8	(0.17-45.37)	0.46
DRB1*07/DRB1*07	6	3.3	2	3.1	0.92	(0.18-4.68)	0.92
DRB1*07/DRB1*08	4	2.2	1	1.5	0.69	(0.07-6.27)	0.74
DRB1*07/DRB1*09	0	-	2	3.1	-	-	1
DRB1*08/DRB1*10	1	0.6	0	-	-	-	1
DRB1*08/DRB1*11	1	0.6	0	-	-	-	1
DRB1*08/DRB1*13	5	2.8	0	-	-	-	1
DRB1*08/DRB1*14	1	0.6	0	-	-	-	1
DRB1*08/DRB1*15	1	0.6	0	-	-	-	1
DRB1*08/DRB1*16	1	0.6	0	-	-	-	1

OR: odds ratio. CI: confidence interval at 95%. Differences were considered significant at P<0.05

Regarding autoantibodies, among the 65 RA patients assessed, 44 (67.7%) had anti-CCP positive results, and 56 (86.1%) tested positive for RF (Table 4).

Table 4 Distribution of patients with RA into serologic subgroups according to anti-CCP and RF antibodies status

Autoantibodies	**RF positive**	**RF negative**	**Total**
	No (%)	**No (%)**	
Anti-CCP positive	42 (64,6)	2 (3.1)	44(67,7)
Anti-CCP negative	14 (21,5)	7 (10,8)	21(32,3)
Total	56 (86,1)	9(13,9)	65

Anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor

In the RF patient subgroup, there was a higher frequency of HLA-DRB1*04 and DQB1*03 in RA patients when compared to the control group (OR: 2.03, 95% CI (1.03-3.99), P=0.039; and 58.9% vs 37.8%, OR: 2.36, 95% CI (1.28-4.36), P=0.006 respectively). The frequency of HLA-DRB1*07, DRB1*15, and QDB1*06 alleles showed a decrease in patients with RA who had anti-CCP antibodies compared to those without (OR=0.46, 95% CI (0.22-0.96) P=0.039; 16.1% vs 31.1% OR=0.42, 95% CI (0.19-0.92), P=0.03; and OR=0.4 95% CI (0.20-0.77), P=0.006 respectively) as shown in Table 5.

On the other hand, the frequency of HLA-DRB1*04 and DQB1*03 alleles was significantly higher in RA patients with anti-CCP antibodies compared to controls (OR=2.7, 95% CI (1;33-5;51), P=0.006 and OR=2;17, 95% CI (1;11-4;23), P=0.0023 respectively). Additionally, the frequency of HLA-DRB1*15 and QDB1*06 alleles showed a decrease in RA patients with anti-CCP antibodies compared to those without (OR=0.42, 95% CI (0.18-0.9), P=0.004 and OR=0.41, 95% CI (0.20-0.85), P=0.016 respectively) (Table 6).

Table 5 Frequency of HLA-DRB1* and -DQB1* gene variants in the group of patients with RA according to RF antibody status

Allelic group of HLA-DRB1 and -DQB1*	FR positive (n=56)		Healthy controls (n=180)		OR	95% CI	P value
DRB1*01	6	10.7	25	13.9	0.74	(0.29-1.91)	0.54
DRB1*03	19	33.9	42	23.3	1.69	(0.88-3.23)	0.11
DRB1*04	18	32.1	34	18.9	2.03	(1.03-3.99)	0.039
DRB1*07	11	19.6	62	34.4	0.46	(0.22-0.96)	0.039
DRB1*08	5	8.9	20	11.1	0.78	(0.28-2.19)	0.644
DRB1*09	2	3.6	1	0.6	6.63	(0.59-74.53)	0.125
DRB1*10	6	10.7	12	6.7	1.68	(0.60-4.70)	0.323
DRB1*11	12	21.4	24	13.3	1.77	(0.82-3.82)	0.145
DRB1*12	1	1.8	1	0.6	3.25	(0.20-52.90)	0.407
DRB1*13	11	19.6	50	27.8	0.63	(0.30-1.33)	0.227
DRB1*14	1	1.8	1	6.1	0.28	(0.035-2.21)	0.227
DRB1*15	9	16.1	56	31.1	0.42	(0.19-0.92)	0.03
DRB1*16	2	3.6	3	1.7	0.35	(0.35-13.41)	0.4
DQB1*1	1	1.8	4	2.2	0.80	(0.09-7.30)	0.747
DQB1*2	28	50	88	48.9	1.04	(0.57-1.90)	0.88
DQB1*3	33	58.9	68	37.8	2.36	(1.28-4.36)	0.006
DQB1*4	8	14.3	17	9.4	1.59	(0.65-3.93)	0.30
DQB1*5	16	28.6	46	25.6	1.65	(0.60-2.27)	0.65
DQB1*6	15	26.8	86	47.8	0.4	(0.20-0.77)	0.006

RF: Rheumatoid factor. OR: odds ratio. CI: confidence interval at 95%. Differences were considered significant at $P < 0.05$

Table 6 Frequency of HLA-DRB1* and -DQB1* gene variants in the group of patients with RA according to anti-CCP antibody status

Allelic group of HLA-DRB1 and -DQB1*	Anti-CCP Positif (n=44)		Healthy controls (n=180)		OR	95% CI	P value
DRB1*01	5	11.4	25	13.9	0.80	(0.28-2.21)	0.66
DRB1*03	13	29.5	42	23.3	1.38	(0.66-2.87)	0.39
DRB1*04	17	38.6	34	18.9	2.70	(1.33-5.51)	0.006
DRB1*07	10	23.7	62	34.4	0.56	(0.26-1.21)	0.14
DRB1*08	2	4.5	20	11.1	0.38	(0.09-1.70)	0.20
DRB1*09	2	4.5	1	0.6	8.52	(0.75-96.22)	0.08
DRB1*10	7	15.9	12	6.7	2.65	(0.98-7.18)	0.056
DRB1*11	7	15.9	24	13.3	1.23	(0.49-3.07)	0.66
DRB1*12	1	2.3	1	0.6	4.16	(0.25-67.90)	0.32
DRB1*13	9	20.5	50	27.8	0.67	(0.30-1.49)	0.32

DRB1*14	0	0	11	6.1	-	-	1
DRB1*15	7	15.9	56	31.1	0.42	(0.18-0.9)	0.04
DRB1*16	2	4.5	3	1.7	2.81	(0.45-17.35)	0.27
DQB1*01	1	2.3	4	2.2	1.02	(0.11-9.39)	0.98
DQB1*02	20	45.5	88	48.9	0.68	(0.45-1.69)	0.870
DQB1*03	24	56.8	68	37.8	2.17	(1.11-4.23)	0.023
DQB1*04	5	11.4	17	9.4	1.22	(0.70-1.23)	0.70
DQB1*05	15	34.1	46	25.6	1.50	(0.74-3.06)	0.26
DQB1*06	12	27.3	86	47.8	0.41	(0.20-0.85)	0.016

Anti-CCP: anti-cyclic citrullinated peptide antibodies. OR: odds ratio. CI: confidence interval at 95%. Differences were considered significant at $P < 0.05$

4. Discussion

Research examining the link between HLA-DRB1 alleles and the risk of developing RA has found that HLA-DRB1 *04 and *10 alleles are linked to a susceptibility to RA in various populations, particularly among Arabs.[12–17] One study examined the distribution of HLA class I and II genes in Morocco. While this study couldn't definitively establish the role of HLA-DRB1* alleles in predisposing individuals to RA, our analysis revealed an increase in the frequency of HLA-DRB1*04 alleles among Moroccan RA patients [10], likely due to the larger sample size. This discovery aligns with findings indicating that different ethnic groups exhibit varying HLA-DRB1 alleles associated with RA. [18] For instance, other predisposing alleles like DRB1*09 in Japanese [19], DRB1*01 in Israeli Jews [20] and DRB1*10 in Saudis [21], Taiwanese [22] and Brazilians [23], have been implicated. Interestingly, some variants of the HLA-DRB1 gene seem to offer protection against the disease.

Studies have observed that certain allele groups, like DRB1*13, DRB1*11, DRB1*07, DRB1*08, and DRB1*15, are notably less common in patients with RA compared to the control group. [24–27] However, our results show that HLA-DRB1*11 is a susceptibility and not a protection allele. This implies that another study on a larger sample may help conclude the role of DRB1*11 in susceptibility to RA.

Our study also explored the connection between the DQB1* gene and RA. Our results point towards a predisposition of the DQB1*03 allele to the disease while suggesting an effect of the DQB1*06 allele. However, concluding is challenging due to the linkage disequilibrium between HLA-DRB1 and HLA-DQB1 loci.

An analysis of HLA-DRB1*/DRB1* genotypes highlighted an association between DRB1*11/DRB1*13 and DRB3*/DRB1*13 and RA among Moroccans. These genotypes could potentially signify an increased risk of developing RA. The findings in our study differ from those of Klimenta [28] , who identified that the DRB1*04/DRB1*04 and DRB1*04/DRB1*03 genotypes were predominant among RA patients.

In our patient cohort, 67.7% tested positive for anti-CCP antibodies and 86.1% for RF antibodies. The prevalence of the anti-CCP positive/RF positive profile was 64.62 %, which aligns with previously published data. [25,29–31] Our investigation also delved into the relationship between HLA-DRB1* alleles and autoantibodies in RA serum samples. As established by previous research, distinct clinical and genetic subtypes exist between anti-CCP positive and negative RA cases. [32] Studies have shown associations between SE alleles and anti-CCP antibody presence in RA patients. [33–35] In our analysis, we observed a link between the HLA-DRB1*04 allele and the presence of both anti-CCP antibodies and RF positivity. This finding is consistent with most previously published reports. [36–38]

Our research found that HLA-DRB1*07 and *15 were negatively associated with anti-CCP-positive RA. Similarly, DRB1*11, which has been noted as an allele of susceptibility to RA, did not affect the serological status, likely because it relates to disease susceptibility rather than severity, unlike HLA-DRB1*04. Moreover, several studies have shown that HLA-DRB1*09 and -DRB1*03 are more prevalent in RA patients without anti-CCP antibodies than those with antibodies. [39–41] Due to a few patients without anti-CCP antibodies in our study, we couldn't establish a connection between HLA-DRB1 alleles and the seronegative status in RA patients.

Overall, research is scarce on HLA-DRB1 alleles and their role in diseases in North Africa. Our study is the first to examine the association between HLA class II and the development of anti-CCP and RF antibodies in Moroccan RA patients. We confirmed the results concerning the HLA-DRB1*04 allele, which was reported as a predisposition allele. It would be interesting to complete the task with specific typing to identify the particular allele carrying the shared epitope on the one hand. On the other hand, a study on a larger sample would be necessary to confirm the different results and gain a better knowledge of the HLA profile of Moroccan patients with RA. Finally, studying HLA-DRB1 alleles in North African populations is essential to understanding the genetic risk factors for RA and developing personalized approaches to prevention and treatment.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare there are no conflicts of interest to report.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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