HLA-DRB1 allele association with rheumatoid arthritis susceptibility and severity in Syria

Jamil Mourad1, Fawza Monem2

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a complex multifactorial chronic disease. The importance of human leukocyte antigen as a major genetic risk factor for RA was studied worldwide. Although it is widely distributed in different Syrian areas, studies of human leukocyte antigen (HLA) alleles’ role are absent. Objective: The aim of our study was to determine the association of HLA-DRB1 alleles with the susceptibility and severity of RA in Syria. Patients and methods: Eighty-six RA patients and 200 healthy controls from Syria were genotyped using polymerase chain reaction with sequence-specific primer (PCR-SSP). Anti-CCP antibodies were measured by ELISA. Rheumatoid factor (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and disease activity score 28 (DAS-28) values were obtained from patients’ medical records. DAS-28 was used to assess the clinical severity of the patients. Results: The HLA-DRB1*01, *04, and *10 frequencies showed a strong association with the disease susceptibility (OR = 2.29, 95% CI = 1.11–4.75, P = 0.022; OR = 3.16, 95% CI = 2.08–4.8, P < 0.0001; OR = 2.43, 95% CI = 1.07–5.51, P = 0.029 respectively), while the frequencies of HLA-DRB1*11, and *13 were significantly lower in RA patients than in controls (OR = 0.49, 95% CI = 0.3–0.8, P = 0.004; OR = 0.32, 95% CI = 0.15–0.69, P = 0.002, respectively). The other HLA-DRB1 alleles showed no significant difference. The frequency of anti-CCP antibodies was higher in shared epitope (SE) positive patients compared with SE-negative patients (OR = 5.5, 95% CI = 2–15.1, P = 0.00054). DAS-28 of RA patients didn’t show significant difference between the SE negative and the SE positive groups. Conclusion: Our results indicate that HLA-DRB1*01, *04, and *10 alleles are related with RA, while HLA-DRB1*11 and *13 protect against RA in the Syrian population.

Keywords: HLA-DR4 antigen, rheumatoid arthritis, disease susceptibility, Syria.

INTRODUCTION

Rheumatoid arthritis (RA) is one of the complex immune-mediated diseases with unknown etiologies and an estimated population prevalence of 1%. It is characterized by chronic inflammation, synovitis, pain, and progressive destruction of both the articular cartilage and bone leading to functional disability. The chance of developing the disease is 2–3 times more frequent in women than men. The peak age of onset of the disease is in the 40s, although it can occur at any age. Genetic and environmental risk factors play key roles in the disease pathogenesis. The inheritance probability of RA is estimated to be around 60%.

The human leukocyte antigen (HLA) is found to be the most important genetic risk factor for RA, which accounts for 30% to 50% of overall genetic susceptibility to RA. The shared epitope (SE) hypothesis described the relationship between HLA-DRB1 and RA. HLA-DRB1 alleles encoding the SE (DRB1*01, *04, *10, and *14) are associated with structural severity of RA and have been more recently related with production of anti-citrullinated peptide autoantibodies (anti-CCP). On the other hand, SE negative genotypes (mainly DRB1*11 and *13) provide protection against RA susceptibility.

The major relationship of particular HLA alleles with RA is not constant in all human populations, different geographical areas, or among different ethnic groups. Despite of the
wide distribution of RA in Syria, the HLA-DRB1 studies are still absent. Hence, the aim of our study is to determine the association of HLA-DRB1 alleles in the disease susceptibility and severity in Syria.

PATIENTS AND METHODS

The study was designed as a case-control study. Blood samples were obtained from 86 patients (mean age 41.41 ± 10.57 years; 69 women, 17 men) admitted to the Department of Rheumatology, Ibn Nafis Hospital, Almowasat and Al-Assad Hospitals, Damascus University, between January 2010 and September 2011. All patients fulfilled the American College of Rheumatology (ACR) criteria.10 Two hundred healthy unrelated volunteers (mean age 40.21 ± 10.11 years; 160 women and 40 men) matched by age, gender, and ethnic origin were allocated as controls. An informed consent was obtained from all patients and healthy individuals. The project was approved by the Ethical Committee of Damascus University.

The detection of anti-CCP IgG antibodies was performed using second-generation ELISA kit (Euroimmun, Lübeck, Germany). Serum samples presenting results > 5 RU/mL were considered to be positive for anti-CCP antibodies. Rheumatoid factor (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and disease activity score 28 (DAS-28) values were adopted from patients’ medical records. DAS-28 was used to assess the clinical severity of the patients.11 Genomic DNA of patients with RA (n = 86) and healthy controls (n = 200) were isolated from 300 μL aliquots of peripheral anticoagulated venous blood samples by using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). Genotyping of HLA-DRB1 was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) using Micro SSPT Generic HLA Class II (DRB) (One Lambda Inc., CA, USA).

Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to estimate the strengths of the associations. Chi-squared and Student’s t-test were used in the statistical analysis. Differences were considered to be significant at P < 0.05.

RESULTS

Demographic data and clinical findings of 86 RA patients diagnosed according to modified ACR criteria are given in Table 1. Frequencies of HLA-DRB1 alleles of RA patients and normal individuals are summarized in Table 2. In RA patients, HLA-DRB1 *01, *04, and *10 allele frequencies were higher than controls (OR = 2.29, 95% CI = 1.11–4.75, P = 0.022; OR = 3.16, 95% CI = 2.08–4.8, P < 0.0001; and OR = 2.43, 95% CI = 1.07–5.51, P = 0.029, respectively). In contrast, DRB1 *11 and *13 alleles were more frequent in controls (OR = 0.49, 95% CI = 0.3–0.8, P = 0.004; OR = 0.32, 95% CI = 0.15–0.69, P = 0.002, respectively). The allele frequency differences of DRB1*03, *07, *08, *09, *12, *14, *15, and *16 were not statistically significant (95% CI of *16 overlapped 1). Compared with controls, frequencies of SE positive alleles (the sum of DRB1*01, *04, *10, *14) were higher in RA patients (OR = 3.41, 95% CI = 2.35–4.95, P < 0.0001).

Anti-CCP antibody was present in 60.46% and RF in 63.95% of the RA patients. Frequencies of anti-CCP antibodies and RF were higher in SE-positive patients compared to SE-negative patients (OR = 5.5, 95% CI = 2–15.1, P < 0.001; OR = 5.45, 95% CI = 2–14.87, P < 0.001, respectively) (Table 3).

Disease severity presented by DAS-28 values showed no significance between SE negative and SE positive RA patients (Figure 1).

DISCUSSION

Different literatures investigated the biogeographic distribution of RA-DRB1 alleles in various ethnicities and races around the world.1,5,12 HLA-DRB1*04 allele has been reported to be linked to RA in many populations.13–25 DRB1*04 was frequent in RA patients in Morocco26 and Zahedan southeast Iran,27 but

Table 1 Demographic and clinical characteristics of patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RA (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (± SD) years</td>
<td>41.41 (10.57)</td>
</tr>
<tr>
<td>Disease duration, mean (± SD) years</td>
<td>11.26 (6.25)</td>
</tr>
<tr>
<td>Women</td>
<td>69 (80.23%)</td>
</tr>
<tr>
<td>Men</td>
<td>17 (19.77%)</td>
</tr>
<tr>
<td>Women:Men ratio</td>
<td>4:1</td>
</tr>
<tr>
<td>RF positive patients</td>
<td>55 (63.95%)</td>
</tr>
<tr>
<td>Anti-CCP positive patients</td>
<td>52 (60.46%)</td>
</tr>
<tr>
<td>Anti-CCP (RU/mL)</td>
<td>110.82 (105.12)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>31.14 (38.4)</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>56.71 (29.67)</td>
</tr>
<tr>
<td>DAS-28, mean (SD)</td>
<td>6.12 (1.4)</td>
</tr>
</tbody>
</table>

Values are mean (SD) or number (%) unless otherwise indicated.

n: number of RA patients; SD: standard deviation; RF: rheumatoid factor; Anti-CCP: anti-citrullinated peptide antibodies; RU: relative units; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS-28: disease activity score 28.
Table 2
The distribution of HLA-DRB1 allele frequencies in RA patients and controls

<table>
<thead>
<tr>
<th>Genotype HLA-DRB1</th>
<th>RA (2n = 172)</th>
<th>Controls (2n = 400)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>AF (%)</td>
<td>n</td>
</tr>
<tr>
<td>DRB1*01</td>
<td>15</td>
<td>9.0</td>
<td>16</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>13</td>
<td>7.8</td>
<td>38</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>60</td>
<td>36.1</td>
<td>58</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>12</td>
<td>7.2</td>
<td>44</td>
</tr>
<tr>
<td>DRB1*08</td>
<td>2</td>
<td>1.2</td>
<td>7</td>
</tr>
<tr>
<td>DRB1*09</td>
<td>1</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>DRB1*10</td>
<td>12</td>
<td>7.2</td>
<td>12</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>24</td>
<td>14.5</td>
<td>99</td>
</tr>
<tr>
<td>DRB1*12</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
</tr>
<tr>
<td>DRB1*13</td>
<td>8</td>
<td>4.8</td>
<td>53</td>
</tr>
<tr>
<td>DRB1*14</td>
<td>10</td>
<td>6.0</td>
<td>23</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>10</td>
<td>6.0</td>
<td>37</td>
</tr>
<tr>
<td>DRB1*16+</td>
<td>5</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>SE positive</td>
<td>97</td>
<td>56.4</td>
<td>110</td>
</tr>
</tbody>
</table>

Values are number (%) unless otherwise indicated. AF: allele frequency; SE positive: the sum of DRB1*01, *04, *10, and *14 alleles; OR: odds ratio; 95% CI: confidence interval at 95%. HLA frequencies observed in patients and controls were compared using the chi-square test. Differences were considered significant at P < 0.05.

Table 3
Association of HLA-DRB1 shared epitopes alleles with anti-CCP and rheumatoid factor antibodies in rheumatoid arthritis patients (n = 86)

| SE status | SE positive (n = 61) | SE negative (n = 25) | OR (95% CI) | P
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Anti-CCP positive</td>
<td>44 (72.13%)</td>
<td>8 (32%)</td>
<td>5.5 (2–15.1)</td>
<td>0.00054</td>
</tr>
<tr>
<td>Anti-CCP negative</td>
<td>17 (27.87%)</td>
<td>17 (68%)</td>
<td>1.00 (0.47–2.17)</td>
<td>0.976</td>
</tr>
<tr>
<td>RF positive</td>
<td>46 (73.77%)</td>
<td>9 (32%)</td>
<td>5.45 (2–14.87)</td>
<td>0.00055</td>
</tr>
<tr>
<td>RF negative</td>
<td>15 (26.23%)</td>
<td>16 (68%)</td>
<td>1.01 (0.47–2.17)</td>
<td>0.976</td>
</tr>
</tbody>
</table>

Values are number (%) unless otherwise indicated. Presence of anti-CCP antibodies and RF in SE positive or SE negative RA patients was compared using the chi-square test. Differences were considered to be significant at P < 0.05.

Figure 1
Relation between shared epitopes and DAS-28 in 86 rheumatoid arthritis patients.
The DAS-28 values were compared between SE negative and SE positive RA patients using Student’s t-test. Differences were considered to be significant at P < 0.05.

surprisingly with no significance. On the other hand, Peruvian and Mexican American populations showed no significant correlation between HLA-DRB1*04 and RA susceptibility. Other alleles were associated with RA proneness as DRB*01 in Brazilians, Mexicans, Spanish, Italians, French, Turkish, Finnish, and Japanese; DRB1*09 in Turkish, Malaysians, and Koreans; DRB1*10 in Brazilians, Iranians, Saudi Arabians, Taiwanese, Asians, and African
Americans, and DRB1*14 in Peruvians, Ecuadorians, and Mexican Americans. Uncommonly, HLA-DRB1*08 was reported for its association with RA in Saudi Arabians and HLA-DRB1*15 in Japanese. In accordance to the nearby populations (Middle Eastern and Mediterranean), our results showed that RA susceptibility is predominantly associated with DRB1*01, *04, and *10 alleles. Albeit not significant, DRB1*09, *14, and *16 were more frequent in RA patients than controls.

The protective effect of certain HLA-DRB1 alleles against RA has been reported in several reviews and revealed in different populations. HLA-DRB1*03 was informed to be protective against RA in Iranians and Asians; DRB1*06 in Saudis; DRB1*07 in Slovaksians; Finnish, and Tunisians; DRB1*08 in Mexican Americans; DRB1*11 in Peruvians and African Americans; whereas DRB1*13 in Turkish, Finnish, and Slovaksians. In this study HLA-DRB1*11 and *13 were negatively associated with RA reflecting a probable protective effect in our population.

The relation between the SEs and the severity of RA has not been clearly verified. The DRB1*0401 allele is indicated to increase the severity of RA in northern Europe, Netherlands, northern Italy, and Caucasians, whereas DRB1*0405 allele is specified in Korea. In contrary, our study showed no significant correlation of disease severity, assessed by mean DAS-28 values, between the SE positive and SE negative patients. These results comply with studies carried out in Turkey and Greece. Our study supported previously reported relationship of SE positive alleles in the productions of anti-CCP and RF sero-positivity. Even less, results in this study may not reflect the relationship between HLA-DRB1 and disease severity because of limited number of patients.

Our study was limited by the inability to perform four-digit subtyping of all DRB1 alleles. However, a significant relation between SE-containing main alleles (the sum of DRB1*01, *04, *10, and *14) in patients with RA was resolute (OR = 3.41, 95% CI = 2.35–4.95, P < 0.0001).

In conclusion, HLA-DRB1*01, *04, and *10 alleles were identified as related with RA and HLA-DRB1*11 and *13 were detected as protective in our population. No significance was observed between SEs alleles and RA severity.
REFERENCES


