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

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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 etiology and an estimated population prevalence of 1%.[1] It is characterized by chronic inflammation, synovitis, pain, and progressive destruction of both the articular cartilage and bone leading to functional disability.[2] The chance of developing the disease is 2–3 times more frequent in women than men. The peak age on onset of the disease is in the 40s, although it can occur at any age.[3] Genetic and environmental risk factors play key roles in the disease pathogenesis.[1,4] The inheritance probability of RA is estimated to be around 60%.[4,5]

The human leukocyte antigen (HLA) is found to be the most important genetic risk factor for RA, which accounts for 30%[1,5] to 50% of overall genetic susceptibility to RA.[6] The shared epitope (SE) hypothesis described the relationship between HLA-DRB1 and RA.[7,8] 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).[5,6] On the other hand, SE negative genotypes (mainly DRB1*11 and *13) provide protection against RA susceptibility.[6,9]

The major relationship of particular HLA alleles with RA is not constant in all human populations, different geographical areas, or among different ethnic groups.[1] Despite of the

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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 Morocco[26] and Zahedan southeast Iran,[27] but

Table 1
Demographic and clinical characteristics of patients with rheumatoid arthritis

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

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

Genotype HLA-DRB 1	RA (2n = 172)		Controls (2n = 400)		Statistical analysis	
	n	AF (%)	n	AF (%)	OR (95% CI)	P
DRB1*01	15	9.0	16	4	2.29 (1.11–4.75)	0.022
DRB1*03	13	7.8	38	10	0.78 (0.40–1.50)	0.455
DRB1*04	60	36.1	58	15	3.16 (2.08–4.80)	< 0.0001
DRB1*07	12	7.2	44	10	0.61 (0.31–1.18)	0.137
DRB1*08	2	1.2	7	1.5	0.66 (0.14–3.21)	0.605
DRB1*09	1	0.6	2	0.5	1.16 (0.10–12.92)	0.901
DRB1*10	12	7.2	12	3	2.43 (1.07–5.51)	0.029
DRB1*11	24	14.5	99	25	0.49 (0.30–0.80)	0.004
DRB1*12	0	0.0	6	1.5	0.00	0.106
DRB1*13	8	4.8	53	13.5	0.32 (0.15–0.69)	0.002
DRB1*14	10	6.0	23	6	1.01 (0.47–2.17)	0.976
DRB1*15	10	6.0	37	9.5	0.61 (0.29–1.25)	0.170
DRB1*16+	5	3.0	3	0.5	3.96 (0.94–16.77)	0.044
SE positive	97	56.4	110	30.5	3.41 (2.35–4.95)	<0.0001

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 $P < 0.05$.
+ Not significant because 95% CI of *16 overlapped 1.

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
Anti-CCP positive	44 (72.13%)	8 (32%)	5.5 (2–15.1)	0.00054
Anti-CCP negative	17 (27.87%)	17 (68%)		
RF positive	46 (73.77%)	9 (32%)	5.45 (2–14.87)	0.00055
RF negative	15 (26.23%)	16 (68%)		

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 $P < 0.05$.
SE: shared epitopes; OR: odds ratio; 95% CI: confidence interval at 95%.

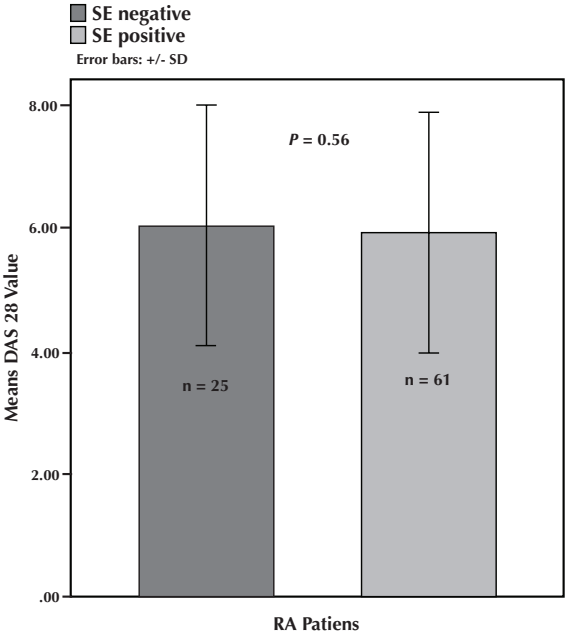

Figure 1
Relation between shared epitopes and DAS-28 in 86 rheumatoid arthritis patients.
The DAS-28 values were compared between SE negative and SE positive RA patients using Student's t-test. Differences were considered to be significant at $P < 0.05$.
n: number of RA patients carrying the alternative genotype.

surprisingly with no significance. On the other hand, Peruvian[28] and Mexican American[29] populations showed no significant correlation between HLA-DRB1*04 and RA susceptibility. Other alleles were associated with RA proneness as DRB*01 in Brazilians,[30] Mexicans,[31] Spanish,[14] Italians,[20] French,[24] Turkish,[25,32] Finnish,[17] and Japanese;[33] DRB1*09 in Turkish,[25] Malaysians,[34] and Koreans;[35] DRB1*10 in Brazilians,[30] Iranians,[27] Saudi Arabians,[16] Taiwanese,[36] Asians,[37] and African

Americans,[22] and DRB1*14 in Peruvians,[28] Ecuadorians,[38] and Mexican Americans.[29] Uncommonly, HLA-DRB1*08 was reported for its association with RA in Saudi Arabians[16] and HLA-DRB1*15 in Japanese.[33] 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[5,12,39,40] and revealed in different populations. HLA-DRB1*03 was informed to be protective against RA in Iranians[27] and Asians;[19] DRB1*06 in Saudis;[16] DRB1*07 in Slovakians,[23] Finnish,[17] and Tunisians;[13] DRB1*08 in Mexican Americans;[29] DRB1*11 in Peruvian[28] and African Americans;[22] whereas DRB1*13 in Turkish,[25,32] Finnish,[17] Asians,[19] and Slovakians.[23] 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.[41] The DRB1*0401 allele is indicated to increase the severity of RA in northern Europe,[42] Netherlands,[43] northern Italy,[44] and Caucasians;[45,46] whereas DRB1*0405 allele is specified in Korea.[47] 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[32] and Greece.[48] Our study supported previously reported relationship of SE positive alleles in the productions of anti-CCP and RF sero-positivity.[5,6,30,43] Even the 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

1. Kochi Y, Suzuki A, Yamada R, Yamamoto K. Genetics of rheumatoid arthritis: underlying evidence of ethnic differences. J Autoimmun 2009; 32(3-4):158–62.
2. Neumann E, Lefèvre S, Zimmermann B, Gay S, Müller-Ladner U. Rheumatoid arthritis progression mediated by activated synovial fibroblasts. Trends Mol Med 2010; 16(10):458–68.
3. Suchomel P, Buchvald P, Choutka O. Rheumatoid Arthritis. In: Suchomel P, Choutka O (eds.). Reconstruction of Upper Cervical Spine and Craniovertebral Junction. Berlin Heidelberg: Springer; 2011, p. 235–46.
4. Hoovestol RA, Mikuls TR. Environmental Exposures and Rheumatoid Arthritis Risk. Curr Rheumatol Rep 2011;1–9.
5. de Vries R. Genetics of rheumatoid arthritis: time for a change! Curr Opin Rheumatol 2011; 23(3):227–32.
6. Bax M, van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: what have we learned? Immunogenetics 2011; 63(8):459–66.
7. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987; 30(11):1205–13.
8. Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. Curr Opin Rheumatol 2010; 22(3):293–8.
9. Gibert M, Balandraud N, Touinssi M, Mercier P, Roudier J, Reviron D. Functional categorization of HLA-DRB1 alleles in rheumatoid arthritis: the protective effect. Hum Immunol 2003; 64(10):930–5.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315–24.
11. Prevoo MLL, Van't Hof MA, Kuper HH, van Leeuwen MA, van De Putte LBA, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38(1):44–8.
12. Newton JL, Harney SM, Wordsworth BP, Brown MA. A review of the MHC genetics of rheumatoid arthritis. Genes Immun 2004; 5(3):151–7.
13. Dhaouadi T, Sfar I, Abdelmoula L, Bardi R, Jendoubi-Ayed S, Makhlouf M, et al. Association of specific amino acid sequence (QRRAA) of HLA-DRB1*0405 with rheumatoid arthritis in a Tunisian population. Arch Inst Pasteur Tunis 2010; 87(1-2):53–9.
14. Balsa A, Minaur NJ, Pascual-Salcedo D, McCabe C, Balas A, Fiddament B, et al. Class II MHC antigens in early rheumatoid arthritis in Bath (UK) and Madrid (Spain). Rheumatology 2000; 39(8):844–9.
15. Hajeer AH, Dababneh A, Makki RF, Thomson W, Poulton K, González-Gay MA, et al. Different gene loci within the HLA-DR and TNF regions are independently associated with susceptibility and severity in Spanish rheumatoid arthritis patients. Tissue Antigens 2000; 55(4):319–25.
16. Al-Swailem R, Al-Rayes H, Sobki S, Arfin M, Tariq M. HLA-DRB1 association in Saudi rheumatoid arthritis patients. Rheumatol Int 2006; 26(11):1019–24.

17. Laivoranta-Nyman S, Möttönen T, Hermann R, Tuokko J, Luukkainen R, Hakala M, et al. HLA-DR-DQ haplotypes and genotypes in Finnish patients with rheumatoid arthritis. Ann Rheum Dis 2004; 63:1406–12.
18. Delgado-veja AM, Anaya JM. Meta-analysis of HLA-DRB1 polymorphism in Latin American patients with rheumatoid arthritis. Autoimmun Rev 2007; 6(6):402–8.
19. Jun KR, Choi SE, Cha CH, Oh HB, Heo YS, Ahn HY, et al. Meta-analysis of the Association between HLA-DRB1 Allele and Rheumatoid Arthritis Susceptibility in Asian Populations J Korean Med Sci 2007; 22(6):973.
20. Bongi SM, Porfirio B, Rombola G, Palasciano A, Beneforti E, Bianucci G. Shared-epitope HLA-DRB1 alleles and sex ratio in Italian patients with rheumatoid arthritis. Joint Bone Spine 2004; 71(1):24–8.
21. Xue Y, Zhang J, Chen YM, Guan M, Zheng SG, Zou HJ. The HLA-DRB1 shared epitope is not associated with antibodies against cyclic citrullinated peptide in Chinese patients with rheumatoid arthritis. Scand J Rheumatol 2008; 37(3):183–7.
22. Hughes LB, Morrison D, Kelley JM, Padilla MA, Vaughan LK, Westfall AO, et al. The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. Arthritis Rheum 2008; 58(2):349–58.
23. Stark K, Rovensky J, Blazickova S, Grosse-Wilde H, Ferencik S, Hengstenberg C, et al. Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls. Arthritis Res Ther 2009; 11(3):R70.
24. Reviron D, Foutrier C, Guis S, Mercier P, Roudier J. DRB1 alleles in polymyalgia rheumatica and rheumatoid arthritis in southern France. Eur J Immunogenet 2001; 28(1):83–7.
25. Uçar F, Karkucak M, Alemdaroglu E, Capkin E, Yücel B, Sönmez M, et al. HLA-DRB1 allele distribution and its relation to rheumatoid arthritis in eastern Black Sea Turkish population. Rheumatol Int 2012; 32:1003-7..
26. Atouf O, Benbouazza K, Brick C, Bzami F, Bennani N, Amine B, et al. HLA polymorphism and early rheumatoid arthritis in the Moroccan population. Joint Bone Spine 2008; 75(5):554–8.
27. Sandoughi M, Fazaeli A, Bardestani G, Hashemi M. Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, southeast Iran. Ann Saudi Med 2011; 31(2):171–3.
28. Castro F, Acevedo E, Ciusani E, Angulo JA, Wollheim FA, Sandberg-Wollheim M. Tumour necrosis factor microsatellites and HLA-DRB1*, HLA-DQA1*, and HLA-DQB1* alleles in Peruvian patients with rheumatoid arthritis. Ann Rheum Dis 2001; 60(8):791–5.
29. del Rincon I, Escalante A. HLA-DRB1 alleles associated with susceptibility or resistance to rheumatoid arthritis, articular deformities, and disability in Mexican Americans. Arthritis Rheum 1999; 42(7):1329–38.
30. Louzada-Junior P, Freitas MVC, Oliveira RDR, Deghaide NHS, Conde RA, Bertolo MB, et al. A majority of Brazilian patients with rheumatoid arthritis HLA-DRB1 alleles carry both the HLA-DRB1 shared epitope and anti-citrunillated peptide antibodies. Braz J Med Biol Res 2008; 41:493–9.
31. Ruiz-Morales JA, Vargas-Alarcón G, Flores-Villanueva PO, Villarreal-Garza C, Hernández-Pacheco G, Yamamoto-Furusho JK, et al. HLA-DRB1 alleles encoding the "shared epitope" are associated with susceptibility to developing rheumatoid arthritis whereas HLA-DRB1 alleles encoding an aspartic acid at position 70 of the beta-chain are protective in Mexican Mestizos. Hum Immunol 2004; 65(3):262–9.
32. Kinikli G, Ates A, Turgay M, Akay G, Kinikli S, Tokgoz G. HLA-DRB1 genes and disease severity in rheumatoid arthritis in Turkey. Scand J Rheumatol 2003; 32(5):277–80.
33. Yukioka M, Wakitani S, Murata N, Toda Y, Ogawa R, Kaneshige T, et al. Elderly-onset rheumatoid arthritis and its association with HLA-DRB1 alleles in Japanese. Rheumatology 1998; 37(1):98–101.
34. Kong KF, Yeap SS, Chow SK, Phipps ME. HLA-DRB1 genes and susceptibility to rheumatoid arthritis in three ethnic groups from Malaysia. Autoimmunity 2002; 35(4):235–9.
35. Lee HS, Lee KW, Song GG, Kim HA, Kim SY, Bae SC. Increased susceptibility to rheumatoid arthritis in Koreans heterozygous for HLA-DRB1*0405 and *0901. Arthritis Rheum 2004; 50(11):3468–75.
36. Liu SC, Chang TY, Lee YJ, Chu CC, Lin M, Chen ZX, et al. Influence of HLA-DRB1 genes and the shared epitope on genetic susceptibility to rheumatoid arthritis in Taiwanese. J Rheumatol 2007; 34(4):674–80.
37. Griffiths B, Situnayake RD, Clark B, Tennant A, Salmon M, Emery P. Racial origin and its effect on disease expression and HLA-DRB1 types in patients with rheumatoid arthritis: a matched cross-sectional study. Rheumatology (Oxford) 2000; 39(8):857–64.
38. Arias MVA, Domingues EV, Lozano RB, Flores CV, Peralta MM, Salinas CZ. Study of Class I and II HLA alleles in 30 Ecuadorian patients with rheumatoid arthritis compared with alleles from healthy and affected subjects with other rheumatic diseases. Rev Bras Reumatol 2010; 50(4):423–33.
39. Perricone C, Ceccarelli F, Valesini G. An overview on the genetic of rheumatoid arthritis: A never-ending story. Autoimmun Rev 2011; 10(10):599–608.
40. Feitsma AL, van der Helm-van Mil AHM, Huizinga TWJ, de Vries RRP, Toes REM. Protection against rheumatoid arthritis by HLA: nature and nurture. Ann Rheum Dis 2008; 67(Suppl 3):iii61–3.
41. Gorman JD, Criswell LA. The shared epitope and severity of rheumatoid arthritis. Rheum Dis Clin North Am 2002; 28(1):59–78.
42. Gorman JD, Lum RF, Chen JJ, Suarez-Almazor ME, Thomson G, Criswell LA. Impact of shared epitope genotype and ethnicity on erosive disease: a meta-analysis of 3,240 rheumatoid arthritis patients. Arthritis Rheum 2004; 50(2):400–12.
43. van Gaalen FA, van Aken J, Huizinga TW, Schreuder GM, Breedveld FC, Zanelli E, et al. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum 2004; 50(7):2113–21.
44. Salvarani C, Macchioni PL, Mantovani W, Bragliani M, Collina E, Cremonesi T, et al. HLA-DRB1 alleles associated with rheumatoid arthritis in Northern Italy: correlation with disease severity. Br J Rheumatol 1998; 37(2):165–9.
45. Mewar D, Marinou I, Coote AL, Moore DJ, Akil M, Smillie D, et al. Association between radiographic severity of rheumatoid arthritis and shared epitope alleles: differing mechanisms of susceptibility and protection. Ann Rheum Dis 2008; 67(7):980–3.
46. Fries JF, Wolfe F, Apple R, Erlich H, Bugawan T, Holmes T, et al. HLA-DRB1 genotype associations in 793 white patients from a rheumatoid arthritis inception cohort: frequency, severity, and treatment bias. Arthritis Rheum 2002; 46(9):2320–9.

47. Kim HY, Min JK, Yang HI, Park SH, Hong YS, Jee WH, et al. The impact of HLA-DRB1*0405 on disease severity in Korean patients with seropositive rheumatoid arthritis. Br J Rheumatol 1997; 36(4):440–3.

48. Boki KA, Drosos AA, Tzioufas AG, Lanchbury JS, Panayi GS, Moutsopoulos HM. Examination of HLA-DR4 as a severity marker for rheumatoid arthritis in Greek patients. Ann Rheum Dis 1993; 52(7):517–9.