Clinical Rheumatology (2020) 39:2875–2879
https://doi.org/10.1007/s10067-020-05060-0

BRIEF REPORT

Role of HLA-DRB1*04 in the susceptibility and HLA-DRB1*08 in the protection for development of rheumatoid arthritis in a population of Southern Mexico: brief report

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Received: 29 December 2019 / Revised: 11 March 2020 / Accepted: 20 March 2020 / Published online: 2 April 2020

Abstract

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease with an increased prevalence in Mexico. Although its etiology is unknown, its development can be influenced by environmental factors such as smoking and viral infections. But among the factors influencing susceptibility, it is the genetic factors that predominate, mainly the HLA-DRB1 genes, and specifically the alleles that have the shared epitope (SE). A transversal study was performed, in which 31 patients (28 women and 3 men) with RA, treated at the autoimmunity clinic of the High Specialty Hospital Ciudad Salud in Tapachula, Chiapas, southern México, were enrolled. Clinical, biochemical, and demographic data were analyzed; ESR (erythrocyte sedimentation rate), CRP (C-reactive protein), RF (rheumatoid factor), and ACPA (anticitrullinated peptide antibody) were recorded. All patients had at least one positive RA biological marker. For HLA alleles frequencies comparison, we enrolled ethnically matched healthy controls in a ratio of 3:1 for 25 cases and 4:1 for 6 cases in order to guarantee the balance between groups regarding the mean of age and proportion of gender (males vs females). HLA-DRB1*04 was found to be significantly increased in patients compared with ethnically matched healthy controls (p 0.0007, OR: 2.8, 95% CI 1.5–5.1); contrarily, DRB1*08 showed a protective effect (p 0.005, OR 0.1). This paper confirmed the involvement of HLA genes on risk determination for RA in a population of Mexican Mestizos from Tapachula, Chiapas.

Key Points

- *HLA-DRB1*04 confirms the increased risk of rheumatoid arthritis.*
- *HLA-DRB1*08 showed a more definite protective effect in southern Mexicans mestizos, a population with more Amerindian ancestry.*

Keywords HLA-DRB1 antigen · Rheumatoid arthritis · Shared epitope · Viral infections

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease of unknown etiology, which not only affects the joints, but also has pulmonary, vascular, and systemic manifestations, through tissue damage mediated by cytokines, immune complexes, and autoantibodies [1]. Globally, it affects 0.5–1% of the adult population, with an annual incidence of approximately 12–1200 per 100,000 people, predominantly affecting women by a rate of 3:1 [2]. We can estimate the prevalence in Mexico of RA around 1.6% and in Yucatan 2.8%.

Approximately two-thirds of RA risk is attributed to genetic factors, with one of the strongest associations being attributed to polymorphisms in the genes for the major histocompatibility complex (MHC), specially the HLA-DRB1 alleles [3]. The remaining one-third of disease susceptibility is attributed to non-genetic mechanisms that are most likely triggered by environmental factors, such as smoking and viral infections [4]. The distribution of the MHC polymorphisms and its risk associations vary geographically. In Mexicans, the HLA-DRB1*04 and DRB1*03 alleles have been described to be associated with increased susceptibility to RA [5].

There is a correlation between DR4 and DR1 with RA, explained by the shared epitope (SE) hypothesis, where these alleles are linked to RA susceptibility, and has been proven in multiple ethnic groups. This theory states that the DRB1 alleles linked to the disease owe it to a 5-amino acid sequence motif in the position 70–74 on the third hypervariable region of the HLA-DRβ1 chain, this sequence is the one known as the SE [6]. There have been reports in the Mexican population that have shown that a copy of a SE allele is enough to markedly increase the susceptibility to RA [7]. Patients with the SE alleles produce anticitrullinated peptide antibodies (ACPA) and show a more severe articular disease, extra-articular manifestations, higher titers of rheumatoid factor (RF), and overall worse prognosis [8, 9]. Among these alleles some carry a higher risk of severe disease, like DRB1*04 [8]. As such, some alleles have demonstrated a protective effect in certain populations, including Mexicans, like HLA-DRB1*07, DRB1*08, and DRB1*11 [10].

Thus, we performed an observational, retrospective study to evaluate the relationship between HLA-DRB1 polymorphisms and other inflammatory markers with clinical course and severity in RA.

Materials and methods

Subjects

Patients with a diagnosis of RA were enrolled between the years 2016–2019, based on the ACR/EULAR 2010 diagnostic criteria. Information was collected retrospectively from clinical records. The demographic variables for this study were age at diagnosis, time of evolution, gender, type of arthritis, levels of RF, ACPA, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), DAS-28, and HLA allelic sequences. For HLA alleles frequencies comparison, we enrolled ethnically matched healthy controls in a ratio of 3:1 for 25 cases and 4:1 for 6 cases in order to guarantee the balance between groups regarding the mean of age and proportion of gender (males vs females). Both patients and controls are residents of Tapachula, Chiapas, Mexico, and their two previous generations were from Tapachula as well.

Autoantibodies and biomarkers of inflammation

The concentrations of the ACPA and RF considered for this analysis were the highest reported in the clinical record. The RF determination was performed by spectrophotometry (Dimension RxL Max ®), and ACPA by solid-phase sandwich ELISA (Novex ®). For the inflammatory biomarkers, CRP was determined by spectrophotometry (Dimension RxL Max®), and the ESR by the Westergren method (Vesmatic®), considering for this study the latest recorded value at patient enrollment.

Disease activity index (DAS-28)

For evaluation of severity, we used the DAS-28 index, a tool validated for RA. We considered the following cut-off points: no joint activity < 2.6; mild activity: > 2.6 to < 3.2; moderate activity: > 3.2 to < 5.1; and severe activity: > 5.1. We used the last DAS-28 index recorded in the clinical record at the moment of enrollment. We calculate DAS-28 considering the inflamed and painful joints, EVA (visual analog scale) and CRP.

HLA typing

To evaluate genetic susceptibility to RA, genomic DNA was obtained from whole blood, and low-resolution HLA typing was performed by Sequence Specific Primers (SSP, TBG technologies) for HLA DRB1 and DQB1 after PCR amplification.

Statistical analysis

Non-parametric statistics were performed (chi-squared test and Fisher's exact test) by using the Epi Info program (version 10.0) and the StatCalc subprogram based on two-by-two contingency tables.

Ethics statement

This study was approved by the Hospital Regional de Alta Especialidad ethics and research committee and has been performed in accordance with the ethical standards of 1964 Declaration of Helsinki, and all subjects were of adult age and signed an informed consent.

Results

Patients

Between 2016 and 2019, 64 new cases of RA were registered in the autoimmunity clinic, but only 31 with HLA typing were included. Of those, 28 were women (90.32%) with a mean age at diagnosis of 43.45 ± 14.72 years, and median years of disease evolution of 5 years (0.08–22). Table 1 shows demographic, clinical, and biochemical data.

Gene frequencies for HLA-DRB1 alleles

Table 2 shows the complete allele frequencies of the HLA-DRB1 gene of 31 patients (62 alleles) and controls. Eleven alleles were identified in the group of cases, being the most frequent HLA-DRB1 *04. In the control group, thirteen haplotypes were identified, HLA-DRB1*04 being also the most frequent.

Table 1 Demographic, clinical, and biochemical and clinical data of RA patients

Characteristic	*N* (%)
Subjects included	31
Gender	Female 28 (90.32%)
	Male 3 (9.68%)
Age at diagnosis in years	43.45 ± 14.72
Disease evolution time: median in years (range)	5 (0.08–22)
Type of arthritis:	
Seropositive	25 (80.65%)
Seronegative	6 (19.35%)
Rheumatoid factor: median of UI/ml (range)	198 (0–3612)
Rheumatoid factor:	
Negative	8 (25.8%)
Low positive	2 (6.45%)
High positive	21 (67.75%)
ACPA: median of U/ml (range)	115.8 (0.5–993.5)
ACPA:	
Negative	12 (38.71%)
Low positive	1 (3.22%)
High positive	18 (58.07%)
DAS-28:	
Remission	16 (51.61%)
Low activity	3 (9.67%)
Moderate activity	9 (29.02%)
High activity	3 (9.67%)

**ACPA* anticitrullinated protein antibodies, *DAS-28* Disease Activity Score-28

The titers of antibodies and rheumatoid factor correspond to the maximum value recorded in the clinical record of each patient

DAS-28 was calculated from the last data registered in the clinical record

*Reference values: rheumatoid factor 0–14 (< 14 UI/ml: negative; 14–42 UI/ml: low positive; > 42 UI/ml: high positive); ACPA 0–17 (< 17 U/ml: negative; 17–51 U/ml: low positive; > 51 U/ml: high positive); DAS-28 0–5.1 (< 2.6: remission; > 2.6 to < 3.2: mild activity; > 3.2 to < 5.1: moderate activity; > 5.1: severe activity)

Shared epitope

In this study, it was found that the shared epitope was present in 27 patients (87.10%), in which the levels of RF and ACPA were strong positive, the former with a median of 200 UL/ml (0–3612) and the latter with a median of 166.7 U/ml (0.5–993.5) in those patients.

Discussion

This research confirmed the increased risk of RA associated with HLA-DRB1*04, and it also showed a protective effect of DRB1*08 in a population of Southern Mexican Mestizos. The protective effect of HLA-DRB1*08 should be analyzed at a molecular level to help clear its mechanism against autoimmunity. A protective effect of HLA-DRB1*07, DRB1*08, and DRB1*11 was previously suggested when analyzed conjointly in a population of Mexican Mestizos from Mexico City [10], but through this research in a population with more Amerindian ancestry, HLA-DRB1*08 individually shows a more definite effect. This protective effect could be explained because of an aspartic acid at position 70, which differentiates it from SE alleles, altering peptide presentation, or through an association with other MHC loci, such as the role of TNF gene, which has been already described in the Mexican population [8].

HLA genes state a high geographical variation related with migration and viral epidemics. Moreno-Estrada et al. demonstrated the vast diversity of genetic substructures within the Mexican population and how it translates into variations of biomedical traits [11]. HLA genes study in different regions allows a better understanding in how influences the pathogenesis. We suggest that this test should be used to aid in the diagnosis of RA. Southern Mexico, which includes Chiapas, has a prevalence of 2.8%, compared with 1% reported in Mexico City [12], given that this region has a high rate of viral infections such as Dengue, Zika, and Chikungunya, which might act as triggers and increased prevalence of RA [13].

Barquera et al. [14] made an analysis of HLA allele distribution among every state in Mexico, including Chiapas. The population of the state of Chiapas is one of the most densely composed by Natives, and their results show that the found frequency of the HLA-DRBI*08 allele is concordant with the frequency described for rural Chiapas, where Tapachula is included, compared with the capital Tuxtla Gutierrez (0.1322 vs. 0.0377 allelic frequencies). It should be noted that HLA-DRB1*08 is the most common allele in indigenous people of Mexico and the Americas, including Central America. Its gene frequency suggests that this allele is a product of

Table 2 Gene frequencies HLA-DRB1 in RA patients from Tapachula, Chiapas, and healthy controls

HLA-DRB1 alleles	Patients ($N = 62$)		Controls ($N = 198$)		P value	OR	95% CI
	n	gf	n	gf			
*04	29	0.468	47	0.237	0.0007	2.8	1.5–5.1
*14	6	0.097	21	0.105	NS		
*01	5	0.080	10	0.050	NS		
*13	5	0.080	10	0.050	NS		
*11	4	0.064	20	0.100	0.4	0.6	0.2–1.8
*07	4	0.064	22	0.111	0.3	0.55	0.1–1.6
*15	3	0.048	13	0.065	NS		
*03	2	0.032	11	0.055	NS		
*08	2	0.032	33	0.165	0.005	0.1	0.03–0.7
*16	1	0.016	5	0.025	NS		
*09	1	0.016	3	0.015	NS		
*12	0	0	2	0.010	NS		
*10	0	0	1	0.005	NS		

**CI* confidence interval, *gf* gene frequency, *NS* not significant, *OR* odd ratio

natural selection favoring those who survived the infections of the sixteenth century in Mexico and that depopulated the country of 22 million inhabitants to only one million at the end of the colonial period.

Additionally, this study reinforced that the subjects who had the shared epitope showed elevated levels of inflammation markers, as well as RF and ACPA, associated with worse prognosis [15].

In conclusion, this paper confirmed the involvement of HLA genes on risk determination for RA in a population of Mexican Mestizos from Tapachula, Chiapas.

Acknowledgments We acknowledge the support of Dr. Fernando Tapia Garduño, Research, Planning and Education Director of the Centro Regional de Alta Especialidad de Chiapas.

Data availability statement The authors confirm that the data supporting the findings of this study are available within the article. For detailed information of data bases, you may contact Dr. Jesus Sepulveda at jesussd52@gmail.com.

Funding information This study was funded with research resources provided by the Federal Government and assigned to the Centro Regional de Alta Especialidad de Chiapas in 2018 and 2019.

Compliance with ethical standards

This study was approved by the Hospital Regional de Alta Especialidad ethics and research committee and has been performed in accordance with the ethical standards of 1964 Declaration of Helsinki, and all subjects were of adult age and signed an informed consent.

Disclosures None.

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