CONCISE REPORT

Influence of human leucocyte antigen-DRB1 on the susceptibility to rheumatoid arthritis and on the production of anti-cyclic citrullinated peptide antibodies in a Portuguese population

D Ligeiro, J E Fonseca, O Abade, I Abreu, M Cruz, P Nero, J Cavaleiro, J Teles, H Trindade, J M Caetano, J Branco

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Objective: To clarify the influence of the HLA-DRB1 locus on the susceptibility to rheumatoid arthritis and the production of anti-cyclic citrullinated peptide antibodies (anti-CCP) in a Portuguese population.

Methods: 141 patients with rheumatoid arthritis fulfilling the American College of Rheumatology 1987 revised criteria for rheumatoid arthritis were compared with 150 healthy controls. Human leucocyte antigen (HLA)-DRB1 locus genotyping was assessed by polymerase chain reaction reverse probing assays and sequence-specific primers. Anti-CCP antibodies were quantified by ELISA in patients with rheumatoid arthritis. Frequencies between groups were compared by the two-sided Fisher's exact test and considered significant if $p<0.05$.

Results: The HLA-DRB1*04 and HLA-DRB1*10 groups were highly associated with rheumatoid arthritis ($p<0.001$ and $p=0.031$, respectively). High titres of anti-CCP antibodies were largely associated with the presence of HLA-DRB1*04/10.

Conclusion: The well-recognised susceptibility alleles to rheumatoid arthritis, HLA-DRB1*04, were associated with rheumatoid arthritis in Portuguese patients. The relatively rare DRB1*10 was also associated with rheumatoid arthritis, as was described previously in other southern European countries. Both groups were associated with high anti-CCP titres, reinforcing its relevance to disease onset.

Rheumatoid arthritis heritability, estimated from twin studies, is 40–60%.[1 2] The only region that has been consistently shown to be associated with rheumatoid arthritis is the major histocompatibility complex (MHC), which contributes approximately 30% to the total genetic effect.[3] Rheumatoid arthritis is associated with specific HLA-DRB1 alleles that encode a conserved sequence of amino acids, known as the shared epitope: DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*0101, DRB1*0102 and DRB1*1001.[4] HLA-DRB1*0401 and HLA-DRB1*0404 alleles were associated with rheumatoid arthritis in northern Europe and western North America.[5] However, in several southern European populations, other HLA-DR antigens (such as HLA-DRB1*0101, HLA-DRB1*1001 and HLA-DRB1*0405) were also associated with rheumatoid arthritis.[5] This fact has been proposed as a partial explanation for the differences in severity of rheumatoid arthritis reported between northern and southern European countries.[6] In Portugal, recent observations were in accordance with the idea that rheumatoid arthritis in southern Europe is less aggressive than in other geographical areas.[7] From a genetic perspective, HLA-DR4 has been previously associated with rheumatoid arthritis susceptibility and with the presence of immunoglobin (Ig)M rheumatoid factors[8] in a Portuguese population. However, no further study has analysed HLA-DRB1 alleles carrying the shared epitope in this population. On the other hand, a recent publication from a northern European population has described that the occurrence of shared epitope alleles in rheumatoid arthritis is associated with the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies.[9] The aim of this work was to characterise the influence of the HLA-DRB1 locus on the susceptibility to rheumatoid arthritis in a Portuguese population and to test whether the association between the shared epitope and anti-CCP antibodies was also present in a southern European population.

METHODS

Patients and controls

A total of 141 Caucasian patients with rheumatoid arthritis and 150 ethnically matched controls were studied. All patients fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for rheumatoid arthritis.[10] Demographic characteristics of the patients were (mean (SD) (range)): age at evaluation 58 (13.8) (20–88) years, age at disease onset 45.5 (14.9) (19–87) years and disease duration 8 (9.6) (0–41) years; 123 (88%) were women and 17 (12%) men.

The control population was recruited from among Portuguese volunteer bone marrow donors after consent and health evaluation to reject autoimmune pathologies.

The study was approved by Hospital Egas Moniz's ethics committee and all patients gave written informed consent.

MHC class II HLA-DRB1 typing

Genomic DNA was isolated from a 200-µl buffy-coat of 5 ml EDTA whole blood with a commercial DNA purification kit, QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

HLA DRB1 genotyping was performed at allele group level (DRB1*01–DRB1*16) with the reverse line probe assay INNO-LiPA DRB1 (Innogenetics, Ghent, Belgium) as recommended by the supplier. Subtyping of shared epitope-carrying alleles (DRB1*01, DRB1*04, DRB*14) was carried out by the polymerase chain reaction with sequence-specific primers (Olerup SSP, Hasselstigen, Sweden).

Anti-CCP antibodies

Anti-CCP_2 IgG antibodies were detected by a commercial ELISA containing synthetic peptides (Inova Diagnostics, San Diego,

Abbreviations: CCP, cyclic citrullinated peptide; HLA, human leucocyte antigen

Table 1 Frequency distribution of HLA-DRB1*04 alleles and shared epitope positive (SE+) genotypes (DRB1*0101/2, *0401/4/5/8/9, *1001) among Portuguese patients with rheumatoid arthritis and controls

Genotype	RA number (%) n = 141	Control number (%) n = 150	Odds ratio (95% CI)	p Value
DRB1*0101/02	38 (14.9)	30 (11.3)	1.48 (0.9 to 2.6)	NS
DRB1*04	63 (25.2)	26 (9.7)		
*0401	24 (8.9)	4 (1.3)	7.5 (2.5 to 22.2)	<0.001
*0402	7 (2.5)	5 (1.7)		NS
*0403	4 (1.4)	2 (0.7)		NS
*0404	14 (5.0)	5 (1.7)	3.2 (1.1 to 9.1)	0.03
*0405	18 (6.4)	10 (3.3)		NS
*0406	0	1 (0.3)		NS
*0407	0	1 (0.3)		NS
*0408	2 (0.7)	0		NS
*0409	1 (0.3)	1 (0.3)		NS
DRB1*0401/4/5/8/9	56 (21.3)	18 (6.7)	4.8 (2.7 to 8.8)	<0.001
DRB1*0402/3/6/7	7 (3.9)	8 (3, 0)		NS
DRB1*1001	17 (6.0)	7 (2.3)	2.8 (1.1 to 7.0)	0.031

OR, odds ratio; RA, rheumatoid arthritis.

California, USA). The ELISA was performed according to the manufacturer's instructions.

Serum samples with a test result of ⩾50 U/ml were considered positive and further designated as the ''standard'' cut-off. All the assays were performed in duplicate.

Statistical analysis

Fisher's exact test was used for testing differences in frequencies of categorical data (genotype direct counting) between groups. Relative risk was calculated as odds ratio with 95% confidence intervals in 2×2 tables with Haldane's correction where appropriate. The calculations were performed using the StatsDirect V. 2.4.6 suite for Windows.

RESULTS

HLA-DRB1 genotyping showed an increased frequency of the DRB1 group of alleles encoding the shared epitope DRB1*0401/04/05/08 and DRB1*1001 in patients with rheumatoid arthritis when compared with controls. In fact, DRB1*04 shared epitope positive alleles were found in 56 (39.7%) patients with rheumatoid arthritis compared with only 18 (12%) controls (OR = 4.8, 95% CI 2.7 to 8.8, p<0.001), whereas DRB1*10 was found in 17 (12%) patients with rheumatoid arthritis and in 7 (4.7%) controls (OR = 2.8, 95% CI 1.1 to 6.9, p = 0.0314). However, there were no differences between patients with rheumatoid arthritis and controls regarding the group DRB1*0101/02 (table 1). In addition, the other HLA-DRB1 groups of alleles were also equally distributed between patients and controls.

Nine alleles of the DRB1*04 group were found in the probed populations. The allele most strongly associated with rheumatoid arthritis susceptibility was DRB1*0401 (OR = 7.5; p<0.001) followed by DRB1*0404 (OR = 3.2; p = 0.03). The most frequent HLA-DRB1*04 allele in the Portuguese control population was DRB1*0405, which was also highly represented in the population with rheumatoid arthritis, although this was not statistically significant.

Anti-CCP antibodies were quantified by ELISA in 131 patients. Of these, 40 were negative (titre <50 UI) and 93 were positive (titre ⩾50 UI). Table 2 shows the association found in this population of patients with rheumatoid arthritis between high titres of anti-CCP antibodies and the carriage status of shared epitope positive DRB1 alleles. In a similar way to HLA genotypes and susceptibility to rheumatoid arthritis, the frequencies of DRB1*04 and DRB1*10 shared epitope positive genotypes were significantly higher (OR = 4.4 (95% CI 4.4 to 11.0) and OR = 15.9 (95% CI 1.8 to 82.7), respectively; p = 0.005) in the group with high titres (⩾50 UI) of anti-CCP antibodies. In contrast, again, HLA- DRB1*0101/02 was not associated with anti-CCP.

Table 2 Distribution of HLA-DRB1 shared epitope positive alleles among 131 patients with rheumatoid arthritis with anti-CCP negative titres (<50 UI) and positive titres (⩾50 UI)

	Anti-CCP <50 UI number (%), n = 40	Anti-CCP ⩾50 UI number (%), n = 93	Odds ratio (95% CI)	p Value
DRB1*0101/02	13 (20.0)	23 (12.9)	0.7 (0.3 to 1.5)	NS
DRB1*04	9 (11.3)	50 (31.2)		
*0401	2 (2.5)	21 (11.8)	5.5 (1.2 to 24.9)	0.013
*0402	2 (2.5)	4 (2.2)		NS
*0403	0	4 (2.2)		NS
*0404	3 (3.8)	10 (5.4)		NS
*0405	2 (2.5)	15 (8.1)		NS
*0406	0	0		NS
*0407	0	0		NS
*0408	0	2 (1.1)		NS
*0409	0	1 (0.5)		NS
DRB1*0401/4/5/8/9		45 (26.9)	4.4 (1.8 to 11.0)	0.001
DRB1*0402/03/06/07	2 (2.5)	5 (4.3)		NS
DRB1*1001	0	15 (16.1)	15.9 (1.4 to 82.7)	0.005

The anti-CCP antibody positive patients had a higher frequency of DRB1*04 shared epitope positive alleles, but this difference reached statistic significance only for the DRB1*0401 allele. Homozygotic shared epitope positive patients were absent in the negative anti-CCP antibody group.

DISCUSSION

HLA-DRB1 genotyping showed that the HLA-DRB1*04 and HLA-DRB1*10 groups were highly associated with rheumatoid arthritis in the studied population. These results show that the well-recognised susceptibility alleles to rheumatoid arthritis, HLA-DRB1*04, were also associated with rheumatoid arthritis in a Portuguese population. In addition, the relatively rare DRB1*10, which had been previously described in association with rheumatoid arthritis in studies performed in Hispanics living in North America and in Mediterranean subjects,[5 11] is also a susceptible marker for rheumatoid arthritis in the Portuguese population. However, the shared epitope positive DRB1*0101/02 alleles, associated with rheumatoid arthritis susceptibility in some Mediterranean populations, do not seem to influence disease susceptibility in Portuguese patients.

Although these results reinforce the fact that the most frequent HLA-DRB1 specificity in European patients with rheumatoid arthritis is DRB1*04, slightly different HLA-DRB1 susceptibility patterns do exist, probably contributing to the previously recorded discrepancies in prevalence and severity of rheumatoid arthritis across Europe.

Both HLA-DRB1 groups linked to susceptibility to rheumatoid arthritis in Portuguese patients were associated with high anti-CCP titres, reinforcing the finding that shared epitope carriage is a risk factor for the presence of anti-CCP antibodies[12] and highlighting their relevance for disease onset. In fact, different studies have shown that these antibodies are highly specific and predictive for rheumatoid arthritis,[13] and that they can be detected years before its onset.[14] Moreover, in DRB1*0401 transgenic mice, citrullination of peptides not only increased peptide–MHC affinity but also led to activation of CD4-T cells,[15] possibly signifying an increased ability of citrulline-containing peptides to bind to certain HLA class II molecules and promoting an antibody response against citrulline-containing antigens.[9]

Authors' affiliations

D Ligeiro, O Abade, H Trindade, Centro de Histocompatibilidade do Sul, Lisboa, Portugal
J E Fonseca, J Cavaleiro, J Teles, Unidade de Artrite Reumatóide, Instituto de Medicina Molecular, Faculdade de Medicina, Lisboa, Portugal
M Cruz, P Nero, J Branco, Unidade de Reumatologia do Hospital de Egas Moniz SA, Lisboa, Portugal
I Abreu, J M Caetano, Departamento de Imunologia da Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisboa, Portugal

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Correspondence to: Professor J E Fonseca, Unidade de Investigação em Reumatologia, Instituto de Medicina Molecular, Edifício Egas Moniz, Faculdade de Medicina de Lisboa, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal; jefonseca@netcabo.pt

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