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BRIEF COMMUNICATION

*Rheumatoid arthritis association in Colombian population is restricted to HLA-DRB1*04 QRRAA alleles*

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*In most ethnic groups genetic susceptibility to rheumatoid arthritis (RA) is associated with certain HLA-DRB1 alleles encoding a similar sequence motif called the 'shared epitope' (SE) spanning amino acid positions 70 to 74 in the third diversity region of the outermost domain of the HLA-DRB1 molecule. We examined the association of the SE and RA in 83 Colombian women with established RA and 90 healthy controls. The group HLA-DRB1*04 was associated with RA with respect to controls (47% vs 18%, respectively. OR: 4.1, 95%CI: 2.1–8.2, $P < 0.001$). HLA-DRB1 alleles carrying the SE QRRAA, but not those carrying QKRAA or RRRAA, were associated with disease (OR: 3.7, 95%CI: 1.73–7.83, $P = 0.0009$). This association was stronger among HLA-DRB1*04 carriers (OR: 23, 95%CI: 1.3–414, $P = 0.002$). In our population, the SE QRRAA expressed in DRB1*04 alleles appears critical in identifying women with increased susceptibility to RA.*
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Rheumatoid arthritis (RA) is a frequent chronic inflammatory disease affecting females two to three times more often than males. Owing to the articular and extra-articular manifestations of the disease and complications such as infections and osteoporosis, RA considerably diminishes the patient's quality of life, having strong physical, psychological and social repercussions.

In Caucasian populations, genetic susceptibility to RA is associated with the histocompatibility antigens of the HLA-DRB1 alleles that share similar amino acid residues at positions 70 through 74 of the DRβ chain called the RA-associated shared epitope (SE).[1,2] These antigens are coded mainly by the HLA-DRB1 *0101, *0102 *0401, *0404, *0405, *0408, *0409, *0410, *1402 and *1001 genes. The alleles DRB1 *0101, *0102 ,*0104, *0105, *0404, *0405, *0408, *0410, and *1402 are characterized by the presence of QRRAA at positions 70–74 while DRB1*0401 and *0409 alleles have a K at the β71 position and DRB1*1001 an R at both β70 and β71. It has been postulated that the presence of the SE influences not only susceptibility towards disease, but also its severity.[3] However, the nature of the association between the SE and RA differs depending on the patients background (ie ethnicity, gender).[3,4] Few studies of human leukocyte antigen (HLA) and RA have been done in South-Americans.[5–7] However, in Colombians, the association of the SE and RA has not been previously investigated. Therefore, we examined the influence of SE on the susceptibility of RA in a northwestern Colombian population (Medellin), whose ancestral origin is more Caucasian with low Amerindian or Negroid contributions.[8]

A total of 83 women with RA[9] and 90 unrelated healthy controls matched to patients by age (± 5 years), gender and geography were analyzed. The mean age ± s.d. of patients was 47 ± 12.7 years, the mean duration of disease was 6.5 ± 5.5 years, rheumatoid factor was positive in 85% (by turbidimetry), and at least one extra-articular manifestation was recorded in 20% of patients. HLA-DRB1 alleles were determined by SSP-PCR using UCLA HLA-DR sequence specific primers (UCLA Tissue Typing Laboratory, Los Angeles, CA, USA). HLA-DR4 alleles not distinguishable using SSP-PCR were identified by reverse dot blot hybridization (INNO-LiPA DRB1*04 kit, Innogenetics, Zwizndrecht, Belgium).

HLA-DRB1*04 group was found in 39 (47%) patients, compared with 16 (18%) controls (OR: 4.1, 95%CI: 2.1–8.2, $P < 0.001$) (Table 1). We observed no association between DRB1*01 (19% *vs* 13%), *14 (9.6% *vs* 24.4%) or *1001 (0% *vs* 3.3%) groups and disease. We tested the existence of population heterogeneity among the case and control sets, by using Wright's F statistics according to the non-biased method of Weir and Cockerham (θ value).[10] There was no stratification between patients and control samples since the θ (FST) was not significantly different from 0 (Table 2). Thus, patients and controls were from a similar genetic background.

Overall, in this study HLA-DRB1 alleles carrying the QRRAA motif were associated with RA. This association

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Table 1 HLA-DRB1 allele frequencies in northwestern Colombian women with RA

DRB1 alleles	*Patients n = 83 (%)*	*Controls n = 90 (%)*
*0101	11 (13.3)	8 (8.9)
*01012	3 (3.6)	0
*0102	1 (1.2)	4 (4.4)
*0103	1 (1.2)	0
*15	18 (21.7)	19 (21.1)
*1601	5 (6.1)	3 (3.3)
*1602	8 (9.6)	7 (7.7)
*03	3 (3.6)	1 (1.1)
*0301	4 (4.8)	7 (7.7)
*0302	5 (6.1)	2 (2.2)
*0303	1 (1.2)	0
*0306	0	2 (2.2)
*0401	3 (3.6)	0
*0402	5 (6.1)	1 (1.1)
*0403	6 (7.2)	0
*0404	8 (9.6)	0
*0405	2 (2.4)	0
*0407	5 (6.1)	2 (2.2)
*0408	2 (2.4)	0
*0410	2 (2.4)	0
*0411	1 (1.2)	5 (5.5)
*0415	1 (1.2)	1 (1.1)
*0419	2 (2.4)	0
*0420	0	1 (1.1)
*0421	2 (2.4)	6 (6.7)
*11	12 (14.5)	11 (12.2)
*12	2 (2.4)	3 (3.3)
*13	8 (9.6)	16 (17.8)
*14	2 (2.4)	9 (10)
*1401	0	1 (1.1)
*1403	5 (6.1)	5 (5.5)
*1404	0	1 (1.1)
*1408	0	1 (1.1)
*1412	0	2 (2.2)
*1414	1 (1.2)	0
*1418	0	1 (1.1)
*1425	0	2 (2.2)
*0701	14 (16.9)	28 (31.1)
*08	10 (12)	15 (16.7)
*0901	4 (4.8)	3 (3.3)
*1001	0	3 (3.3)

Table 2 Weir and Cockerham estimates of population subdivision

Bound	*f*	*F*	*Theta-P*
Upper	0.387231	0.390764	0.016262
Lower	–0.084668	–0.079820	0.003590
No of replicas	10000	10000	10000
CI	95.0	95.0	95.0

θ, f and F estimates represent the non-biased estimates of Wright classical Fis, Fit and Fst subdivision coefficients; CI = Confidence interval of 95% of the estimates after the bootstrap process of 10.000 replicas. It is shown that there was no subdivision among individual–subpopulation level, individual–total population level (patients and controls put together) and subpopulation–total population level.

was stronger among HLA-DRB1*04 carriers (Table 3). Thus, in our population RA is linked to HLA-DRB1*04-QRRAA alleles. As previously reported,[5] we observed a gene dosage effect in the association of the SE with RA (Table 3). This was a cross-sectional study in which the sample size as well as the low number of positive patients for extra-articular manifestations precludes an analysis to test the severity with respect to SE. Other studies from South America have reported that the SE is a susceptibility factor for RA but it does not significantly contribute to severity of disease. In Chilean patients from Santiago, the most frequently expressed DRB1 alleles were DRB1*0404 or DRB1*0408.[5] However it was demonstrated that in 46% of the cases the susceptibility towards disease and its severity were independent of the SE.[5] In Argentinean patients from Buenos Aires, susceptibility to RA was associated with DRB1*0404 but the SE alleles failed to correlate with more severe disease with the exception of DRB1*1001 which, although infrequent, was significantly associated with radiological damage.[6] In Peruvians patients from Lima, a weak association of disease susceptibility with DRB1*1402 was reported, but the SE did not influence disease severity.[7] Factors that may explain the lack of significant influence of the SE on disease susceptibility in these studies include the methods used (ie cross-sectional analysis, different radiographic measurements, lack of statistical power) as well as the low prevalence of DRB1*0401 allele among those patients (6.2% in Chileans,[5] 16% in Argentineans,[6] and 1.9% in Peruvians[7]). It has been suggested that there is a hierarchy of DRB1 alleles in their effect on RA outcome, with HLA-DRB1*0401, an allele particular to whites and Eskimos, at the top of the list.[11] Recently, the Chilean group has studied prospectively the previous reported cohort and found an influence of the SE on hand erosions.[12]

Table 3 Association between the SE and RA in northwestern Colombian women

RA Shared Epitope	*RA n = 83 (%)*	*Controls n = 90 (%)*	*OR*	*95% CI*	*P*
QRRAA	30 (36)	12 (13)	3.7	(1.73–7.83)	0.0009
QKRAA	5 (6)	6 (7)	–	–	NS
RRRAA	0	3 (3)	–	–	NS
QRRAA Homozygotes	9 (11)	1 (1)	10.8	(1.34–87.46)	0.007
DRB1*04-QRRAA	16/39 (41)	0/16	23.2	(1.3–414.3)	0.002

Functionally HLA-DRB1 genes influence the types of antigens recognized by immune T cells. The β1 chain of HLA-DR molecules contain polymorphic residues contributing to five binding pockets: P1, P4, P6, P7, and P9. These pockets control the peptide-binding specificity of different class II molecules. The P4 pocket, formed by the amino acid residues at positions β13, β70, β71 and β74, is critical in antigen presentation.[13] The last three residues are in contact with the T cell receptor, therefore, they are important in determining T-cell recognition of the peptide-DR complex. HLA-DRB1*04-QRRAA alleles have a positively charged P4 pocket, and thus they can only present neutral or negative peptides to T cells. As recently summarized, this could occur in the periphery and allow CD4 T cell recognition of particular antigens presented by HLA-DR, and/or could occur in the thymus, which could lead to selection of a particular repertoire by a positively charged peptide presented by HLA-DR.[14] However, RA is a polygenic disease and other

genes as well as HLA may contribute to disease risk or prognosis.

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