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THE ROLE OF HLA-DRB1*04 ALLELES AND THEIR ASSOCIATION WITH HLA-DQB GENES IN GENETIC SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS IN HUNGARIAN PATIENTS*

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We investigated the HLA-DRB, and DQB polymorphism and haplotypes in RA subjects of Hungarian origin by PCR typing using sequence-specific primers. Molecular subtyping of HLA-DRB1*04 alleles in RA patients showed strongest association with highest relative risk with DRB1*0404. A significantly decreased frequency of DRB1*0403 was observed in patients compared to controls. A significant number of patients carried DR4 haplotypes on DQB1*0302 (54%) relative to DQB1*0301 which was present on 36% of the haplotypes. When compared to controls, the frequency was higher in the latter allele only. Few unique DRB-DQB haplotypes were observed in Hungarian RA patients. In spite of the fact, that the Hungarian population has been isolated linguistically over centuries, a considerable racial admixture has occurred following immigration and invasions, thus the present study confirms in Hungarian patients with RA, previous findings for RA and HLA in European countries.

Keywords: rheumatoid arthritis, Hungarian population, HLA-DRB alleles, HLA-DQB alleles, PCR-SSP

Introduction

Rheumatoid arthritis (RA) is currently considered as an autoimmune disease in which a pathologic immune response attacks synovial cells, cartilage, and bone resulting in joint destruction and permanent disability. RA is an example of a group of diseases based on common genetic disorders and is characterized by a complex genetic trait. It is unknown how many genetic factors are involved in susceptibility to RA. The best-known susceptibility genes in RA are HLA genes. Available data support the

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concept that HLA molecules are crucial in determining disease progression and disease severity but are not required for disease initiation [1].

RA is associated with particular HLA-DRB1 alleles which share highly conserved amino acid sequences in their third hypervariable region (HVR3), the shared epitope [2]. These alleles include HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408 and *1001 [3]. A contrasting hypothesis has been recently proposed, suggesting that, in general, the DRB1 locus is associated with protection to RA and that the RA-associated DRB1 alleles are not responsible for the primary disease association but merely permissive for the susceptibility conferred by the HLA-DQ alleles with which they are in linkage disequilibrium [4]. DR4 positive haplotypes carry preferentially the DQB1 encoded variants *0301 or *0302.

In general, 75% of Caucasian RA patients are HLA-DR4 positive, compared with a normal background frequency of 30%. The HLA-DRB1*0401 and *0404 are the predominant alleles found in Caucasian RA patients, whereas *0405 is associated with the disease in the Japanese population [5].

The aim of this study was first to investigate the involvement of HLA-DRB1*04 alleles in a Hungarian population with RA and, second, to evaluate the importance of DR and DQ types as markers of RA in Hungarian patients.

Because the major RA-associated DR4 alleles, DRB1*0401 and DRB1*0404, are in strong linkage equilibrium with the DQB1*0301 and DQB1*0302 alleles, our analyses naturally focus on these genes.

Materials and methods

Subjects for the study

We investigated two-hundred-and-nine Hungarian RA patients classified as having RA, according to the 1987 revised criteria of the American College of Rheumatology (ARA) [6]. The patients received medical care at the National Institute of Rheumatology and Physiotherapy in Budapest. The patients were 36 men and 173 women, with a mean age of 50.1 years. Three-hundred-and-seven unrelated healthy blood donors served as controls with the same mean age and ethnic origin as the patients.

Polymerase chain reaction with sequence specific primers

Analysis of PCR-SSP products

Genetic polymorphism of the HLA class II loci including the DRB1, DQB1 genes and DRB1*04 alleles was investigated by the polymerase chain reaction with sequence specific primers (PCR-SSP) method. Genomic DNA was isolated from buffy coats of EDTA anticoagulated blood using a modified salting out method [7]. DNA was resuspended in distilled water at a concentration of 0.1–0.5 μg/ml (DNA concentrations were not measured precisely as the PCR-SSP method works well over a wide range of DNA concentrations). Genomic DNA was amplified for the exon 2 of DRB1 and DQB1 genes by the 30 cycles of PCR using a thermal cycler and Taq DNA polymerase [8]. DR4-positive DNAs were further amplified using the DRB1*0401 to *0424 specific primers derived from exon 2 of the HLA-DRB1 [9]. Then, DNA samples were subjected to electrophoresis in 2% agarose gels, and HLA genotypes were determined on the basis of the obtained PCR pattern.

Statistical analysis

Allele and genotype frequencies were determined by direct counting and a comparison between patients and controls was performed. Hardy-Weinberg equilibrium was calculated by standard method and tested by chi-square goodness of fit or Fisher's exact test for discrete data. The intensity of association was estimated by calculating relative risk (RR) by using Woolf's formula and the modified method described by Haldane. Two-locus associations were determined by direct counting of samples with a particular combination of alleles and significance of these associations was determined by a chi-square test with Yates' correction. The association was considered to be significant when the p value was less than 0.05.

Results

Distribution of HLA-DR genotypes

The most frequent HLA-DRB1*04 allele was identified in 23.40% of the RA patients compared with 15.31% of the controls. Comparisons of HLA-DR alleles in RA and controls showed that HLA-DR4, -DR1 and surprisingly -DR15 were significantly more frequent (23.40%, 14.89% and 12.76%), and HLA-DR14 less frequent in RA (1.06%), but there were no other significant differences.

*Distribution of DRB1*04 alleles*

Table I shows the distribution of DRB1*04 alleles among DR4-positive patients and that in the Hungarian normal population. Ninety-five patients expressed HLA-DRB1*04 on one allele and only 3 expressed DRB1*04 on both alleles. These 3 carried *0401/*0402, *0401/*0404 or *0403/*0404. Nine control subjects were homozygous in DR4 antigen and were typed as *0401/*0401 (3 of 9), *0401/*0407 (3 of 9), *0401/*0402 (2 of 9) or *0401/*0404 (1 of 9). The DRB1*0406, *0409, *0410, *0411, *0413, *04014, *0416–*0424 alleles were absent from the Hungarian control population, the DRB1*0412 and *0415 were absent in patients, but present at low frequency in the control group (2.22%). DRB1*0401 allele was the most common in patients and in controls (46.94%, and 53.33%). DRB1*0404 had an increased frequency in the patients and was significantly associated with RA (20.41%, RR=2.05, $p<0.01$). Eighty-four percent of the DR4-positive patients carried the conserved amino acid sequence in the third hypervariable region (HVR3) of the DR β chain, as compared with 73% of the DR4-positive controls.

Table I

Frequency of DRB1*04 alleles in DR4-positive patients with rheumatoid arthritis (RA) and controls

DRB1*04 alleles	RA (%) n=98	Controls (%) n=90	RR	p<
*0401	46.94	53.33	0.77	0.001
*0402	6.12	6.66	0.91	0.001
*0403	4.08	6.66	0.59	0.5
*0404	20.41	11.11	2.05	**0.01**
*0405	6.12	2.22	2.86	0.9
*0407	6.12	8.88	0.66	0.2
*0408	10.20	6.66	1.59	0.7
*0412	–	2.22	–	–
*0415	–	2.22	–	–

Most frequent alleles in RA are underlined.
n=number of subjects with the allele
RR=relative risk

Distribution of DQB alleles

The distribution of DQB alleles within the controls was as expected on the basic of the DR specificities assigned. However, in RA patients there was an unusual DQB-DRB linkage disequilibrium: DQB1*0501–DRB1*0403, DQB1*0501–DRB1*0404,

DQB1*0201–DRB1*0404. Table II shows heterogeneity of some of the DRB1*04–DQB1* haplotypes in RA patients and controls. Whereas most of the haplotypes had DQB1*03. In patients DRB1*0402, *0403, *0404, *0405 occurred in association with DQB1*0302. On the other hand, DRB1*0401, *0407 were found to be linked with DQB1*0301 and *0302, DRB1*0408 was in associated with DQB1*0301, *0302, 0304.

Table II

DRB1*04 and DQB1* allele combinations in RA patients and controls

DRB1*04 Alleles RA patients	DQB1 Alleles	Haplotype Frequencies	Inclusion (%)	χ^2	p<
*0401	*0301	0.3061	83.33	7.56	0.01
	*0302	0.1632	30.76	15.28	0.001
*0402	*0302	0.0612	11.53	3.16	0.1
*0403	*0302	0.0204	3.84	7.86	0.01
	*0501	0.0204	100	98.01	0.001
*0404	*0302	0.1428	26.92	0.63	0.5
	*0201/0501	0.0408	100	5.47	0.02
	*0401	0.0204	100	27.76	0.001
*0405	*0302	0.0612	11.53	3.16	0.1
*0407	*0301	0.0204	5.55	3.97	0.05
	*0302	0.0408	7.69	3.25	0.1
*0408	*0301	0.0408	11.11	0.57	0.5
	*0302	0.0408	7.69	0.33	0.7
	*0304	0.0204	100	44.56	0.001
Controls					
*0401	*0301	0.1555	70	0.31	0.01
	*0302	0.3777	51.51	8.75	0.01
*0402	*0302	0.0666	9.09	10.13	0.01
*0403	*0302	0.0444	6.06	7.14	0.01
	*0305	0.0222	100	81.16	0.001
*0404	*0302	0.1111	15.15	3.82	0.1
*0405	*0401	0.0222	100	223.23	0.001
*0407	*0301	0.0444	20	2.82	0.1
	*0302	0.0444	6.06	2.49	0.2
*0408	*0302	0.0666	9.09	10.13	0.01
*0412	*0301	0.0222	10	33.45	0.001
*0415	*0302	0.0222	3.03	43.64	0.001

Most frequent haplotypes are underlined.

When we looked for susceptibility caused by a specific haplotype, significant differences were observed in two of the total of haplotypes observed. DRB1*0401-DQB1*0301 was significantly higher in patients than in controls, whereas DRB1*0401–DQB1*0302 occurred more frequently in controls. Fifty-four percent of RA patients had DQB1*0302 as compared to 74% of controls and 36% of RA patients

had DQB1*0301 as compared to 22% of control subjects, although the differences were not significant statistically. DQB1*0305 was only observed in the control group, and DQB1*0304 only in the patient group, respectively.

Discussion

Since the early observations by Stastny [10], it has been clear that some HLA-DRB1 alleles, i.e. DRB1*01, *04 and *1001, or their corresponding haplotypes predispose to RA. All these alleles share the similar motifs QKRAA, QRRAA or RRRAA in their HV3 region [11]. This observation has been the basis of the Shared Epitope (SE) hypothesis. But, the SE hypothesis is not able to explain several observations reported in RA patients. Thus, some authors suggest that the HLA and RA association was the result of DQ and DR allele combinations. In this model DQ predisposes to while some DRB1 alleles protect against RA [12–15].

It appears that in specific racial or ethnic groups, either DR4 or DR1, or both, mediate susceptibility to RA. The Hungarian population is of particular interest, because no association of HLA class II antigen with RA has been reported previously. In addition, since the prehistoric times until the Middle Ages, the small Hungarian nation has been isolated linguistically and genetically in the Carpathian Basin for centuries [1–7]. The aim of the present study was to analyze the relationship between the presence of HLA-DRB1*04 alleles and the occurrence of RA in a group of Hungarian patients. Furthermore, we investigated the role of HLA-DQB polymorphic epitopes in conferring susceptibility to the disease in Hungarian RA patients.

Rheumatoid arthritis affects almost all human populations and has a population prevalence of 1% in Caucasians. In many Caucasian populations, HLA-DR4 is found in approximately 20% to 30% of individuals (15.31% in Hungarians) [16]. Out of the Hungarian RA patients 23.40% had HLA-DR4 antigen. It shows lower frequency relative to other Caucasian populations [17], but significantly higher, than in the control group. In DR4-negative patients, we found positive association of RA with HLA-DR1 and DR15. Conversely, DR6 subtypes as DR13 and DR14 were found negatively associated with RA.

In DR4-positive RA patients a negative association was found with DRB1*0403 and *0407 alleles (Table II) whereas the strongest association with the highest risk occurred with *0404 and *0405. Most of the haplotypes (54%) (Fig. 2) with DRB1*0401, *0402, *0403, *0404, *0405, *0407 and *0408 among patients were associated with DQB1*0302, although it showed higher frequency in controls (74%) (Fig. 1). In addititon, DRB1*0401, *0407 and *0408 were found to be heterogeneous in their association with DQB1*03. The highest relative risk for RA was observed with

haplotype DRB1*0401-DQB1*0301. The involvement of DQB locus is still controversial and our results reinforce this idea. Because of the extremely strong linkage disequilibrium between DQB1 and DRB1 alleles, it is difficult to discriminate their relative importance in RA predisposition.

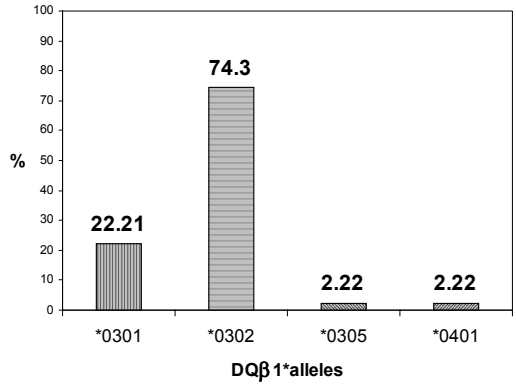

Fig. 1. Distribution of DQB1 alleles in DRB1*04-positive Hungarian controls

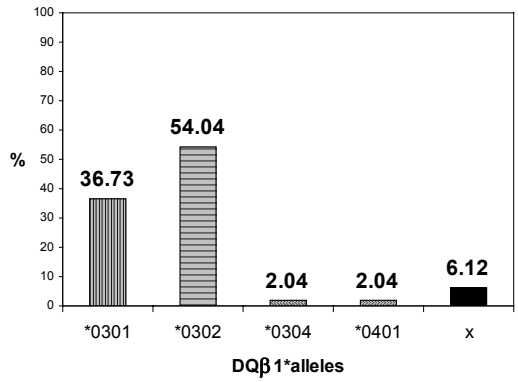

Fig. 2. Distribution of DQB1 alleles in DRB1*04-positive Hungarian patients with RA. X= DQB1*0501, *0201 alleles

The correlation between HLA status and clinical disease extend from mild to severe RA. Generally, our data confirm that a gene dose effect is functional in RA [18]: homozygosity for the HLA-DRB1*04 (especially DRB1*0401/0404) allele appeared to be the strongest predictor for progression of the disease to rheumatoid vasculitis. Thus,

the diagnostic and prognostic values of HLA-DRB1 *04 markers in RA are important in clinical practice.

In the present study, we have analysed the distribution of HLA-DRB and -DQB alleles in a Hungarian RA patients compared to controls from the same region and have made a comparison between the HLA-related alleles of Hungarian patients and other Europeans. We observed some different HLA genetic constitutions between Hungarians and other Europeans (considerably lower frequency of HLA-DR4 allele in Hungarian patients vs in other populations, low frequency of RA-associated DR10 allele and increased frequency of DR15). Nevertheless, the HLA-DRB1*0404 allele, which is classically associated with RA in Caucasians, exhibited statistically significant increase in our patients, too. When DR4 subtypes were studied in association with DQB1 only one combination (HLA-DRB1*0401-DQB1*0301) was associated with the disease risk in opposition with DRB1*0401-DQB1*0302 which showed a striking association in other populations. The present study, in fact, confirms previous findings for RA and HLA in European countries, but it should be kept in mind that together with the genetic background of this population, different environmental factors could possibly determine the HLA alleles finally implicated in RA disease.

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References

1. Wagner U, Kaltenhauser S, Sauer H, Arnold S, Seidel W, Hantzschel H, Kalden JR, Wassmuth R: HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. Arthritis Rheum **40**:341 (1997).
2. Gregersen PK, Silver J, Winchester RJ: The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum **30**:1205-1213 (1987).
3. Winchester R, Dwyer E, Rose S: The genetic basis of rheumatoid arthritis. The shared epitope hypothesis. Rheum Dis Clin North Am **18**:761-766 (1992).
4. Lanchbury JSS, Sakkas LI, Marsh SGE, Bodmer JG, Welsh KI, Panayi GS: HLA-DQB 3.1 allele is a determinant of susceptibility to DR4-associated rheumatoid arthritis. Hum Immunol **26**:59-71 (1989).
5. Winchester R: The molecular basis of susceptibility to rheumatoid arthritis. Adv Immunol **56**:389-466 (1994).
6. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, SC, Haller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum **31**:315-324 (1988).

7. Miler SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res **16**:1215 (1998).
8. Olerup O, Zetterquist H: HLA-DR typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practise including donor-recipient matching in cadaveric transplantations. Tissue Antigens **39**:225-235 (1992).
9. Zetterquist H, Olerup O: Identification of the HLA-DRB1*04, -DRB1*07, and -DRB1*09 alleles by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Hum Immunol **34**:64-74 (1992).
10. Stastny P, Ball EJ, Khan MA, Olsen N, Pincus T, Gao X: HLA-DR4 and other genetic markers in rheumatoid arthritis. Br J Rheumatol **27**:132-138 (1988).
11. Wordsworth BP, Stedeford J, Rosenberg WMC, Bell JI: Limited heterogeneity of the HLA class II contribution to susceptibility to rheumatoid arthritis is suggested by positive associations with HLA-DR4, DR1 and DRw10. Br J Rheumatol **30**:178-180 (1991).
12. Eberhardt K, Fex E, Johnson U, Wollheim FA: Associations of HLA-DRB and -DQB genes with two and five years outcome in rheumatoid arthritis. Ann Rheum Dis **55**:34-39 (1996).
13. Fugger L, Svejgaard A: The HLA-DQ7 and -DQ8 associations in DR4-positive rheumatoid arthritis patients. A combined analysis of data available in the literature. Tissue Antigens **50**:494-500 (1994).
14. Seidl C, Donner H, Petershofen E, Usadel KH, Seifried E, Kaltwasser JP, Badenhoop K: An endogenous retroviral long terminal repeat at the HLA-DQB1 gene locus confers susceptibility to rheumatoid arthritis. Hum Immunol **60**:63-68 (1999).
15. van der Horst-Bruinsma IE, Visser H, Hazes JMW, Breedveld FC, Verduyn W, Schreuder GMTh, de Vries RRP, Zanelli E: HLA-DQ-associated predisposition to and dominant HLA-D-associated protection against rheumatoid arthritis. Hum Immunol **60**:152-158 (1999).
16. Gjertson DW, Geer L, Lee S-H, Kawatw J, Sutrisno R: Population studies. Petri IB, Varga É, Endreffy E, Samu G, Szekeres É: Caucasian Hungarian normal; in Terasaki PI, Gjertson DW (eds): HLA 1997, UCLA Tissue Typing Laboratory, Los Angeles, 1997, pp 174-425.
17. Gjertson DW, Geer L, Lee S-H, Locke A, Tsai E, Lei C-P, Yeh C: Population studies; in Gjertson DW, Terasaki PI (eds): HLA 1998, American Society for Histocompatibility and Immunogenetics, Lenexa, 1998, pp 104-326.
18. Nepom GT, Nepom BS: Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. Rheum Dis Clin North Am **18**:785 (1992).