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The Protective Effect of HLA-DRB1 and HLA-DQB1 Alleles to ACPA-Positive and RF-Positive RA in Albanian Population

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Abstract

BACKGOUND: The prevalence of rheumatoid arthritis (RA) and its specific autoantibodies varies in different populations. This variability depends on the genetic polymorphism of the immune response genes among which the HLA system plays a major role.

AIM: We conducted a preliminary study of the distribution of HLA-DRB1 and HLA-DQB1 first field level alleles in a sample of 100 Albanian patients with RA.

METHODS: In this context, we studied the HLA-DRB1 and HLA-DQB1 first-level allele frequencies in 100 Albanian patients with RA and considering their rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) serologic subgroups. We compared them with the respective frequencies in a population of 191 Albanian individuals without known pathology.

RESULTS: No differences were found between the controls and the RA patient group, but three statistically significant differences were found: an increase in DRB1*04 among ACPA-positive, RF-positive and ACPA-positive/RF-positive patients, a significant decrease in DRB1*11 among ACPA-positive/RF-positive, and also a decrease in DRB1*13 among RF-positive patient subgroups. The frequencies of DRB1 allotypes in ACPA+ and RF+ patients compared to their counterpart ACPA− and RF−patient subgroups showed the predisposing effect of HLA-DRB1*04 for ACPA and RF seropositivity (p, respectively, 0.0008 and 0.0017) and the protective role of HLA-DRB1*11 for ACPA and RF positivity (p, respectively, 0.007 and 0.02). The same protective role from the RF positivity is also found with the HLA-DRB1*13 alleles (p = 0.007). As far as the DQB1 locus is concerned, a protective association has been found between the HLA-DQB1*06 alleles with both RF+ and RF+ ACPA+ positivity (p, respectively, 0.05 and 0.04) when comparing the control group with the respective RA patient subgroups. In ACPA+ and RF+ patients compared to the ACPA− and RF− patient subgroups, the only detected difference was between RF (+) and RF (−) patients (p =0.04).

CONCLUSION: The relatively low frequencies of DRB1*04 and high (DRB1*11 and DRB1*13) in the Albanian population might explain the rather low positivity rate of ACPA and RF antibodies among the Albanian RA patients. Our study demonstrates that DRB1*11, DRB1*13 and DQB1*06 may be negatively associated with RA. Conversely, DRB1*04 may confer susceptibility to RA in Albanian population.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that is reported to have a worldwide prevalence between 0.5% and 1 % of the population [1], [2]. This prevalence varies in different populations, presumably in the function of a complex interplay between the environmental factors related to specific geographical areas and the polymorphism of the immune response genes of the populations living in these areas [3], [4]. The HLA class II genes contribute to at least one-third of the total RA genetic susceptibility [5], [6], and their associations with the RA have been an important subject of study concerning pathogenesis and especially the autoantibody production [7]. Specific autoantibodies such as rheumatoid factor (RF) and the anti-citrullinated peptide antibodies (ACPA) constitute important markers for RA diagnosis [8].

They may be present years before the disease onset [9], [10] and are usually associated with a more severe disease progression [11], [12]. RF, a classical RA autoantibody, is not very specific for this disease as it may be present in other autoimmune disorders as well as in healthy individuals [13]. In contrast, ACPA has shown a higher specificity for this disease, and they seem to play a pivotal role in the pathogenesis of RA [14], [15], [16]. Recent research has established a strong association between the ACPA positivity and the presence of the so defined “shared epitopes” (SE) in HLA-DRB1 alleles [17], [18]. [19].

Since the frequency distribution of these alleles varies in different populations, it is of interest to assess these associations in distinct populations. In this context, we conducted a preliminary study of the distribution of HLA-DRB1 and HLA-DQB1 first field level alleles in a sample of 100 Albanian patients with RA. Taking into consideration the specific autoantibody

subgroups in these patients, we compared them with a control sample of 191 Albanian individuals without known pathology. Furthermore, we decided to look for the presence of any eventual relationship between the production of these autoantibodies and the distribution of the so-called predisposing and protective HLA-DRB1 alleles among different European populations, as well as with RA prevalence in these populations.

Materials and Methods

This included 100 consecutive patients with established RA diagnosis who were sent to our laboratory for serologic ACPA and RF testing. The RA diagnosis was defined as following the 1987 ACR/EULAR Rheumatoid Arthritis Classification Criteria [20]. The presence of RA clinical data was checked in all patients' files, including their specific autoantibodies, during all the disease courses. The control sample consisted of 191 Albanian individuals without known disease, mainly blood donors.

HLA-DRB1 and HLA-DQB1 genotypes, defined at the first field level, were determined using molecular biology methods. The DNA was extracted from the blood buffy coat using QIAamp@ DNA Blood Mini kit (Qiagen Inc, Valencia, CA, USA). HLA genotyping was carried out with a generic SSP method (One Lambda Inc, Canoga Park, CA, USA), and in case of ambiguities or for confirmation purposes, it was followed by a SSOP reverse line method (Innogenetics, Gent, Belgium) or a bead-based Luminex method (Tepnel Lifecodes, Stamford, CT, USA). The term "allele" or "allotype" used throughout this article in reference to our results implies the first field as well as the second field level alleles. The following autoantibodies were studied among the RA patients: Serum levels of ACPA were measured using an anticyclic citrullinated peptide high-sensitive IgG ELISA method (Orgentec, Mainz, Germany). This kit uses mutated citrullinated vimentin as antigen [21], [22]. RF screen (IgG, IgM, and IgA autoantibodies anti-human IgG Fc fragment) was also measured using a commercial ELISA method (Orgentec, Mainz, Germany). The patients were considered positive for ACPA or RF antibodies if their ELISA testing results were superior to the respective cut-off values recommended from the kit manufacturer (>20 U/mL for ACPA and >25 U/mL for RF).

Statistical methods

The allele frequencies and sample summaries for control and RA patient samples were calculated using the Gene(rate) programs [23]. The differences between allele frequencies in both groups were assessed using the R statistical software [24]. The odds ratio calculation was carried out with Med-Calc software (MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium). The correlations between allele frequencies and RA prevalence rates among European populations were assessed using the nonparametric coefficient of Spearman and its significance was tested with R [24], [25].

Results

The general characteristics of the RA patient population, including specific autoantibody positivity data, are presented in Table 1.

Table 1: Baseline characteristics and specific autoantibody positivity rates in the 100 RA patients studied

Characteristics of RA patients	Number (%)
Age in years (mean ± SD)	49.9 ± 10.4
Disease duration in months (mean ± SD)	54.9 ± 48.1
Female patients	83 (83)
ACPA-positive	46 (46)
RF-positive	48 (48)
ACPA-positive/RF-positive	35 (35)

ACPA: Anticitrullinated peptide antibodies, RF: Rheumatoid factor, SD: Standard deviation.

No significant variations in the specific autoantibody levels have been detected among the RA patients throughout their disease course. The DRB1 and DQB1 allele frequencies in all 100 RA patients compared to the control group are presented in Table 2.

Table 2: HLA-DRB1 and HLA-DQB1 allele frequency distribution in normal controls and RA patient group

HLA alleles DR-B1	Normal controls	RA patients
DRB1		
01	0.0491	0.0630
03	0.0538	0.0550
04	0.0807	0.0985
07	0.0644	0.0300
08	0.0278	0.0350
10	0.0209	0.0200
11	0.2621	0.2319
12	0.0288	0.0100
13	0.1248	0.0954
14	0.0755	0.0600
15	0.0759	0.0985
16	0.1283	0.1761
Blank	0.0079	0.0210
DQB1		
02	0.1125	0.0800
03	0.3821	0.3631
04	0.0314	0.0350
05	0.2748	0.3229
06	0.1989	0.1933
, Blank	0.0002	0.0056

For the DRB1 locus, the test yields a p value of 0.071, and the controls as well as the RA patient group are not significantly different. At the DQB1 locus, there is a significant difference between RA and controls (p = 0.004). The DRB1 and DQB1 allele frequencies in the control group were compared with the respective frequencies of the following RA patient serologic subgroups: ACPA-positive, RF-positive, and ACPA-positive/RF-positive. The results with the corresponding calculated odds ratios, 95% confidence intervals of the odds ratios, and the respective p values are presented in Table 3. A statistically significant difference was found for the DRB1*04 between the controls and

Table 3: HLA-DRB1 and HLA-DQB1 allele frequency rates of the RA patient serologic subgroups compared to the normal controls and of the ACPA and RF positive RA patients

Normal controls (N = 191)	No of alleles (%)	RA patients subgroups					
		ACPA-positive (N = 46)		FR-positive (N = 48)		ACPA-psitive/FR-positive (N = 35)	
		No of alleles (%)	OR (CI 95%) p value	No of alleles (%)	OR (CI 95%) p value	No of alleles (%)	OR (CI 95%) p value
DRB1							
01	18 (9.4%)	8 (17.4%)	2.0 (0.8–5.0) 0.12	8 (16.6%)	1.9 (0.8–4.7) 0.15	7 (20%)	2.4 (0.9–6.3) 0.07*
03	18 (9.4%)	5 (10.8%)	1.2 (0.4–3.3) 0.8	5 (10.4%)	1.1 (0.4–3.2) 0.8	5 (14.3%)	1.6 (0.5–4.6) 0.4
04	30 (15.7%)	18 (39%)	3.4 (1.7-7.0) 0.0006**	16 (33.3%)	2.7 (1.3–5.5) 0.0006**	15 (43%)	4.0 (1.8–8.7) 0.0004**
07	23 (12%)	2 (4.3%)	0.3 (0.1–1.5) 0.14	2 (4.2%)	0.3 (0.1–1.4) 0.12	2 (5.7%)	0.4 (0.1–2) 0.3
08	10 (5.3%)	4 (8.7%)	1.7 (0.5–5.8) 0.4	3 (6.2%)	1.2 (0.3–4.6) 0.8	3 (8.6%)	1.7 (0.4–6.5) 0.4
10	8 (4.2%)	2 (4.3%)	1.0 (0.2–5.2) 0.9	2 (4.2%)	1.0 (0.2–4.8) 1.0	1 (2.8%)	0.7 (0.1–5.5) 0.7
11	86 (45%)	14 (30.4%)	0.5 (0.3–1.0) 0.07*	16 (33.3%)	0.6 (0.3–1.3) 0.2	8 (23%)	0.4 (0.2–0.8) 0.01*
12	11 (5.8%)	0 (0%)	0.2 (0.01–2.9) 0.2	0 (0%)	0.2 (0.01–2.8) 0.2	0 (0%)	0.2 (0.01–2.8) 0.3
13	45 (23.5%)	6 (13%)	0.5 (0.2–1.2) 0.12	3 (6.3%)	0.2 (0.06–0.7) 0.01*	2 (5.7%)	0.2 (0.04–0.9) 0.03*
14	27 (14.1%)	5 (10.8%)	0.7 (0.3–2) 0.6	8 (16.7%)	1.2 (0.5–3) 0.7	5 (14.3%)	1.0 (0.4–2.8) 1.0
15	29 (15.2%)	9 (19.6%)	1.4 (0.6–3) 0.5	9 (18.7%)	1.3 (0.6–3) 0.5	6 (17.1%)	1.2 (0.4–3) 0.8
16	48 (25.1%)	15 (32.6%)	1.4 (0.7–3) 0.3	18 (37.5%)	1.8 (0.9–3.5) 0.08	11 (31.4)	1.4 (0.6–3) 0.4
DQB1							
02	39 (20.4%)	17 (37%)	0.6 (0.2–1.5) 0.3	6 (12.5%)	0.6 (0.2–1.4) 0.2	6 (17.1%)	0.8 (0.3–2) 0.6
03	121 (63.4%)	29 (63%)	1.0 (0.5-1.9) 1.0	29 (60.4%)	0.9 (0.5–1.7) 0.7	20 (57%)	0.8 (0.4–1.6) 0.5
04	12 (6.3%)	7 (15.2%)	1.4 (0.4–4.6) 0.5	3 (6.2%)	1.0 (0.3–3.7) 1.0	3 (8.6%)	1.4 (0.4–5.2) 0.6
05	91 (47.6%)	26 (56.5%)	1.4 (0.7–2.7) 0.3	29 (60.4%)	1.7 (0.9–3.2) 0.12	21 (60%)	1.6 (0.8–3.4) 0.2
06	72 (37.7%)	13 (28.3%)	0.6 (0.3–1.3) 0.2	11 (23%)	0.5 (0.2–1.0) 0.05*	7 (20%)	0.4 (0.2–1) 0.04*

OR: Odds ratio; 95% CI: Confidence interval; *Statistically significant; **Statistically significant after Bonferroni adjustment (p-value×number of alleles) <0.05.

ACPA-positive (p = 0.0006), RF-positive (p = 0.0006), and ACPA-positive/RF-positive (p = 0.0004) patient subgroups as shown in Table 3. A similar difference was observed for DRB1*11 between the controls and ACPA-positive/RF-positive (p = 0.01) and also for DRB1*13 between the controls and RF-positive (p = 0.01) as well as ACPA-positive/RF-positive (p = 0.03) patient subgroups. The frequencies of DRB1 allotypes in ACPA-positive and RF-positive patients compared to their counterpart ACPA-negative and RF-negative patient subgroups are also shown in Tables 4 and 5. Here, again we find the predisposing effect of HLA-DRB1*04 for ACPA and RF seropositivity (p, respectively, 0.0008 and 0.0017) and the protective role of HLA-DRB1*11 for ACPA and RF positivity (p, respectively, 0.007 and 0.02). The same protective role from the RF positivity is also found with the HLA-DRB1*13 alleles (p = 0.007). As far as the DQB1 locus is concerned, a protective association has been found between the HLADQB1*06 alleles with both RF-positive and RF-positive/ACPA-positive (p, respectively, 0.05 and 0.04) when comparing the control group with the respective RA patient subgroups as shown in Table 3. In ACPA-positive and RF-positive patients compared to the ACPA-negative and RF-negative patient subgroups, the only detected difference was between RF-positive and RF-negative patients (p = 0.04) as shown in Table 5.

Discussion

We have found in the RA Albanian patients, a strong increase in the HLA-DRB1*04 allele frequency among ACPA+ and RF+ patients compared with the control population and with their counterpart ACPA− and RF− patients. The same association trend, but

Table 4: Frequency of HLA-DRB1 and -DQB1 alleles in ACPA-positive individuals compared to ACPA-negative individuals

HLA-DRB1 Alleles												
	01	03	04	07	08	10	11	12	13	14	15	16
OR	2.63	0.97	34.0	0.6	1.6	1.2	0.32	0.2	0.5	0.7	1.0	0.96
CI 95%	0.7–9.4	0.3–3.4	4.3–0.2.7	0.1–3.3	0.34–8	0.2–8.7	0.14–0.7	0.01–4.8	0.2–1.5	0.2–2.3	0.4–3.0	0.4–2.2
p value	0.14	0.96	0.0008**	0.5	0.54	0.9	0.007*	0.34	0.23	0.6	0.9	0.9
HLA-DQB1 Alleles												
	02	03	04	05	06							
OR	0.6	1.0	1.6	1.3	0.6							
CI 95%	0.2–1.7	0.5–2.4	0.34–7.6	0.6–2.9	0.3–1.6							
p value	0.3	0.8	0.5	0.5	0.35							

OR: Odds ratio; 95% CI: Confidence interval; *Statistically significant; **Statistically significant after Bonferroni adjustment (p-value×number of alleles) <0.05.

Table 5: Frequency of HLA-DRB1 and -DQB1 alleles in RF-positive individuals compared to RF-negative individuals

HLA-DRB1 Alleles												
	01	03	04	07	08	10	11	12	13	14	15	16
OR	2.4	0.9	8.1	0.5	0.8	1.0	0.4	0.2	0.2	1.9	1.0	1.5
CI 95%	0.7–8.5	0.3–3.1	2.2–30	0.1–3.0	0.2–3.8	0.2–8.0	0.2–0.9	0.01–4.4	0.04–0.6	0.6–6.2	0.4–2.6	0.6–3.4
p value	0.17	0.85	0.0017**	0.5	0.8	0.9	0.02*	0.3	0.007*	0.3	0.9	0.4
HLA-DQB1 Alleles												
	02	03	04	05	06							
OR	0.5	0.9	0.8	1.8	0.4							
CI 95%	0.2–1.6	0.4–2.0	0.17–3.8	0.8–4.0	0.2–1.0							
p value	0.3	0.75	0.8	0.2	0.04*							

OR: Odds ratio; 95% CI: Confidence interval; *Statistically significant; **Statistically significant after Bonferroni adjustment (p-value×number of alleles) <0.05.

without reaching a statistical significance, has been observed between DRB1*01 and double-positive ACPA RF patients. RA protective (RAP) alleles such as HLA-DRB1*11:02, 11:03, 13:01, 13:02, or 01:03 have been reported to play a protective effect from the ACPA+ and RF+ RA disease [26], [27]. In our RA Albanian patient population, we observed similar findings with the HLA-DRB1*11 and DRB1*13 alleles which seem to be associated with a protective effect from both ACPA and RF positivity. A similar protective role is also observed with HLA-DQB1*06, but apparently, this is due to the strong linkage of this allele with HLA-DRB1*13 [28].

The HLA-DRB1*11 genotype profile detected in our RA patients belongs to the HLA-DRB1*11:01/04 but not to the HLA-DRB1*11:02/03 allele groups as reported in the literature [7]. The alleles HLA-DRB1*01:03 and HLA-DRB1*04:02 have also been reported to play a protective role in RA. It is interesting to mention that the predisposing HLA-DRB1*01 and HLA-DRB1*04 alleles if calculated together are found in 29.0 % of our Albanian RA patients, whereas the corresponding result for the HLA-DRB1*11 and HLA-DRB1*13 alleles is 56.0%. The high frequency of these protective alleles, combined with the low frequency of the predisposing alleles in this population, can probably explain the rather low positivity rates of ACPA and RF antibodies among Albanian RA patients (46% and 48%, respectively).

We analyzed different alleles for the investigated groups by the Bonferroni corrected p-value and we found smaller number of significant associations [29]. The comparisons with the published associations were not possible because not all published results are with Bonferroni corrected values.

Conclusion

We can confirm that the two auto-antibodies ACPA and RF, although with a lower sensitivity than other peoples of Western or Northern Europe, can provide a very valuable support for the diagnosis of RA in the Albanian population. These findings significantly relate to the varying genetic background as well as serology between different populations.

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