Rheumatology International (2019) 39:2147–2157
https://doi.org/10.1007/s00296-019-04429-y

Rheumatology
INTERNATIONAL

GENES AND DISEASE

Association of biomarkers of inflammation and HLA-DRB1 gene locus with risk of developing rheumatoid arthritis in females

Biljana Klimenta[1] · Hilada Nefic[2] · Nenad Prodanovic[3] · Radivoj Jadric[4] · Fatima Hukic[5]

Received: 13 July 2019 / Accepted: 19 August 2019 / Published online: 26 August 2019

Abstract

Rheumatoid arthritis (RA) is an autoimmune disease causing chronic inflammation of the joints. Multiple factors, including *HLA-DRB1* gene variants, influence the susceptibility to RA. The *HLA-DRB1* gene is part of a family of genes called the human leukocyte antigen (HLA) complex. In this study, we compared the inflammatory biomarkers values, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), between patients with RA and healthy control group of females of the Public Institution Health Centre of Sarajevo Canton. In addition, we estimated the frequencies of the *HLA-DRB1* gene variants and their association with the risk for RA development in females. The haematological and biochemical tests were completed on automated analyzers. To assess the association between the *HLA-DRB* genes and the risk of RA in females, low-resolution genotyping of the *HLA-DRB1*, *DRB3, DRB4,* and *DRB5* gene loci was performed by the sequence-specific polymerase chain reaction method (PCR-SSP). ESR and CRP were the most sensitive acute-phase reactants in females with RA and there was a correlation between ESR and CRP values in RA patients. There was significantly positive association between of the *HLA-DRB1*03*, **04*, **08*, **10*, **11,* and **14* variants and elevated values of ESR in RA patients, but negative between *HLA-DRB1*03*, **13* and **15* alleles and elevated CRP values. Furthermore, our results confirm genetic susceptibility to RA in a female population to the members of the *HLA-DRB1*04* and **03* allelic groups, the *DRB1*04/DRB1*04* and *DRB1*03/DRB1*04* genotypes, and the *DRB1*04-DRB4** or *DRB1*03-DRB3** haplotypes, which, therefore, represent risk factors for the development of this disease. According to our results, the *DRB1*01/DRB1*15* and *DRB1*07/DRB1*16* genotypes and the *HLA-DRB5* gene locus represent a protective factor for RA. The presence of specific *HLA-DRB1* gene variants increases the risk of developing RA, while other variants provide protection against disease. Therefore, HLA typing could be helpful in the prediction of RA development and establishing and confirming a definitive diagnosis of autoimmune diseases in some subjects. A strong association with the higher levels of ESR and CRP could be used to establish definitive diagnosis and introduce of early treatment of RA to prevent the occurrence of RA symptoms.

Keywords Rheumatoid arthritis (RA) · C-reactive protein (CRP) · Erythrocyte sedimentation rate (ESR) · *HLA-DRB1* gene

Introduction

Rheumatoid arthritis [RA (OMIM: 180300)] or susceptibility to rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints. RA is characterized by joint swelling, tenderness, and destruction of synovial joints leading to severe disability and premature mortality [1, 2]. Elevation of the acute-phase reactants erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) indicates the maintenance of activity in patients with RA. The precise aetiology of RA has not yet been established. There is no known specific therapy for RA, but treatment can slow the progression of the disease, indicating the importance for earlier diagnosis and effective disease-suppressing therapy to prevent or minimize the occurrence of the symptoms of RA.

The prevalence of RA in Caucasians (North America and Europe) is 0.5–1% of the general population [2–4], but the prevalence varies between 3 and 8% depending on the environmental and genetic risk factors (2–3 times more common in females than in males). Approximately 80% of RA patients develop the disease between 35 and 50 years. The mortality rate of these patients is at least twice as high as in the healthy population.

✉ Hilada Nefic
hnefic@gmail.com

Extended author information available on the last page of the article

Stastny [5] first established that RA was associated with HLA-DRw4, while subsequent studies confirmed an association with the *HLA-DRB1* gene [6] and the strongest association with *HLA-DRB1*04* [7]. This association is observed in many populations; however, in different ethnic groups, a connection has been established between other variants of the *HLA-DRB1* gene locus and RA, which may occur together with *HLA-DRB1*04* or separately. A particular subset of *HLA-DRB1* gene variants (alleles at *DRB1*01*, **04*, and **10* groups), called shared epitope (SE) alleles, is the most important genetic contributor to the risk of developing RA. The genetic variants that are negatively linked to the development of RA are *DRB1*01*, *DRB1*07,* and *DRB1*13*, which are considered to have a protective role in increasing RA.

The *HLA-DRB1* gene is part of a family of genes called the human leukocyte antigen (HLA) complex. The HLA complex is the human version of the major histocompatibility complex (MHC). Researchers have identified at least 2479 different versions of the *HLA-DRB1* gene (the Immuno Polymorphism Database (IPD)—the international ImMunoGeneTics (IMGT); IPD-IMGT/HLA Release 3.36.0, http://www.ebi.ac.uk/imgt/hla/).

The aims of this study were to compare inflammation biomarkers values, the ESR and CRP among the other haematological and biochemical parameters, between patients with RA and a healthy control group of females of the Public Institution Health Centre of Sarajevo Canton (Bosnia and Herzegovina) and estimate the frequency of the *HLA-DRB1* gene variants, the possible contributions of the predisposing and protective gene variants for the risk of developing RA in females using a low-resolution (two-digit) polymerase chain reaction with sequence-specific primers (PCR-SSP) technique.

Patients and methods

Study groups

The peripheral blood samples were collected from 83 subjects with a diagnosis of RA and 164 healthy donors of the Hospital Novo Sarajevo (the Public Institution Health Centre of Sarajevo Canton, Bosnia and Herzegovina) without a diagnosis of RA and inflammation who came for a medical check-up between April 2015 and June 2018. A total of 98.8% of the participants were females. When collecting samples, ethical principles of research were considered. Data relating to the age and sex were reported by donors, and all the participants included in this analysis signed written informed consent. The study was approved by the Research Ethics Committee of the Public Institution Health Centre of Sarajevo Canton, Bosnia and Herzegovina (01-08-5080-2/16 from 25.08.2016).

Haematological and biochemical analyses

A cross-sectional study was performed on 80 RA patients and 82 healthy control subjects. All haematological and biochemical analyses were carried out in the Department of Laboratory Diagnostics of the Hospital Novo Sarajevo. The haematology tests included the CBC (complete blood count) with measurements of the red blood cells (RBC), the white blood cells (WBC) and the platelets (PLT) counts, hemoglobin (HGB), and hematocrit (HCT). We additionally investigated the erythrocyte constants mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), and red cell distribution width standard deviation (RDW-SD) along with the platelet constant mean platelet volume (MPV). The differential blood count (DBC) included the number of neutrophils (NE), eosinophils (EO), basophils (BA), monocytes (MO), and lymphocytes (LY). The ESR and CRP levels were also analysed. Moreover, the neutrophil and lymphocyte ratio (NLR) and the platelets and lymphocyte ratio (PLR) were determined.

The samples of peripheral blood were taken in accordance with good laboratory practice standards. For general haematological examination, 3 mL of blood was collected in a vacuum tube containing anticoagulant ethylenediaminetetraacetic acid (EDTA). Haematological parameters were measured on the automated Beckman Coulter DxH 800 Haematology Analyzer. For ESR, 1.8 mL of blood was collected into a vacuum tube with sodium citrate and placed in a 150 mm graduated pipette. The samples for analysis of CRP were collected in a vacuum tube, centrifuged for 10 min at 3500 rpm, and separated from the serum. The CRP was measured by a standard CRP test on the Roche/Hitachi Cobas C311 Chemistry Analyzer (Boehringer Mannheim, Germany).

HLA genotyping

The molecular analysis was carried out on a group of 83 patients with a diagnosis of RA ($n = 83$; $2n = 166$) and 82 control subjects ($n = 82$; $2n = 164$) in the Laboratory of Genetics in the Department of Biology (Faculty of Science of the University Sarajevo, Bosnia and Herzegovina). The control group included healthy subjects who did not have a diagnosis of RA.

Genomic deoxyribonucleic acid (DNA), previously isolated from whole blood collected in EDTA using the salting out procedure [8], was subjected to PCR amplification. For the amplification of the *HLA-DRB1* gene locus, the HLA-Ready Gene Class II test system (HLA-Ready Gene DR,

Inno-Train, Germany) was used according to the manufacturer's instructions. This system enables low-resolution typing of *HLA-DRB1*, *DRB3*, *DRB4,* and *DRB5* gene loci.

Each sample was genotyped by a set of 24 PCRs. PCR samples in which the primer binds to its specific target have a specific amplification following PCR, while samples without this primer-specific binding do not. The positive control amplicon (HGH, human growth hormone) in each reaction tube is present in two different sizes (430 bp and 800 bp), with the exception of the first tube containing a negative control (NC).

The DNA samples (approximately 50 ng/μL of concentration) were added to the master mix containing distilled water, Ready PCR (deoxyribonucleotide triphosphates—dNTPs, PCR buffer, cresol red and glycerine) and Taq polymerase (5 U/μL, Axi-Taq DNA Polymerase, Inno-Train, Germany). The program has been validated with a thermocycler Eppendorf Mastercycler gradient (Hamburg, Germany). The PCRs were carried out in 10 μL volumes. Samples were first denatured at 96 °C for 2 min, followed by 10 cycles of 96 °C for 15 s, 65 °C for 60 s, 20 cycles of 96 °C for 15 s, 61 °C for 50 s, and 72 °C for 30 s. Evaluation of the results was performed by 2% agarose gel electrophoresis in Tris/Borate/EDTA (TBE) buffer at 70 V for 15–20 min. The bands were visualized under ultraviolet light (UV) by intercalating ethidium bromide (EtBr, 0.7 μg/μL). Interpretation of the results was performed using kit manufacturer's data sheets.

Statistical analysis

Results were expressed as median and range of values (minimum and maximum), the number, mean ± standard deviation (mean ± SD), decimal or percentage. Kolmogorov–Smirnov test for results distribution and Student's *t* test were performed to determine the statistically significant differences in haematological and biochemical biomarkers between the patients with RA and a healthy control group of females using the NCSS 2019 Statistical Software version 19.0.2 with a significance level of 0.05. The correlation analysis between observed parameters and ESR or CRP between groups was performed using Pearson's coefficient of correlation *r* and 95% confidence intervals (95% CI). Results with values ≤ 0.05 were considered statistically significant.

The OpenEpi computer program version 3.01 (Open Source Epidemiologic Statistics for Public Health) was used to evaluate the statistical significance of the differences in the frequency of genetic variants between the group of RA patients and the control group by Fisher's exact test and 2 × 2 contingency tables. A two-tailed or one-tailed *P* value less than 0.05 was considered significant in all tests. Statistical significance was improved by the Mantel–Haenszel Chi-squared (χ^2) test because of the small sample size. The strength of the association between the presence of a particular genetic variant within the *HLA-DRB1* gene locus and the occurrence of the disease was estimated by calculating the odds ratio (OR) and 95% CI for the OR value. An OR < 1 indicates protection, whereas an OR > 1 indicates an increased risk.

The software package PowerMarker, version 3.25, was used to analyse parameters for polymorphism estimation within the *HLA-DRB1* gene locus, heterozygosity (*H*), gene diversity and polymorphic information content (PIC), as well as to assess whether the population was in Hardy–Weinberg equilibrium (HWE) among the patients with RA and control group subjects.

Results

The haematological and biochemical analyses

The mean age of the control group subjects was 46.50 ± 8.52 years (between 30 and 59 years of age), and the mean age of females with RA was 52.51 ± 7.26 years (between 34 and 60 years of age).

All values of the haematological–biochemical parameters of the control group were within the normal range for females (Table 1). The values of haematological parameters in patients with RA were also normal except for ESR and CRP. The values for ESR were elevated in RA patients (median 21, range 1–88 and average 23.94 ± 16.54) in comparison with the normal rate and with the control (median 5, range 2–9 and average 5.02 ± 1.92). Similarly, the CRP in RA patients (median 3, range 1–70 and average 9.01 ± 13.44) was considerably higher than normal CRP values and higher that in the control group (median 2, range 0–4 and average 1.98 ± 1.03).

To determine the statistical significance of the differences in haematological–biochemical parameters between patients with RA and a healthy control group of females, we used the Kolmogorov–Smirnov test. The values of WBC ($P = 0.0256$), MCV ($P = 0.0212$), MCH ($P = 0.0045$), RDW ($P < 0.0001$), RDW-SD ($P < 0.0001$), MO ($P = 0.0048$), and EO ($P = 0.0225$) were significantly increased in RA patients (Table 1). Similarly, the values of the ESR ($P < 0.0001$) and CRP ($P < 0.0001$) were statistically increased in RA patients compared to those in the control group. According to Student's *t* test, the relative risk related to ESR (95% CI 15.30–22.54; $t = 10.31$; $P < 0.0001$) was 2.7 times higher than that related to CRP (95% CI 4.09–9.97; $t = 4.72$; $P < 0.0001$). The values of NE and BA were also increased in RA patients but not significantly. The values of RBC ($P = 0.0216$) and HCT ($P = 0.0123$) were significantly reduced in patients with RA compared with the control, while the values of HGB, MCHC, PLT, MPV, and LY were reduced but not significantly. The NLR (median 1.86, range 0.26–5.92, and average 2.12 ± 1.17)

Table 1 Frequencies of the haematological and biochemical parameters and their distribution in the control group and the group of females with RA using Kolmogorov–Smirnov test

Parameters	Normal values for women	Control Mean ± SD	RA patients Mean ± SD	Control Median (min–max)	RA patients Median (min–max)	Largest difference Criterion value	*P* value
WBC (10^3/μL)	3.4–9.7	7.00 ± 1.38	7.83 ± 2.47	**6.85** (4.1–9.9)	**7.5** (3.5–16)	0.2256	**0.0256**
RBC (10^6/μL)	3.86–5.08	4.73 ± 0.32	4.59 ± 0.33	**4.74** (4–5.48)	**4.58** (3.87–5.37)	0.2308	**0.0216**
HGB (g/dL)	11.9–15.7	13.52 ± 0.96	13.16 ± 1.21	**13.3** (11.6–15.8)	**13.2** (10–16.6)	0.1530	0.2634
HCT (%)	35.6–47.0	42.37 ± 2.75	41.14 ± 3.41	**42.1** (36.7–49.4)	**40.9** (33–50.5)	0.2451	**0.0123**
MCV (fL)	83.0–97.2	89.64 ± 3.68	89.91 ± 6.07	**89.4** (80.9–98.6)	**91.2** (70.7–103.3)	0.2314	**0.0212**
MCH (pg)	27.4–33.9	28.65 ± 1.48	28.75 ± 2.25	**28.7** (24.3–32.8)	**29.4** (21.3–32.8)	0.2686	**0.0045**
MCHC (g/dL)	32.0–34.5	32.00 ± 0.61	31.94 ± 0.70	**32.1** (30–33.8)	**32** (29–33.5)	0.1814	0.1171
RDW (%)	9.0–15.0	13.51 ± 0.85	14.63 ± 1.64	**13.4** (12–18.2)	**14.3** (12.1–20.8)	0.4037	**0.0000**
RDW-SD (fL)	36.5–45.9	41.90 ± 2.46	44.98 ± 3.53	**41.6** (37.6–51.6)	**44.6** (38.5–57.8)	0.3924	**0.0000**
PLT (10^3/μL)	158–424	248.09 ± 47.9	255.38 ± 72.33	**244** (163–408)	**234** (116–471)	0.1540	0.2561
MPV (fL)	6.8–10.4	8.93 ± 0.86	8.95 ± 1.02	**8.9** (6.9–10.6)	**8.8** (6.6–11.2)	0.1076	0.6743
NE (10^3/μL)	1.1–7.0	3.96 ± 1.09	4.54 ± 1.96	**3.8** (2.3–6.4)	**4.2** (1.1–10.9)	0.2070	0.0514
LY (10^3/μL)	0.7–4.5	2.28 ± 0.58	2.30 ± 0.74	**2.3** (1.2–3.5)	**2.2** (1.1–4.9)	0.0750	0.9578
MO (10^3/μL)	0.0–1.2	0.53 ± 0.14	0.65 ± 0.24	**0.5** (0.1–0.9)	**0.6** (0.2–1.6)	0.2671	**0.0048**
EO (10^3/μL)	0.0–0.7	0.18 ± 0.12	0.26 ± 0.20	**0.2** (0–0.7)	**0.2** (0–0.9)	0.2296	**0.0225**
BA (10^3/μL)	0.0–0.2	0.05 ± 0.05	0.08 ± 0.06	**0.1** (0–0.1)	**0.1** (0–0.2)	0.2009	0.0629
ESR (mm/h)	0.0–10.0	5.02 ± 1.92	23.94 ± 16.54	**5** (2–9)	**21** (1–88)	0.8000	**0.0000**
CRP (mg/L)	0.0–5.0	1.98 ± 1.03	9.01 ± 13.44	**2** (0–4)	**3** (1–70)	0.4018	**0.0000**
NLR		1.85 ± 0.68	2.12 ± 1.17	**1.65** (0.79–3.76)	**1.86** (0.26–5.92)	0.1158	0.5961
PLR		115.47 ± 34.48	120.75 ± 48.23	**109.27** (56–194.38)	**107.56** (50.28–253.64)	0.1030	0.7333

P values ≤ 0.05 were significant (bold)

and the PLR (median 107.56, range 50.28–253.64, and average 120.75 ± 48.23) were increased in patients with RA compared with healthy controls, but these differences were not statistically significant (Table 1).

Using the Pearson's coefficient of correlation, no statistically significant correlation was found between the analysed haematological parameters and ESR or CRP in the control group. However, in patients with RA, a statistically significant negative correlation between ESR values and HGB ($P = 0.04$) was found, but there was a positive association between ESR and RDW ($P = 0.0013$), RDW-SD ($P = 0.0001$), PLT ($P = 0.0043$), or NE ($P = 0.0055$) (Table 2). There was a statistically significant positive correlation between CRP values on one side and WBC ($P = 0.009$), RDW-SD ($P = 0.0351$), PLT ($P = 0.0041$) or NE ($P = 0.0001$) values on the other side. The correlation between ESR and CRP in patients with RA was also established. With the ESR value increased, the CRP value of CRP ($P < 0.0001$) also increased (Table 2).

The frequency of allele groups and genotypes of the *HLA-DRB1* gene locus in patients with RA and control females

The mean age of the control group subjects was 40.52 ± 14.06 years (range 21–68 years), and the age of patients with RA was 52.89 ± 7.63 years (age range between 34 and 77).

Among the subjects of the control group, 13 different allelic groups of the *HLA-DRB1* gene locus (*DRB1*01*, **03*, **04*, **07*, **08*, **09*, **10*, **11*, **12*, **13*, **14*, **15,* and **16*) were present all 13. The most common allelic groups among the control subjects were *DRB1*15* (15.2%), **01* (14.6%), and **16* (12.8%), followed by **07* and **13* (11%) and **11* (9.8%) allelic groups. The other allele groups included **04*, **03*, **14*, **08*, **10*, **12,* and **09* are less represented among subjects in the control group (Table 3). The most prevalent genotypes in the control subjects were *DRB1*01/*15* (8.5%), followed by *DRB1*01/*04*, *DRB1*11/*16*, *DRB1*13/*15,* and *DRB1*07/*16* (6.1%), *DRB1*01/*07* (4.9%), *DRB1*07/*13*, *DRB1*03/*16,* and *DRB1*15/*15* (3.7%). The remaining 34 different genotypes had a frequency under 2.5% (Table 4).

Among patients with RA, of the 13 allele groups of the *HLA-DRB1* gene, the most common were *DRB1*04* (15.7%), **03,* and **15* (12.6%) after that *DRB1*01* (11.4%), **11,* and **16* (10.2%). The other allele groups were **07*, **13*, **14*, **08*, **10*, **12,* and **09* (Table 3). The most frequent genotypes in RA patients were *DRB1*01/*07* and *DRB1*03/*04* with 6% *DRB1*01/*14*, *DRB1*11/*15,* and *DRB1*04/*04* with 4.8%, respectively. The genotypes *DRB1*07/*13*,

Table 2 Comparison of haematological parameters with ESR and CRP in the group of RA females using Pearson's correlation coefficient (*r*)

Parameters	ESR (mm/h)		CRP (mg/L)	
	r (95% CI)	*P* value	*r* (95% CI)	*P* value
WBC (10^3/μL)	**0.186** (−0.04 to 0.39)	0.0994	**0.290** (0.075 to 0.48)	**0.0090**
RBC (10^6/μL)	**−0.166** (−0.37 to 0.06)	0.1417	**0.062** (−0.16 to 0.28)	0.5879
HGB (g/dL)	**−0.230** (−0.43 to −0.01)	**0.0400**	**0.007** (−0.21 to 0.23)	0.9486
HCT (%)	**−0.206** (−0.41 to 0.01)	0.0672	**0.006** (−0.21 to 0.22)	0.9575
MCV (fL)	**−0.092** (−0.31 to 0.13)	0.4169	**−0.070** (−0.28 to 0.15)	0.5390
MCH (pg)	**−0.127** (−0.34 to 0.10)	0.2609	**−0.060** (−0.28 to 0.16)	0.5963
MCHC (g/dL)	**−0.177** (−0.38 to 0.04)	0.1172	**0.026** (−0.19 to 0.24)	0.8183
RDW (%)	**0.354** (0.15 to 0.53)	**0.0013**	**0.168** (−0.05 to 0.37)	0.1359
RDW-SD (fL)	**0.435** (0.24 to 0.60)	**0.0001**	**0.236** (0.02 to 0.43)	**0.0351**
PLT (10^3/μL)	**0.316** (0.10 to 0.50)	**0.0043**	**0.317** (0.10 to 0.50)	**0.0041**
MPV (fL)	**−0.152** (−0.36 to 0.07)	0.1778	**−0.110** (−0.32 to 0.11)	0.3294
NE (10^3/μL)	**0.308** (0.09 to 0.49)	**0.0055**	**0.419** (0.22 to 0.58)	**0.0001**
LY (10^3/μL)	**−0.159** (−0.37 to 0.06)	0.1578	**−0.183** (−0.39 to 0.04)	0.1043
MO (10^3/μL)	**−0.019** (−0.24 to 0.20)	0.8641	**0.108** (−0.11 to 0.32)	0.3395
EO (10^3/μL)	**0.034** (−0.19 to 0.25)	0.7665	**0.065** (−0.16 to 0.28)	0.5655
BA (10^3/μL)	**−0.101** (−0.31 to 0.12)	0.3738	**0.058** (−0.16 to 0.27)	0.6058
ESR (mm/h)			**0.472** (0.28 to 0.63)	**0.0001**

P values ≤ 0.05 were significant (bold)

Table 3 Comparative analysis of the frequency of allelic groups of the *HLA-DRB1* gene locus between patients with RA and control group of females

Allelic group of HLA-DRB1	Control ($2n = 164$)		RA patients ($2n = 166$)		OR (95% CI)	*P* value
	No	*f*	No	*f*		
DRB1*01	**24**	0.146	**19**	0.114	**0.755** (0.37–1.51)	0.3904
DRB1*03	**10**	0.061	**21**	0.126	**2.225** (0.96–5.48)	**0.0416**
DRB1*04	**12**	0.073	**26**	0.157	**2.346** (1.09–5.31)	**0.0177**
DRB1*07	**18**	0.110	**14**	0.084	**0.748** (0.33–1.66)	0.4360
DRB1*08	**5**	0.030	**6**	0.036	**1.192** (0.34–4.32)	0.7750
DRB1*09	**1**	0.006	**1**	0.006	**0.988** (0.01–78.02)	0.9932
DRB1*10	**4**	0.024	**3**	0.018	**0.737** (0.11–4.43)	0.6909
DRB1*11	**16**	0.098	**17**	0.102	**1.055** (0.48–2.32)	0.8835
DRB1*12	**2**	0.012	**2**	0.012	**0.988** (0.07–13.78)	0.9903
DRB1*13	**18**	0.110	**12**	0.072	**0.633** (0.27–1.44)	0.2379
DRB1*14	**8**	0.049	**7**	0.042	**0.859** (0.26–2.78)	0.7734
DRB1*15	**25**	0.152	**21**	0.126	**0.806** (0.41–1.58)	0.4971
DRB1*16	**21**	0.128	**17**	0.102	**0.778** (0.37–1.62)	0.4663

P values ≤ 0.05 (bold) were statistically significant

*DRB1*03/*11*, *DRB1*03/*15*, *DRB1*01/*08*, *DRB1*07/*15*, *DRB1*13/*16*, *DRB1*15/*16*, *DRB1*03/*03*, *DRB1*04/*15,* and *DRB1*04/*16* had frequencies of 3.6%, respectively. The remaining 24 different genotypes had frequencies under 2.5% (Table 4).

The analyses of allelic groups of *HLA-DRB3*, *DRB4,* and *DRB5* genes (Table 5) showed that the *DRB3* gene variants were most common in the control group (39.5%) as well as in RA patients (45.3%).

The *HLA-DRB1* gene locus is highly polymorphic and can be observed in the control group and in the group of patients with RA. It shows a high degree of heterozygosity (0.93 and 0.89), gene diversity (0.89 and 0.89), and PIC (0.88 and 0.88). We observed 43 different genotypes of the *DRB1* gene locus in the control group and 39 in the RA patient group. The control group was in HWE, and the group of RA patients was not ($P = 0.0285$).

Table 4 Comparative analysis of the frequency of genotypes of the *HLA-DRB1* gene locus between patients with RA and the control group of females

Genotype	Control ($n=82$)		RA patients ($n=83$)		OR (95% CI)	*P* value
	No	*f*	No	*f*		
DRB1*01/DRB1*15	**7**	0.085	**0**	0	**0.143** (0.003–1.18)	**0.0074**
DRB1*01/DRB1*04	**5**	0.061	**2**	0.024	**0.382** (0.04–2.42)	0.1210
DRB1*11/DRB1*16	**5**	0.061	**2**	0.024	**0.382** (0.04–2.42)	0.1210
DRB1*13/DRB1*15	**5**	0.061	**2**	0.024	**0.382** (0.04–2.42)	0.1210
DRB1*07/DRB1*16	**5**	0.061	**1**	0.012	**0.189** (0.003–1.75)	**0.0471**
DRB1*01/DRB1*07	**4**	0.049	**5**	0.060	**1.248** (0.26–6.54)	0.3733
DRB1*07/DRB1*13	**3**	0.037	**3**	0.036	**0.988** (0.13–7.60)	0.4940
DRB1*03/DRB1*16	**3**	0.037	**2**	0.024	**0.652** (0.05–5.85)	0.3204
DRB1*15/DRB1*15	**3**	0.037	**1**	0.012	**0.323** (0.01–4.12)	0.1545
DRB1*03/DRB1*11	**2**	0.024	**3**	0.036	**1.496** (0.17–18.36)	0.3303
DRB1*03/DRB1*15	**2**	0.024	**3**	0.036	**1.496** (0.17–18.36)	0.3303
DRB1*04/DRB1*07	**2**	0.024	**1**	0.012	**0.490** (0.01–9.58)	0.2771
DRB1*04/DRB1*13	**2**	0.024	**1**	0.012	**0.490** (0.01–9.58)	0.2771
DRB1*01/DRB1*16	**2**	0.024	**0**	0	**0.198** (0.01–4.05)	0.2929
DRB1*07/DRB1*11	**2**	0.024	**0**	0	**0.198** (0.01–4.05)	0.2929
DRB1*13/DRB1*14	**2**	0.024	**0**	0	**0.198** (0.01–4.05)	0.2929
DRB1*14/DRB1*16	**2**	0.024	**0**	0	**0.198** (0.01–4.05)	0.2929
DRB1*03/DRB1*04	**1**	0.012	**5**	0.060	**5.149** (0.56–248.6)	**0.0500**
DRB1*01/DRB1*14	**1**	0.012	**4**	0.048	**4.071** (0.39–204.4)	0.0895
DRB1*11/DRB1*15	**1**	0.012	**4**	0.048	**4.071** (0.39–204.4)	0.0895
DRB1*01/DRB1*08	**1**	0.012	**3**	0.036	**3.019** (0.24–161.4)	0.1594
DRB1*07/DRB1*15	**1**	0.012	**3**	0.036	**3.019** (0.24–161.4)	0.1594
DRB1*13/DRB1*16	**1**	0.012	**3**	0.036	**3.019** (0.24–161.4)	0.1594
DRB1*15/DRB1*16	**1**	0.012	**3**	0.036	**3.019** (0.24–161.4)	0.1594
DRB1*01/DRB1*11	**1**	0.012	**2**	0.024	**1.992** (0.10–119.4)	0.2842
DRB1*04/DRB1*11	**1**	0.012	**2**	0.024	**1.992** (0.10–119.4)	0.2842
DRB1*10/DRB1*11	**1**	0.012	**2**	0.024	**1.992** (0.10–119.4)	0.2842
DRB1*01/DRB1*13	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*08/DRB1*13	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*08/DRB1*15	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*09/DRB1*14	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*10/DRB1*16	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*11/DRB1*13	**1**	0.012	**1**	0.012	**0.988** (0.01–78.48)	0.4966
DRB1*01/DRB1*01	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*03/DRB1*12	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*03/DRB1*13	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*04/DRB1*08	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*07/DRB1*10	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*08/DRB1*14	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*10/DRB1*13	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*11/DRB1*11	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*12/DRB1*16	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*14/DRB1*15	**1**	0.012	**0**	0	**0.329** (0.01–7.97)	0.4945
DRB1*04/DRB1*04	**0**	0	**4**	0.048	**6.361** (0.49–410)	**0.0367**
DRB1*03/DRB1*03	**0**	0	**3**	0.036	**6.917** (0.36–131.84)	0.1985
DRB1*04/DRB1*15	**0**	0	**3**	0.036	**6.917** (0.36–131.84)	0.1985
DRB1*04/DRB1*16	**0**	0	**3**	0.036	**6.917** (0.36–131.84)	0.1985
DRB1*01/DRB1*03	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039
DRB1*01/DRB1*12	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039

Table 4 (continued)

Genotype	Control ($n=82$)		RA patients ($n=83$)		OR (95% CI)	*P* value
	No	*f*	No	*f*		
DRB1*03/DRB1*08	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039
DRB1*04/DRB1*12	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039
DRB1*07/DRB1*14	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039
DRB1*11/DRB1*14	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039
DRB1*16/DRB1*16	**0**	0	**1**	0.012	**2.964** (0.12–71.73)	0.5039

P values ≤ 0.05 (bold) were statistically significant

Table 5 Frequencies of *HLA-DRB3*, *HLA-DRB4* and *HLA-DRB5* gene variants and the association of *HLA-DRB* haplotypes with susceptibility to RA

Locus	Control		RA patients		OR (95% CI)	*P* value
	No	*f*	No	*f*		
DRB3	**45**	0.3947	**48**	0.4528	**1.268** (0.72–2.25)	0.1923
DRB4	**28**	0.2456	**32**	0.3019	**1.326** (0.70–2.52)	0.1751
DRB5	**41**	0.3596	**26**	0.2453	**0.580** (0.31–1.08)	**0.0331**
DRB-allele haplotype	No	%	No	%		
DRB1*01	**24**	15.29	**19**	12.5	**0.792** (0.39–1.59)	0.4799
DRB1*03-DRB3	**10**	6.37	**20**	13.16	**2.222** (0.95–5.52)	**0.0443**
DRB1*04-DRB4	**12**	7.64	**24**	15.79	**2.26** (1.04–5.17)	**0.0259**
DRB1*07-DRB4	**17**	10.83	**11**	7.24	**0.643** (0.26–1.52)	0.2735
DRB1*08	**4**	2.55	**6**	3.95	**1.57** (0.36–7.72)	0.4877
DRB1*09-DRB4	**0**	0	**1**	0.66	**1.703** (0.02–16)	0.6655
DRB1*10	**4**	2.55	**3**	1.97	**0.771** (0.11–4.64)	0.7350
DRB1*11-DRB3	**15**	9.55	**17**	11.18	**1.191** (0.54–2.67)	0.6388
DRB1*12-DRB3	**2**	1.27	**2**	1.32	**1.033** (0.07–14.42)	0.9741
DRB1*13-DRB3	**17**	10.83	**10**	6.58	**0.581** (0.23–1.40)	0.1870
DRB1*14-DRB3	**8**	5.1	**7**	4.60	**0.899** (0.27–2.92)	0.8414
DRB1*15-DRB5	**25**	15.92	**18**	11.84	**0.71** (0.35–1.43)	0.3026
DRB1*16-DRB5	**19**	12.1	**14**	9.21	**0.738** (0.33–1.62)	0.4114

P values ≤ 0.05 (bold) were statistically significant

The frequency of *HLA-DRB1* gene variants in patients with RA in comparison with control female subjects

The distribution of allelic groups of the *HLA-DRB1* gene locus in RA patients was compared with controls (Table 3). The frequency of the *DRB1*04* ($P=0.0177$) and **03* ($P=0.0416$) allele groups in RA patients was significantly higher than in the control group. The frequencies of *DRB1*08* and **11* allele groups were slightly higher in RA patients than in the control group, but were not statistically significant. In contrast, the frequencies of allele groups *DRB1*01*, **07*, **10*, **13*, **14*, **15,* and **16* were higher in the control group than in RA patients but not significantly.

The frequencies of the *DRB1*04/*04* ($P=0.0367$) and *DRB1*03/*04* ($P=0.05$) genotypes were significantly higher among RA patients than in the control group (Table 4). A significantly increased frequency of *DRB1*01/*15* ($P=0.0074$) and *DRB1*07/*16* genotypes ($P=0.0471$) was found in control subjects compared with RA patients. These genotypes have not been observed in patients with RA (*DRB1*01/*15*) or were reduced (*DRB1*07/*16*).

Homozygous genotypes *DRB1*04/*04*, *DRB1*03/*03,* and *DRB1*16/*16* were present in RA patients, but not detected in the control group, whereas homozygous genotypes *DRB1*01/*01* and *DRB1*11/*11* were present in the control group, but were not detected in RA patients.

The frequency of allelic groups of the *HLA-DRB5* gene was significantly increased in the control group when compared with RA patients ($P=0.0331$). The frequencies of allelic groups of the *DRB3* and *DRB4* gene loci were higher among RA patients than in the control group but

not significantly (Table 5). Furthermore, we examined haplotypes of the *HLA-DRB* genes (*DRB1* alone and *DRB1-DRB3/4/5* haplotypes). The occurrence of *DRB3*, *DRB4,* and *DRB5* is correlated with two-digit alleles of *DRB1*. We estimated 10 haplotypes (Table 5). The frequencies of the *DRB1*03-DRB3** ($P=0.0443$) and *DRB1*04-DRB4** ($P=0.0259$) haplotypes were significantly higher among RA patients than in the control group.

In this study, we found there was significantly higher expression of alleles of the *HLA-DRB1*03* ($P=0.0061$), **04* ($P=0.0001$), **08* ($P=0.0270$), **10* ($P=0.0254$), **11* ($P=0.0024$), and **14* ($P=0.0100$) groups among RA patients with ESR values above 10 mm/h (Table 6). The *HLA-DRB1*04*, **09* and **14* variants were expressed more in RA females with CRP values above 5 mg/L but not significantly. RA patients with normal CRP values had significantly higher frequency of *DRB1*03* ($P=0.0008$), **13* ($P=0.0165$), and **15* alleles ($P=0.0008$).

Discussion

The cause of RA is still unknown. An important role for the development of RA belongs to the immune complexes that occur in the injured cells of the synovia and the inflamed blood vessels. To date, no specific biochemical or immunological marker has been found for RA. Some laboratory tests serve only to screen the activity and therapeutic effect in RA.

In our study, we showed that the values of the ESR ($P<0.0001$) and CRP ($P<0.0001$) were significantly increased in RA patients compared to those in the control group and was largely above the upper normal limit. ESR and CRP were also increased in RA patients when compared with control in the research accomplished by Sokolovic et al. [9]. We found a strong association between ESR and CRP in patients with RA ($P<0.0001$), but the relative risk related to ESR was more than twofold (2.7 times) than that related to CRP. The results of this study indicate that ESR and CRP were the most sensitive indicators of disease in patients with RA. The persistence of a high serum CRP concentration is usually a severe prognostic sign. The classification criteria for RA, including ESR and CRP measurements, allow early aggressive treatment of RA [10].

Multiple factors, including *HLA-DRB1* gene variants, influence the susceptibility to RA. In this study, we showed that the alleles of the *HLA-DRB1*04* ($P=0.0177$) and *HLA-DRB1*03* ($P=0.0416$) groups were the most frequent in RA patients and, therefore, represent a risk factor for the development of this disease. In RA, the HLA supports an odds ratio (OR) of ~ 2.8, whereas most non-HLA loci have an OR in the range of 1.1–1.4 [11]. The role of allele groups *HLA-DRB1*08*, **09*, **10*, **11*, **12,* and **14* in the development of RA was not completely defined in this study based on HLA typing. Furthermore, the frequencies of the *DRB1*04/*04* ($P=0.0367$) and *DRB1*03/*04* ($P=0.05$) genotypes were significantly higher among RA patients than in the control group and, therefore, represent risk genotypes for the occurrence of RA. However, Fejzic and co-workers [12] found that alleles of HLA-DRB1*01 and *04 group as well as DRB1*01/*13 genotype were the most frequent in RA patients of the Federation of Bosnia and Herzegovina. Homozygous genotype *DRB1*04/*04* has not been observed in the control group of females along with *DRB1*03/*03* and *DRB1*16/*16* genotypes. Correspondingly, the strong

Table 6 Frequencies of *HLA-DRB1* gene variants in the group of RA females with normal and increased values of ESR and CRP

Allelic group of HLA-DRB1	ESR (mm/h)		OR (95% CI)	*P*	CRP (mg/L)		OR (95% CI)	*P*
	≤ 10	> 10			≤ 5	> 5		
	No	No (%)			No	No (%)		
DRB1*01	**8**	**11** (57.9)	1.859 (0.5–7.1)	0.3368	**11**	**8** (42.1)	0.538 (0.1–1.0)	0.3368
DRB1*03	**6**	**15** (71.4)	5.945 (1.6–24.8)	**0.0061**	**16**	**5** (23.8)	0.105 (0.02–0.4)	**0.0008**
DRB1*04	**5**	**18** (78.3)	12.06 (3.1–54.9)	**0.0001**	**10**	**13** (56.5)	1.671 (0.5–5.6)	0.3816
DRB1*07	**5**	**8** (61.5)	2.466 (0.5–13.24)	0.2493	**9**	**4** (30.8)	0.212 (0.04–1.1)	0.0544
DRB1*08	**1**	**5** (83.3)	16.6 (1.1–696)	**0.0270**	**4**	**2** (33.3)	0.2834 (0.02–3.2)	0.2700
DRB1*09	**1**	**0**	9.0 (0.0–190)	0.3195	**0**	**1** (100)	9 (0.1–831)	0.3865
DRB1*10	**0**	**3** (100)	49 (0.7–3236)	**0.0254**	**2**	**1** (33.3)	0.322 (0.01–9.5)	0.4561
DRB1*11	**4**	**13** (76.5)	9.686 (2.1–54.7)	**0.0024**	**11**	**6** (35.3)	0.309 (0.1–1.3)	0.0911
DRB1*12	**0**	**2** (100)	25 (0.3–1831)	0.1361	**1**	**1** (50)	1 (0.01–76.5)	0.5000
DRB1*13	**4**	**8** (66.7)	3.758 (0.7–23.6)	0.1099	**9**	**3** (25)	0.1242 (0.02–0.8)	**0.0165**
DRB1*14	**1**	**6** (85.7)	23.5 (1.7–949.2)	**0.0100**	**2**	**5** (71.4)	5.377 (0.6–73.7)	0.1223
DRB1*15	**8**	**13** (61.9)	2.578 (0.7–9.5)	0.1274	**16**	**5** (23.8)	0.105 (0.02–0.4)	**0.0008**
DRB1*16	**6**	**9** (60)	2.188 (0.5–10.2)	0.2828	**8**	**7** (46.7)	0.772 (0.2–3.4)	0.7196

P values ≤ 0.05 were significant (bold)

genetic associations between the *HLA-DRB1*04-DRB4** ($P=0.0259$) or *HLA-DRB1*03-DRB3** ($P=0.0443$) haplotypes and a risk for the development of RA have been established in females.

On the other hand, the most frequent variants in the control group of females were *HLA-DRB1*15*, **01*, **16*, **07,* and **13* and possibly have a protective role in the formation of RA. However, the differences observed between the control group and patients suffering from this disease were not statistically significant in this research study. Nevertheless, the *DRB1*01/*15* ($P=0.0074$) and *DRB1*07/*16* ($P=0.0471$) genotypes can be considered protective factors for RA, i.e., prevent the development of the disease. These genotypes were not observed in patients with RA, or their number was reduced. The frequency of allelic groups of the *HLA-DRB5* gene was significantly increased in the control group when compared with RA patients ($P=0.0331$). According to our results, the *HLA-DRB5* gene represents a protective factor for RA.

In addition, we observed a significantly increased frequency of alleles of the *HLA-DRB1*03*, **04*, **08*, **10*, **11,* and **14* groups in RA patients with ESR values above 10 mm/h and decreased frequency of *DRB1*03*, **13,* and **15* alleles in RA patients with CRP values above 5 mg/L, suggesting that certain *HLA-DRB1* gene variants could be associate with milder or severe symptoms of disease.

The results of the H, gene diversity and PIC obtained in this study indicate that the *HLA-DRB1* gene locus was highly polymorphic in the control group and in patients with RA. We observed a greater number of homozygous females in the group of patients with RA (~ 11%) than in healthy controls (~ 7% homozygotes).

HLA-DRB1 is the most important gene locus for RA susceptibility, particular for *HLA-DRB1* gene variants at the **01*, **04*, and **10* groups, which encode a conserved five amino acid sequence motif (QRRAA/RRRAA/QKRAA) at 70–74 in the third hypervariable region (*HVR*3) of the DRβ1 chain that are described as the shared epitope (SE). The SE-coding alleles include members of the *HLA-DRB1*04* group (**04:01, *04:04, *04:05, *04:08,* and **04:210*), *HLA-DRB1*01:01* or **01:02*, *HLA-DRB1*14:02,* and *HLA-DRB1*10:01*. The protective HLA alleles (*HLA-DRB1*01:03*, **04:02*, **08:02*, **11:02*, **11:03*, **13:01*, **13:02,* and **13:04*) have, instead of the SE motif, a different, but shared sequence at the same location in the beta chain of HLA-DR molecules, consisting of the amino acid residues DERAA.

In different ethnic groups, RA-associated *HLA-DRB1* alleles differ: *HLA-DRB1*04:01*, **04:04,* and **04:08* are the predominant RA-associated alleles in Caucasians [13], **01:01* and **01:02* in Israeli Jews [14] and **01:01*, **04:01*, **04:04*, and **04:05* in Latin Americans [15]. Shared epitope (SE) alleles and *DRB1*09:01* were significantly associated with RA susceptibility in the Japanese population [16]. In addition, the alleles of the *HLA-DRB1*04* group dominated in RA populations of Finland [17], the England, the Netherlands [18], and Slovakia [19]. The allele groups *DRB1*04* and **01* are equally represented in the British and the Spanish population [20], while *DRB1*01* and **10* dominated in the Mediterranean populations [21, 22].

In contrast, there are other *HLA-DRB1* gene variants that are negatively associated with RA and, therefore, have a protective role. The allele group *HLA-DRB1*13* was found to be significantly lower in RA patients compared to the control group in many studies [17, 18, 21], but in the control group of Mexican Americans, the *HLA-DRB1*08* allele group is statistically significantly more frequent and is considered a protective allelic group [23]. Alleles of the *DRB1*07* and **13* groups showed protective effects in a Slovak population [19].

The presence of specific *HLA-DRB1* gene variants increases the risk of developing RA, while other variants provide protection against disease. Therefore, HLA typing could be helpful in prediction of RA development and establishing and confirming a definitive diagnosis of autoimmune diseases in some subjects. Because there is not specific therapy for RA, treatment could be slow down the progression of the disease indicating the importance for earlier diagnosis. A strong association ESR and CRP with RA could be used to establish definitive diagnosis and introduce of early treatment of RA to prevent the occurrence of RA symptoms.

Author contributions All authors contributed to the design of the research and interpretation of the data, and critical revisions to the content. BK and HN contributed to data collection. HN analysed the data. The first draft of the manuscript was written by HN and all authors commented on the previous versions of the manuscript. All authors read and approved the final version of manuscript and take full responsibility for the accuracy or integrity of all parts of it. Writing review and editing: Springer Nature Research Editing Service.

Funding No funding was received for this study.

Compliance with ethical standards

Conflict of interest Author Biljana Klimenta, author Hilada Nefic, author Nenad Prodanovic, author Radivoj Jadric, and author Fatima Hukic declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (the Research Ethics Committee of the Public Institution Health Centre of Sarajevo Canton, Bosnia and Herzegovina: 01-08-5080-2/16) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JMW, Hobbs K, Huizinga TWJ, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovský J, Wolfe F, Hawker G (2010) 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism Collaborative Initiative. Arthritis Rheum 62(9):2569–2581. https://doi.org/10.1002/art.27584
2. Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, Kavanaugh A, McInnes IB, Solomon DH, Strand V, Yamamoto K (2018) Rheumatoid arthritis. Nat Rev Dis Primer 4:18001. https://doi.org/10.1038/nrdp.2018.1
3. Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423:356–361. https://doi.org/10.1038/nature01661
4. Choy E (2012) Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology 51:v3–v11. https://doi.org/10.1093/rheumatology/kes113
5. Stastny P (1978) Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 298(16):869–871. https://doi.org/10.1056/NEJM197804202981602
6. Gregersen PK, Silver J, Winchester RJ (1987) The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 30(11):1205–1213. https://doi.org/10.1002/art.1780301102
7. Viatte S, Plant D, Raychaudhuri S (2013) Genetics and epigenetics of rheumatoid arthritis. Nat Rev Rheumatol 9(3):141–153. https://doi.org/10.1038/nrrheum.2012.237
8. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16(3):1215. https://doi.org/10.1093/nar/16.3.1215
9. Sokolovic S, Dervisevic V, Fisekovic S (2014) Mental health status can reflect disease activity in rheumatoid arthritis. Eur J Rheumatol 1:55–57. https://doi.org/10.5152/eurjrheumatol.2014.020
10. Kim KW, Kim BM, Moon HW, Lee SH, Kim HR (2015) Role of C-reactive protein in osteoclastogenesis in rheumatoid arthritis. Arthritis Res Ther 17(1):41. https://doi.org/10.1186/s13075-015-0563-z
11. Nigrovic PA, Raychaudhuri S, Thompson SD (2018) Review: genetics and the classification of arthritis in adults and children. Arthritis Rheum 70(1):7–17. https://doi.org/10.1002/art.40350
12. Fejzic E, Sahovic A, Sisic S, Alijagic A, Suljevic D (2017) Crosslinks between human leukocyte antigen DRB1*01 and human leukocyte antigen DRB1*13 allelic variants and occurrence of rheumatoid arthritis in patients from Federation of Bosnia and Herzegovina. Arch Rheumatol 32(4):290–297. https://doi.org/10.5606/ArchRheumatol.2017.6258
13. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, Li Y, Kurreeman FA, Zhernakova A, Hinks A, Guiducci C, Chen R, Alfredsson L, Amos CI, Ardlie KG, BIRAC Consortium, Barton A, Bowes J, Brouwer E, Burtt NP, Catanese JJ, Coblyn J, Coenen MJ, Costenbader KH, Criswell LA, Crusius JB, Cui J, de Bakker PI, De Jager PL, Ding B, Emery P, Flynn E, Harrison P, Hocking LJ, Huizinga TW, Kastner DL, Ke X, Lee AT, Liu X, Martin P, Morgan AW, Padyukov L, Posthumus MD, Radstake TR, Reid DM, Seielstad M, Seldin MF, Shadick NA, Steer S, Tak PP, Thomson W, van der Helm-van Mil AHM, van der Horst-Bruinsma IE, van der Schoot CE, van Riel PL, Weinblatt ME, Wilson AG, Wolbink GJ, Wordsworth BP, YEAR Consortium, Wijmenga C, Karlson EW, Toes RE, de Vries N, Begovich AB, Worthington J, Siminovitch KA, Gregersen PK, Klareskog L, Plenge RM (2010) Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 42(6):508–514. https://doi.org/10.1038/ng.582
14. de Vries N, Rønningen KS, Tilanus MGJ, Bouwens-Rambouts A, Segal R, Egeland T, Thorsby E, van de Putte LB, Brautbar C (1993) HLA-DR1 and rheumatoid arthritis in Israeli Jews: sequencing reveals that DRB1*0102 is the predominant HLA-DR1 subtype. Tissue Antigens 41(1):26–30. https://doi.org/10.1111/j.1399-0039.1993.tb01973.x
15. Cruz-Tapias P, Rojas-Villarraga A, Maier Moore S, Anaya JM (2012) HLA and Sjögren's syndrome susceptibility A meta-analysis of worldwide studies. Autoimmun Rev 11(4):281–287. https://doi.org/10.1016/j.autrev.2011.10.002
16. Shimane K, Kochi Y, Suzuki A, Okada Y, Ishii T, Horita T, Saito K, Okamoto A, Nishimoto N, Myouzen K, Kubo M, Hirakata M, Sumida T, Takasaki Y, Yamada R, Nakamura Y, Kamatani N, Yamamoto K (2013) An association analysis of HLA-DRB1 with systemic lupus erythematosus and rheumatoid arthritis in a Japanese population: effects of *09:01 allele on disease phenotypes. Rheumatology 52(7):1172–1182. https://doi.org/10.1093/rheumatology/kes427
17. Tuokko J, Nejentsev S, Luukkainen R, Toivanen A, Ilonen J (2001) HLA haplotype analysis in Finnish patients with rheumatoid arthritis. Arthritis Rheum 44(2):315–322. https://doi.org/10.1002/1529-0131(200102)44:2%3c315:AID-ANR48%3e3.0.CO;2-G
18. Van Jaarsveld CHM, Otten HG, Jacobs JWG, Kruize AA, Brus HLM, Bijlsma JWJ (1998) Association of HLA-DR with susceptibility to and clinical expression of rheumatoid arthritis: re-evaluation by means of genomic tissue typing. Br J Rheumatol 37(4):411–416. https://doi.org/10.1093/rheumatology/37.4.411
19. Stark K, Rovenský J, Blažičková S, Grosse-Wilde H, Ferencik S, Hengstenberg C, Straub RH (2009) Association of common polymorphisms in known susceptibility genes with rheumatoid arthritis in a Slovak population using osteoarthritis patients as controls. Arthritis Res Ther 11(3):R70. https://doi.org/10.1186/ar2699
20. Balsa A, Minaur NJ, Pascual-Salcedo D, McCabe C, Balas A, Fiddament B, Vicario JL, Cox NL, Martín-Mola E, Hall ND (2000) Class II MHC antigens in early rheumatoid arthritis in Bath (UK) and Madrid (Spain). Rheumatology 39(8):844–849. https://doi.org/10.1093/rheumatology/39.8.844
21. Kinikli G, Ateş A, Turgay M, Akay G, Kinikli S, Tokgöz G (2003) HLA-DRB1 genes and disease severity in rheumatoid arthritis in Turkey. Scand J Rheumatol 32(5):277–280. https://doi.org/10.1080/03009740310003901
22. Fathi NA, Ezz-Eldin AM, Mosad E, Bakry RM, Hamed HB, Ahmed S, Mahmoud M, Rashed HA, Abdullah F (2008) Diagnostic performance and predictive value of rheumatoid factor, anti-cyclic-citrullinated peptide antibodies and HLA-DRB1 locus genes in rheumatoid arthritis. Int Arch Med 1(1):20. https://doi.org/10.1186/1755-7682-1-20
23. Del Rincón I, Escalante A (1999) HLA–DRB1 alleles associated with susceptibility or resistance to rheumatoid arthritis, articular deformities, and disability in Mexican Americans. Arthritis Rheum 42(7):1329–1338. https://doi.org/10.1002/1529-0131(199907)42:7%3c1329:AID-ANR5%3e3.0.CO;2-1

Affiliations

Biljana Klimenta[1] · Hilada Nefic[2] · Nenad Prodanovic[3] · Radivoj Jadric[4] · Fatima Hukic[5]

Biljana Klimenta
biljanaklimenta@hotmail.com

Nenad Prodanovic
nenad.prodanovic@med.unibl.org

Radivoj Jadric
radivoj.jadric@mf.unsa.ba

Fatima Hukic
fatima.hukic@ukctuzla.ba

[1] Department of Laboratory Diagnostics, The Public Institution Health Centre of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina

[2] Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

[4] Department of Medical Biochemistry, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[5] Department of Biochemistry, Clinical University Centre of Tuzla, Tuzla, Bosnia and Herzegovina