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10.2478/AMB-2019-0002

HLA DRB1 AND HLA DQB1 ALLELES IN BULGARIAN PATIENTS WITH PRIMARY AND SECONDARY ANTIPHOSPHOLIPID SYNDROME

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Abstract. *Antiphospholipid syndrome (APS) is an autoimmune disease with multifactorial and polygenic pathogenesis. Recently, the genetic predisposition in APS has been subjected to wide discussion. The aim of this study is to determine the prevalence of DRB1 and DQB1 loci in Bulgarian population of healthy persons and patients with primary (PAPS) and secondary (SAPS) APS. Patients are divided in 5 groups: I-29 patents with systemic lupus erythematosus (SLE) with SAPS, II-35 patients with PAPS, III-32 women with spontaneous abortions without aPL, IV-15 patients with different thrombosis (deep venous thromboses, pulmonary embolism, mesenterial thrombosis, myocardial infarction, stroke) without laboratory data for APS, and V-16 SLE patients without clinical and laboratory data for APS. SAPS patients have more frequently DRB1*03 and DQB1*02 and more rarely DRB1*11 and DQB1*03 in comparison with healthy subjects and patients with PAPS. Patents with PAPS, those with spontaneous abortions and patients with thrombotic events but without antiphospholipid antibodies (aPL) have DRB1*03, DRB1*11, DQB1*02 and DQB1*03 alleles similar to the general population. There are no differences between group I (SLE+APS) and group V (SLE) in DRB1* and DQB1*alleles.*

Key words: *antiphospholipid syndrome, spontaneous abortions, systemic lupus erythematosus, HLA DRB1, HLA DQB1*

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INTRODUCTION

APS is observed in association with different diseases and conditions, such as SLE, high-risk pregnancy, pulmonary embolism, deep venous thromboses, stroke, myocardial infarction, etc. APS affects heterogeneous population of patients and its genetic basis is difficult to evaluate. A wide variety of antiphospholipid antibodies (aPL) can be detected in APS patients, including lupus anticoagulant (LA), anticardiolipin (aCL), anti-beta-2-glycoprotein I (aB2GPI), antiprothrombin (aPT), antiphosphatidyl serine (aPS), etc. This impedes the formation of more homogenous groups of patients.

Many authors have suggested that in parallel to SLE, HLA alleles in APS are just one predisposing genetic factor and there are probably many more. Some HLA alleles probably increase the risk for the production of aPL but do not determine the clinical manifestations of APS.

The aim of this study was to determine the possible association of DRB1 and DQB1 alleles in Bulgarian patients with primary (PAPS) and secondary (SAPS) APS.

MATERIAL AND METHODS

We analyzed DRB1 and DQB1 distribution in 204 healthy volunteers and 127 patients tested for antiphospholipid antibodies (aPL-aCL and aB2GPI) using ELISA method (Orgentec), divided in 5 groups: group I – 29 patents with systemic lupus erythematosus (SLE) with SAPS; II – 35 patients with PAPS; III – 32 women with spontaneous abortions without aPL; and IV – 15 patients with different thrombotic events (deep venous thromboses, pulmonary embolism, mesenterial thrombosis, myocardial infarction, stroke) without laboratory data for APS, and V – 16 SLE patients without clinical and laboratory data for APS. All SLE patients fulfilled the American College of Rheumatology criteria for SLE [1], and patients with PAPS and SAPS fulfilled the Sidney criteria [2]. DRB1 and DQB1 loci were genotyped with PCR-SSP technique.

The **statistical analysis** was performed using standard statistical packages – SPSS 16.0 (IBM Corp., New York, USA), Arlequin v3.5 (University of Berne, Berne, Switzerland) and GeneRATE (Université de Genève, Geneva, Switzerland). The following statistical methods were used: Chi square and Fischer's exact test were used to evaluate differences between groups. Maximum-likelihood and descriptive statistic were also calculated. In all tests $p \leq 0.050$ was considered statistically significant.

RESULTS

In group I (SLE+APS) we detected DRB1*03 more frequently than in the control group ($p < 0,0001$; O.R. 4,04; CI 95%), group II ($p = 0,025$; O.R. 0,32; CI 95%) and group III ($p = 0,02$; O.R. 0,3; CI 95%). On the other hand, the patients in group I had lower prevalence of DRB1*11 compared to control group ($p = 0,014$; O.R. 0,29; CI 95%) and group III ($p = 0,012$; O.R. 4,5; CI 95%). There were no differences between group I (SLE+APS) and group V (SLE) in DRB1* and DQB1*alleles. Moreover, the patients from group I had more frequently DQB1*02 compared to the control group ($p = 0,02$; O.R. 2,88; CI 95%) and patients from group II ($p = 0,029$; O.R. 0,35; CI 95%), but patients from group I had lower prevalence of DQB1*03 ($p = 0,01$; O.R. 0,42; CI 95%) compared to group II ($p = 0,05$; O.R. 2,37; CI 95%) and group III ($p = 0,028$; O.R. 2,62; CI 95%). There were no statistically significant differences with the other groups. The data for HLA-DRB1*03, DRB1*11, DQB1*02 and DQB1*03 are presented in Table 1.

DISCUSSION

Different HLA associations have been investigated in APS and in aPL-positive individuals. Some authors have found association between certain HLA loci and APS and/or the presence of aPL. The association of aPL with DR and DQ loci has been subjected to wide discussions. Yet, very often the interpretation of the results is difficult due to the small number of patients investigated [3]. Kapitany et al. [4] investigated Hungarian patients divided in the following groups: SLE, SLE+APS and PAPS patients that subsequently progress to SLE. The authors discovered that the SLE-associated alleles DRB1*03/DQB1*02 have higher prevalence in SLE and SLE+APS. In the third group (PAPS progressing subsequently to SLE) the allele prevalence was different: DRB1*03 and DQB1*0201 are less prevalent and DRB1*13, DQB1*06, and DQB1*0302 alleles are more prevalent.

Table 1. Distribution (%) of DRB1*03, DRB1*11, DQB1*02 and DQB1*03 alleles in the analyzed groups

HLA alleles	I group (SLE+APS)	II group (PAPS)	III group (spontaneous abortions without aPL)	IV group (different thrombotic events without aPL)	V group (SLE)	controls
DRB1*03	29,31 %	4,41%	10,93%	13,33%	21,87%	9,31%
DRB1*11	6,89%	22%	25%	23%	15,62%	20,34%
DQB1*02	32,75%	14,7 5	17,18%	20%	25%	14,45%
DQB1*03	20,68	38,23	40,62%	26,66%	31,25%	38,55%

Trabace et al. [5] investigated Italian women with recurrent spontaneous abortions and found that half of them were aCL-positive, and half were aCL-negative. HLA-DR7 was detected in 28% of the controls and in 24.5% of the investigated patients, but the prevalence of HLA-DR7 was much higher in the a CL-positive women (40%), compared to that in the aCL-negative (8,3%). Therefore, the authors concluded that in female patients with recurrent spontaneous abortions of unknown cause HLA-DR genes could play a role in the production of specific autoantibodies. Camps et al. found that PAPS from the South of Spain had higher prevalence of HLA-DQ7, DR4 and DRw53 [6]. In Canada, Goldstein et al. [7] investigated Caucasian patients with SLE and PAPS and found significant association between aPL (LA and aCL) and HLA-DR53 haplotypes. HLA-B8, DR17 and DQ2 haplotypes, related to SLE, had lower prevalence in aPL-positive and PAPS patients. The authors concluded that from immunogenetic point of view the synthesis of aPL in SLE and PAPS differs from that in SLE itself.

Asherson et al. [8] investigated 13 English patients with PAPS and positive LA and aCL and found higher prevalence of DR4 and DRw53 compared to the controls. In the US, Arnett et al. [9] showed that 70% of the LA-positive patients have HLA-DQw7 (DQB1*0301) linked to HLA-DR5 and – DR4 haplotypes, that was much higher prevalence than that in the control group. Moreover, SLE patients with positive LA had higher prevalence of DQB1*0301 compared to these with negative LA with positive antibodies to nuclear antigens. Several years later, Arnett et al. [10] showed that HLA class II haplotypes can influence the formation of aB2GPI. These authors found that DQ8 (DQB1*0302) was associated with aB2GPI when compared to both aB2GPI-negative SLE patients and to healthy controls of Mexican descent and to a lesser extent to Caucasian Americans. The combined analysis of three ethnic groups – Caucasians, African Americans, and of Mexican descent, HLA-DQB1*03:02, as well as DQB1*03:03 alleles overall (DQB1*03:01, *03:02 and *03:03) correlated significantly with aB2GPI. The prevalence of HLA DR6 (DRB1*13:02), DQB1*06:04/*06:05 was also increased, mainly in African Americans. The prevalence of HLA DR7 was not increased in any of the three ethnic groups, and the frequency of HLA-DR53 (DRB4*01:01) was increased only in the Americans of Mexican descent. The authors concluded that HLA class II haplotypes determine the expression of aB-2GPI with some variations related to the ethnic group.

The investigations in another ethnic group – British Caucasoid, made by Caliz et al., revealed comparable results and underlined the presence of a positive correlation between DQ B1*06:04/06:05/06:06/06:07/06:09-DQA1*01:02-DRB1*13:02 and DQB1*03:03-DQA1*02:01-DRB1*07:01 haplotypes and APS (PAPS and SAPS), that increased in PAPS with positive aB-2GPI [11]. The studies of Sebastiani et al. [12] in Italy showed the presence of positive association between APS and certain HLA alleles – HLA-DR4, DR7, DRw53 and DQB1*0302 with aCL in both PAPS and SAPS. Moreover, other aPL (LA, aB2GPI, aPS/aPT) also showed similar HLA-associations in both PAPS and SAPS. The studies of Granados et al. [13] revealed high prevalence of HLA DR 3, HLA DR 7 and HLA DQ2 antigens in aCL-positive SLE patients in Mexico. Moreover, the first-degree relatives of these aCL-positive patients also exhibited higher prevalence of DR 7 but the difference did not reach statistical significance. The aCL-negative SLE patients showed moderately increased prevalence of HLA DR 3 and DQ2, but not of DR 7. The SLE patients with APS and those with probable APS have higher prevalence of DR 7. These data are in contrast with the findings in patients with disputable or negative APS, who do not differ from the control group for the prevalence of this allele. According to the authors, these data support the role of DR 7 for the synthesis of aCL in SLE patients and their relatives.

Davies et al. [14] investigated Caucasian patients with SLE and found the following prevalence of HLA haplotypes: 89% of the SLE patients with positive aB-2GPI were positive for HLA DRB1*04:01/04:04/04:08, DR11 or DRB1*13:02. This prevalence was significantly higher than that in controls. The frequency of the described alleles in the second group (aCL-positive patients) was 43%, and in the third group (aCL-negative SLE patients) it was 38%, compared to 48% in the control group. In aCL-positive Dannish and Czech women with recurrent miscarriages the prevalence of HLA DR3 phenotype was higher than that in controls [15].

According to the studies performed by Ioannidis et al. [16] the aB2GPI-response is associated with HLA-DQA1*03 (in particular *03:01) and HLA-DRB1*13:02-DQB1*06:04 haplotype, whereas HLA-DRB1*01:01-DQA1*01:01 haplotype and HLA-DRB1*11:01 allele are protective against the synthesis of aB2GPI, and these findings do not vary in different ethnic groups (Greeks, Caucasian Americans, African Americans and Mexican Americans).

In Brazil, Freitas et al. [17] investigated several groups of patients (PAPS, SLE/APS, SLE without APS and controls) and found that in contrast to controls PAPS patients had non-significantly increased frequency of DRw53, SAPS patients had significantly higher preva-

lence of DRB1*03 allele. The latter was over-represented in SAPS patients with aCL and in SLE patients in general. The prevalence of aCL was similar in patients with and without DRB1*03 allele. The authors observed a trend towards an increase in the frequency of DQB1*0604 allele and DQB1*03:02 allele in SAPS. Therefore, the investigators concluded that the association of SAPS with HLA-DRB1* 03 is due to the association with SLE and is not due to aCL.

Galeazzi et al. [18] investigated 577 European patients with SLE and reached the following conclusions: 1) aCL showed positive association with HLA DRB1*04, DRB1*04:02, DRB1*04:03, DRB1*07, DRB3*03:01, DQA1*02:01, DQA1*03:01, DQB1*03:02 and negative association with DQA1*05:01, DRB3*02:02; 2) antibodies to beta-2 – GPI showed positive association with DRB1*04:02, DRB1*04:03, DQB1*03:02; 3) DRB1*04:02 allele has the highest relative risk for the presence of both aCL and aB2GPI.

The approaches towards the investigation of the relation between HLA class II loci and APS and/or aPL differ significantly in various studies. Some studies investigate the relation between APS and HLA class II alleles [4, 6], others – the relation to LA [9], to aCL [5, 13, 15, 17], LA and aCL [7, 8], aB2GPI [10, 11, 14, 16, 18], or aCL, LA, aB2GPI, aPS/aPT [12]. Moreover, different ethnic groups have been studied – Hungarians [4], Italians [5, 12], Southern Spain [6], Canadians [7], Caucasions [14], Americans [9], Caucasians, African Americans and Mexican Americans [10, 16], British [8, 11], Mexicans [13], Dannish and Czech [15], Brazilians [17], Europeans [18], Greek, Caucasian Americans, African Americans and Mexican Americans [16]. Different comparative analyses have been performed – SLE, SAPS, PAPS [4, 6, 7, 8, 9, 11, 12, 17], SLE [10, 13, 14, 18], patients with problematic pregnancy [5, 15].

In our study we compared the prevalence of HLA DRB1 and HLA-DQB1 alleles in aCL- and/or B2GPI-positive patients with SAPS to that in patients with PAPS, in patients with SLE without APS, in female patients with problematic pregnancy without APS, and in patients with different thrombotic events without APS. We found statistically significant differences for the following alleles: DRB1*03, DRB1*11, DQB1*02 and DQB1*03. The highest prevalence of HLA-DRB1* 03 allele was detected in SAPS patients and the lowest – in PAPS patients and in control subjects. The differences between SAPS and controls, between SAPS and PAPS, SAPS and aPL-negative patients with abortions reached statistical significance. We found no significant differences between SAPS patients and those with SLE without APS. In our previous studies we have found that Bulgarian SLE patients had higher prevalence of HLA-DRB1* 0301 compared to controls [19]. The higher prevalence of HLA-DRB1* 03 allele in SLE and SLE+APS has been noted by Kapitany et al. in Hungarians [4]. In Brazil, Freitas et al. [17] have found significantly higher prevalence of DRB1*03 allele in SAPS that was also over-represented in SAPS patients with aCL and in SLE patients in general. The authors suggest that PAPS and SAPS patents have different HLA II profiles. In the Bulgarian population the prevalence of DRB1*03 is relatively low – 9.31%, and is comparable in PAPS, in aPL-negative women with spontaneous abortions, and in patients with thrombotic events without aPL (Table 1).

The results of our study show that HLA-DRB1*11 is found in 20.34% of the controls. Its prevalence is comparable in groups II, III and IV and in controls. On the other hand, the prevalence of HLA-DRB1*11 is much lower in SAPS and SLE. The difference between group I and controls and between group I and groups II and III is statistically significant. The prevalence of HLA-DRB1*11 in groups I and V is comparable, as is in controls and group V. Our previous studies in the Bulgarian population found protective role of DRB1*11 allele group for different autoimmune diseases, including SLE [19]. According to Ioannidis et al. [16] HLA-DRB1*11:01 is one of the protective alleles against the production of aB2GPI in Greeks, Caucasian Americans, African Americans, and Mexican Americans.

According to our data, patients with SLE and APS (SAPS) more frequently were DQB1*02 positive compared to the control group and patients with PAPS. The prevalence among groups II, III and IV is comparable to the control group. In SLE patients (group II) the frequency is higher and close to that in SAPS. Bulgarian population shows tendency towards higher prevalence of DQB1*02:01 in SLE patients compared to controls [19]. The results of Kapitany et al. [4] show that DQB1*02 is more prevalent both in SLE and in SLE+APS (SAPS) patients. In patients with PAPS that subsequently progresses to SLE the allele frequency is much different: less prevalent DQB1*02:01. According to Goldstein et al. [7] one of the alleles related to SLE is DQ2. This allele has significantly lower prevalence in aPL-positive and PAPS patients, which, according to the authors, suggests different mechanism of aPL formation in SLE and PAPS. J. Granados et al. [13] show high prevalence of HLA DQ2 antigens in aCL-positive SLE patients in Mexico.

Our data show that patients with SAPS have lower frequency of DQB1*03 compared to controls, patients with PAPS and with spontaneous abortions

without aPL. On the other hand, DQB1*03 alleles in patients in group II, III, IV and V have similar frequency compared to the control group. Kapitany et al. [4] showed that PAPS patients have higher prevalence of DQB1*0302 allele. Arnett et al. [9] found that a major part of LA-positive patients carry HLA – DQw7 (DQB1*03:01). Several years later the same authors revealed the connection between DQB1*03:01, *03:02 and *0303 alleles and aB2GPI [10]. According to Caliz et al. [11], DQB1*03:03 is one of the alleles related to the production of aB2GPI. Freitas et al. [17] detected higher prevalence of DQB1*03:02 allele in SAPS, and M. Galeazzi et al. [18] suggested that DQB1*03:02 is one of the alleles related to increased production of aCL and aB2GPI. Frequently, the same alleles are found in both PAPS and SAPS – i.e., HLA DR4, DRw53, DQB1 03:02 (detected also in combination with aCL in PAPS and in SLE+APS), as compared to SLE, where the more common loci are DR2, DR3, and DRw52 [20]. The results of our study in Bulgarian patients and healthy persons showed some differences between patients with SLE/APS, PAPS, spontaneous abortions and control subjects, concerning mainly the distribution of DRB1*11, DRB1*03, DQB1*02, and DQB1*03 alleles.

HLA alleles seem to be one of the many genetic and other factors determining the risk for the development of APS. Many authors suggest that HLA loci probably define the risk for aPL production, not the development of certain clinical symptoms. The results of our study reveal significant differences between SAPS and PAPS patients concerning the prevalence of DRB1*03, DRB1*11, DQB1*02, and DQB1*03. The prevalence of these alleles among PAPS patients is comparable to controls, to aPL-negative women with spontaneous abortions and in aPL-negative subjects with thrombotic events. We detected no significant differences in the prevalence of the HLA alleles among SLE patients with and without APS. These results support the hypothesis that the underlying genetic mechanisms of aPL production in SAPS and in PAPS are different [7, 13, 17].

Uthman et al. [21] stated the following difficulties and limitations related to the genetic studies in APS: 1.Despite the standardization of the methods for aPL detection, there are substantial differences among the studies, especially in relation to the positive values. 2. The different definitions of APS and, therefore, the differences in patient selection, especially having in mind that substantial part of the APS patients have lupus. 3. The concomitant medication and the inborn thrombophilic conditions (i.e., factor VLeiden, 20210 prothrombin gene mutation, etc.). 4. Disease activity. 5. Geographical/ethnic and racial differences.

The genetic predisposition for APS has intrigued the scientists for many years. Many aspects of APS remain unclear, including its relation to autoimmune and other diseases, its etiology and pathogenesis. We are still in need for multi-center, multinational studies that could elucidate the genetic basis, etiology and pathogenesis of APS.

Conflict of interest

None to declare

REFERENCES

1. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 1982, 25 (11), 1271-1277.
2. Miyakis S, Lockshin M.D, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. J Thromb Hemost, 2006, 4 (2), 295-306.
3. Castro-Marrero J, Balada E, Vilardell-Tarrés M, Ordi-Ros J. Genetic risk factors of thrombosis in the antiphospholipid syndrome. Br J Haematol, 2009, 147 (3), 289-296.
4. Kapitany A, Tarr T, Gytvai A, et al. Human leukocyte antigen-DRB1 and -DQB1 genotyping in lupus patients with and without antiphospholipid syndrome. Ann NY Acad Sci, 2009, 1173, 545-551.
5. Trabace S, Nicotra M, Cappellacci S, et al. of HLA-DR and DQ antigens and anticardiolipin antibodies in women with recurrent spontaneous abortions. Am J Reprod Immunol, 1991, 26 (4), 147-149.
6. Camps MT, Cuadrado MJ, Ocon P, et al. Association between HLA class II antigens and primary antiphospholipid syndrome from the South of Spain. Lupus, 1995, 4 (1),51-55.
7. Goldstein R, Moulds JM, Smith CD, Sengar DP. MHS studies of the primary antiphospholipid antibody syndrome and of antiphospholipid antibodies in systemic lupus erythematosus. J Rheumatol, 1996, 23 (7), 1173-1179.
8. Asherson RA, Doherty DG, Vergani D, et al. Concise communication: major histocompatibility complex associations with primary antiphospholipid syndrome. Arthr Theum, 1992, 35 (1),124-125.
9. Arnett FC, Olsen ML, Anderson L, Reveille JD. Molecular analysis of major histocompatibility complex alleles associated with the lupus anticoagulant. J Clin Invest, 1991, 87 (5), 1490-1495.
10. Arnett FC, Thiagarajan P, Ahn C, Reveille JD. Association of anti-B2 – glycoprotein I autoantibodies with HLA class II alleles in three ethnic groups. Arthr Rheum, 1999, 42 (2), 268-274.
11. Caliz R, Atsumi T, Kondeatis E, et al. HLA class II gene polymorphisms in antiphospholipid syndrome: haplotype analysis in 83 Caucasoid patients. Rheumatology, 2001, 40 (1), 31-36.
12. Sebastiani D, Minisola G, Galeazzi M. HLA class II alleles and genetic predisposition to the antiphospholipid syndrome. Autoimmune Rev, 2003, 2 (6), 387-394.
13. Granados J, Vargas-Alarcon G, Drenkard C, et al. Relationship of anticardiolipin antibodies and antiphospholipid syn-

drome to HLA DR7 in Mexican patients with systemic lupus erythematosus (SLE). Lupus, 1997, 6 (1), 57-62.
14. Davies ML, Young SP, Welsh K, et al. Immune response to native B2-glycoprotein I in patients with systemic lupus erythematosus and antiphospholipid syndrome. Rheumatology, 2002, 41 (4), 395-400.
15. Christiansen OB, Ulcova-Gallova Z, Mohapeloa H, Krauz V. Studies on associations between human leukocyte antigen (HLA) class II alleles and antiphospholipid antibodies in Danish and Czech women with recurrent miscarriages. Human Reproduction 1998, 123 (12), 3326-3331.
16. Joannidis JPA, Tektonidou MG, Vlachoyiannopoulos PG et al. HLA associations of anti-beta2 glycoprotein I response in a Greek cohort with antiphospholipid syndrome and meta-analysis of four ethnic groups. Hum Immunol, 1999, 60 (12), 1274-1280.
17. Freitas MV, da Silva LM, Deghaide NH, et al. Is HLA II susceptibility to primary antiphospholipid syndrome different from susceptibility to secondary antiphospholipid syndrome? Lupus, 2004, 13 (2), 125-131.
18. Galeazzi M, Sebastiani GD, Tincani A, et al. HLA class II associations of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. Lupus, 2000, 9 (1),47-55.
19. Marintchev L, Naumova E, Rashkov R, et al. HLA class II alleles and autoantibodies in Bulgarians with systemic lupus erythematosus. Tissue Antigens, 1995, 46 (5), 422-425.
20. Sebastiani D, Iuliano A,Cantarini L, Galeazzi M. Genetic aspects of antiphospholipid syndrome. An update. Autoimmune Rev, 2016, 15 (5), 433-439
21. Uthman I, Khamashta M. Ethnic and geographical variation in antiphospholipid (Hughes) syndrome. Ann Rheum Dis 2005, 64 (12), 1671-1676.