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Short Communication

The Protective Role of the HLA-DR Locus in Patients with Various Clinical Types of Alopecia Areata

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Abstract. One of the genetic factors associated with the development of alopecia areata (AA) is the HLA locus. The study comprised 52 patients with AA aged 10 to 64 years. The frequences of HLA-DRB alleles in the patients and controls were compared. The control group comprised 152 healthy persons. Familial occurrence of AA was seen in 7 (13.5%) cases. A significantly lower frequency of HLA-DRB1*03 was observed in patients with AA comparison with the control group. In all patients with AA, alleles HLA-DRB1*15/*16 occurred more frequently than in the control group, but this was not significant after correction. In the group of patients with more severe forms of alopecia areata (alopecia areata totalis/alopecia areata universalis) there was no significant difference in HLA-DR allele distribution.

Key words: alopecia areata; familial inheritance; HLA locus.

Introduction

The etiopathogenesis of alopecia areata (AA) has not been explained so far. The disease is thought to be immunologically controlled. Its occurrence depends on many genetic factors, nervous system disorders, the presence of an internal inflammatory focus, hormonal factors and coexisting atopic diseases[6, 9, 12, 14, 17].

The frequency of familial AA ranges between 3 to 42%[13], mainly in subjects with early onset of the disease. COLOMBE et al.[2] describe familial inheritance in 37% of patients in whom AA appeared before 30 years of age, and only in 7.1% of those older than 30, including monozygotic twins, for whom the number is as high as 55%[11, 15].

One of genetic factors associated with the development of AA is the HLA locus. Genes of the HLA system are located on the short arm of chromosome 6. Within the HLA region, genes of classes I, II and III can be distinguished. The genes of HLA classes I and II encode molecules which are responsible for presenting peptides derived from degraded proteins to lymphocytes[12, 19].

Studies on HLA locus in patients with AA concern genes of classes I and II. Frequencies of HLA-DR4, DR5, DR11 and DQ3 were found to be increased in

Abbrevations used: AA – alopecia areata, AAT – alopecia areata totalis, AAU – alopecia areata universalis, AAV – alopecia areata vulgaris.

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patients with AA[2, 4, 7, 18], DR5 was specifically associated with an earlier onset and a more severe course of disease[7], and DRB1*0401 and DQB1*0301 (DQ7) were even more frequent in alopecia areata totalis/alopecia areata universalis (AAT/AAU) than in alopecia areata vulgaris (AAV)[2, 3, 5, 7].

Reports on the correlation between AA and histocompatibility antigens are controversial. Also, no studies on patients with AA in the Polish population have been conducted so far. Thus, the aim of the study was an evaluation of the association between HLA-DRB alleles and various clinical types of AA.

Materials and Methods

The study comprised 52 patients with AA (39 males, 13 females) aged 10–64 years, with a mean age of 33.4 years. The patients were divided into two groups. Group I comprised 36 patients with AAV, and group II 16 patients with more severe forms of alopecia: AAT and AAU.

In the group of patients with AAV, alopecia appeared in 23 persons before the age for 30, and in 13 after 30, while in the AAT/AAU group, in 13 and 3 cases, respectively. Familial occurrence of AA was seen in 7 patients, i.e. in 13.5% of the 52 patients studied.

The control group comprised 152 healthy unrelated persons from families of organ donors.

Genomic DNA was isolated from peripheral blood cells using the "Easy Blood DNA Prep" (A&A Biotechnology, Poland). Molecular typing of groups of HLA-DRB alleles at a low-resolution level was done using the Dynal RELI SSO HLA-DRB Test (Dynal, Norway) set of 36 molecular probes. Polymorphic fragments (second exon) of genes DRB1, DRB3, DRB4 and DRB5 were amplified in PCR reaction: 35 cycles – 15 s at 95˚C, 45 s at 60˚C, 15 s at 72˚C and 5 min at 72˚C, using Taq polymerase and PCR buffer. After denaturation the PCR products were hybridized in SSPE buffer at 50˚C with a set of specific molecular probes coating nylon stripes. The reaction results were read using the system of horse radish streptavidin-peroxidase. The results of typing were analyzed using the Dynal RELI SSO Pattern Matching Program.

The frequencies of HLA-DRB alleles in patients and controls were compared using the two-sided Fisher's exact test. Values of p less than 0.05 were considered significant. To correct for incidental significance, the p value was multiplied by the number of alleles compared (p_{cor}). An odds ratio (OR) was calculated as the cross product in a 2×2 table within 95% confidence intervals (95% CI).

Results

Familial occurrence of AA was seen in 7 cases, i.e. in 13.5% of the 52 cases studied. This mainly concerned subjects in whom the disease developed before 30 years of age (6 out of 7).

Coexisting autoimmune diseases of the thyroid were found in 4 patients, in one case albinism, in 5 cases atopy. Atopic diseases in families of patients were reported by 3 patients.

A significantly lower frequency of HLA-DRB1*03 (OR = 0.17; 95% CI = 0.04–0.73; p = 0.004; $p_{cor.}$= =0.05) was observed in patients with AA in comparison with the control group. A lower frequency of this allele was also found in a group of patients with AAV (OR = 0.12; 95% CI = 0.02–0.92; p = 0.016, p_{cor} = n.s.; Table 1). In all patients with AA, alleles HLA-DRB1*15/16 occurred more frequently than in the control group (p = 0.04), however, this was not significant after correction (Table 1). A similar association was found in patients with AAV (p = 0.02; p_{cor} = n.s.; Table 2), while in patients with more severe forms of alopecia this association was not present.

In the group of patients with more severe forms of alopecia (AAT/AAU) there was no significant difference in HLA-DR allele distribution in comparison with the control group (Table 3). Similarly, in patients with early onset of alopecia (before 30 years of age) we did not observe a significant difference in HLA-DR allele frequencies (Table 4).

Discussion

Familial occurrence of AA was seen in 13.5% of our patients' cases. This mainly concerned patients with early onset of the disease (before 30 years of age). Similar results are reported by other authors[2, 12].

Immunological factors probably play an important role in the etiopathogenesis of AA. This fact is confirmed by abnormalities in humoral and cellular response, reported by the majority of authors, and the coexistence with other autoimmune diseases[10]. Numerous studies confirmed a more frequent occurrence of thyroid diseases in patients with AA (8–11.8%) as compared with the general population (2%)[16]. According to literature data, patients with AA suffer from vitiligo 4-times more frequently[1]. In 4 of our patients, concomi-

Table 1. Frequency of HLA-DRB1 alleles in patients with AA compared with the control group

HLA-DRB1	Frequency of alleles		OR	95% CI	p
	AA n=104	controls n=304			
*01	0.154	0.102			n.s.
*15*16*	0.260	0.168	1.74	1.02–2.96	0.04; p_{cor} = n.s.
*03	0.019	0.102	0.17	0.04–0.73	0.004; p_{cor} = 0.05
*04	0.135	0.125			n.s.
*07	0.106	0.145			n.s.
*08	0.009	0.023			n.s.
*09	0.000	0.010			n.s.
*10	0.009	0.023			n.s.
*11	0.183	0.138			n.s.
*12	0.029	0.046			n.s.
*13	0.067	0.099			n.s.
*14	0.020	0.020			n.s.

n – number of chromosomes, n.s. – not statistically significant.

Table 2. Frequency of HLA-DRB1 alleles in patients with AAV compared with the control group

HLA-DRB1	Frequency of alleles		OR	95% CI	p
	AAV n=172	controls n=304			
*01	0.153	0.102			n.s.
*15*16*	0.292	0.168	2.04	1.13–3.69	0.02; p_{cor} = n.s.
*03	0.014	0.102	0.12	0.02–0.92	0.016; p_{cor} = n.s.
*04	0.125	0.125			n.s.
*11	0.167	0.138			n.s.

n – number of chromosomes, n.s. – not statistically significant.

Table 3. Frequency of HLA-DRB1 alleles in patients with AAT/AAU compared with the control group

HLA-DRB1	Frequency of alleles		p
	AAT/AAU n=32	controls n=304	
*01	0.156	0.102	n.s.
*15*16*	0.188	0.168	n.s.
*03	0.031	0.102	n.s.
*04	0.156	0.125	n.s.
*11	0.219	0.138	n.s.

n – number of chromosomes, n.s. – not statistically significant.

Table 4. Frequency of HLA-DRB1 alleles in patients with AA in relation to onset of disease (before or after 30 years of age)

HLA-DRB1	Frequency of alleles		p
	before 30 years n=72	after 30 years n=32	
*01	0.111	0.250	n.s.
*15*16*	0.278	0.219	n.s.
*03	0.014	0.031	n.s.
*04	0.111	0.188	n.s.
*11	0.222	0.094	n.s.

n – number of chromosomes, n.s. – not statistically significant.

tant autoimmune thyroid diseases were seen, and in one case vitiligo.

A number of studies confirm the frequent concurrence of AA and atopic diseases[16]. The presence of atopy precludes a more severe course of the disease[2]. Among our patients, atopy was seen in 5 cases, and in 3 other cases atopy in family members.

Studies of histocompatibility antigens in patients with AA in the American population revealed more frequent occurrence of alleles HLA-DR4 and HLA-DR11 in the studied groups[2, 4, 5, 12, 18]. HLA-DR4 was more frequent in severe form and long-standing AA[3].

COLOMBE et al.[2] claim that HLA-DRB1*1104 is connected with more severe forms of AA, but it is not significantly more frequent in patients with AAV. DUVIC et al.[5] in a study group of 86 patients found that among the DR11 alleles, DRB1*1104 was significantly more frequent in patients with patchy AA (AAV).

Our study does not confirm significantly higher occurences of HLA-DR4 and HLA-DR11 alleles in patients with AA; however, HLA-DR11 occurred slightly more frequently than other alleles, especially in patients with more severe forms of AA (n = 0.219; control group n = 0.138). This would possibly be stronger if the sample were larger.

In our study group of 52 patients, we observed a lower occurrence of the allele HLA-DRB1*03. We have not found other studies concerning HLA-DRB1*03 occurence in alopecia areata in accessible literature.

In our study group we found a more frequent, albeit nonsignificant, occurrence of allele HLA-DRB1*15/ /*16. COLOMBE et al.[2] observed a high occurrence of this allele in recently diagnosed patients, though this was not statistically significant (either). The fact that HLA-DR11 occurs slightly more frequently in AAT/ /AAU, whereas HLA-DR*15/*16 is associated rather with AAV, may result from genetic heterogeneity between clinical forms of AA. This trend was also emphasized by GALBRAITH and PANDEY[8] in their study concerning TNF-α polymorphisms. The observed associations of HLA-DRB1 alleles with alopecia areata may result rather from linkage disequilibrium with HLA-DQ alleles, which seem to be strongly associated with the disease.

HLA-association studies carried out so far were made on the American population and, hence, there is no data to make comparisons with European countries.

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References

1. BERTOLINO A. P. (2000): Alopecia areata. Postgrad. Med., **107**, 81–90.
2. COLOMBE B. W., PRICE V. H., KHOURY E. L., GAROVOY M. R. and LOU C. D. (1995): HLA class II antigen associations help to define two types of alopecia areata. J. Am. Acad. Dermatol., **33**, 757–764.
3. COLOMBE B. W., PRICE V. H., KHOURY E. L. and LOU C. D. (1995): Class II alleles in long-standing alopecia totalis/alopecia universalis and long-standing patchy alopecia areata differentiate these two clinical groups. J. Invest. Dermatol., **104** (suppl.), 4S–6S.
4. DUVIC M., HORDINSKY M. K., FIEDLER V. C., O'BRIEN W. R., YOUNG R. and REVEILLE J. D. (1991): HLA-D locus associations in alopecia areata: DRRw52a may confer disease resistance. Arch. Dermatol., **127**, 64–68.
5. DUVIC M., WELSH E. A., JACKOW C., PAPADOPOULOS E., REVEILLE J. D. and AMOS C. (1995): Analysis of HLA-D locus alleles in alopecia areata patients and families. Arch. Dermatol., **104** (suppl.), 5S–6S.
6. FIEDLER V. C. (1992): Alopecia areata. A review of efficacy, safety and mechanism. Arch. Dermatol., **128**, 1519–1529.
7. FRENTZ G., THOMSEN K., JACOBSEN B. K. and SVEJGAARD A. (1986): HLA-DR4 in alopecia areata (letter). J. Am. Acad. Dermatol., **14**, 129.
8. GALBRAITH G. M. and PANDEY J. P. (1995): Tumor necrosis factor α (TNF-α) gene polymorphism in alopecia areata. Hum. Genet., **96**, 433–436.
9. GARCIA-HERNANDEZ M. J., TORRES M. J., PALOMARES J. C., RODRIGUEZ-PICHARDO A., AZNAR J. and CAMACHO F. (1998): No evidence of cytomegalovirus DNA in alopecia areata. J. Invest. Dermatol., **110**, 185.
10. HOFFMANN R. and HAPPLE R. (1999): Alopecia areata. 1: Clinik, etiology, pathogenesis. Hautarzt, **50**, 222–232.
11. JACKOW C., PUFFER N., HORDINSKY M., NELSON J., TARRAND J. and DUVIC M. (1998): Alopecia areata and cytomegalovirus infection in twins: genes versus environment? J. Am. Acad. Dermatol., **38**, 418–425.
12. MADANI S. and SHAPIRO J. (2000): Alopecia areata update. J. Am. Acad. Dermatol., **42**, 549–566.
13. MCDONAGH A. J. G. and MESSENGER A. G. (1996): The pathogenesis of alopecia areata. Dermatol. Clin., **14**, 661–670.
14. MITCHELL A. J. and KRULL E. A. (1984): Alopecia areata: pathogenesis and treatment. J. Am. Acad. Dermatol., **11**, 763–775.
15. SCERRI L. and PACE J. L. (1992): Identical twins with identical alopecia areata. J. Am. Acad. Dermatol., **27**, 766–767.
16. SHELLOW W. V., EDWARDS J. E. and KOO J. Y. (1992): Profile of alopecia areata: a questionnaire analysis of patient and family. Int. J. Dermatol., **31**, 186–189.
17. WEISE K., KRETZSCHMAR L., JOHN S. M. and HAMM H. (1996): Topical immunotherapy in alopecia areata: anamnestic and clinical criteria of prognostic significance. Dermatology, **192**, 129–133.
18. WOSZCZEK G. (2000): Rola głównego układu zgodności tkankowej (MHC) w odpowiedzi immunologicznej. In KOWALSKI M.: Immunologia kliniczna. Mediton, Łódź, **2**, 33–48.

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