Med Sci Monit, 2000; 6(2): 336-341

HLA class I haplotypes in families of children with coeliac disease

Anna Kędzierska, Gabriel Turowski

Independent Laboratory of Clinical Immunology, Faculty of Medicine, Collegium Medicum, Jagiellonian University, Cracow, Poland

key words: HLA system, haplotype frequency, coeliac disease

SUMMARY

The purpose of the study was the analysis of HLA-AB haplotypes frequency in the families of children with coeliac disease. Haplotypes present in 46 probands' families including 69 affected children, 49 healthy siblings and 91 parents were verified. HLA antigens were typed by Terasaki and McClelland's routine two-step-microcytotoxic assay in NIH modification. Among 138 haplotypes, the following were significantly more frequent in affected children: HLA-A1-B8 (3116×10^{4}), HLA-A3-B8 (290×10^{4}) and HLA-A2-B8 (217×10^{4}). Total frequency of haplotypes including HLA-B8 antigen in comparison to control population equalled 3986×10^{4} vs. 763×10^{4}. HLA-A1-B8 haplotype frequency was twice lower in probands' healthy siblings and parents, equalling 1837×10^{4} and 1868×10^{4}, respectively. Highly significantly more frequent HLA-A1-B8 haplotype found in probands' families may indicate the correlation between inherited gene products and increased risk of coeliac disease incidence.

INTRODUCTION

The discovery of highly polymorphic histocompatibilty complex antigens (HLA) made it possible to conduct multiple studies on the way of inheriting HLA antigens in families and on their prevalence in various populations and ethnic groups [1,2,3]. HLA system genes are codominant, which means that two alleles of specific locus are manifested simultaneously in the phenotype of heterozygote. Genetic products of these loci are inherited in compliance with Mendel's law as autosomal features. Each person, then, possesses two 'half-sets' (haplotypes) of genes, each of them inherited from one parent. Each haplotype undergoes expression to the same extent. Haplotypes contain sets of genes of all sites of HLA region, and their expected frequency in population is a derivative of the probability of accidental meeting of specific alleles [4].

Multiple studies showed that the presence of specific HLA antigens in phenotype may lead to increased or decreased risk in the incidence of specific diseases. It was showed that some HLA haplotypes may protect from infectious factors and own antigens. The comparison of HLA haplotypes related to severe anaemia in course of malaria revealed that DRB1*1302-DQB1*0501 haplotype, which is widespread in Western Africa, prevents lethal consequences of *Plasmodium falciparum* infections. The DRB1*1302 particles bind different peptides than DRB1*1301. This depends on the difference in one amino acid in β chain which may influence the response to malaria parasite [5].

The relation of HLA system with increased incidence of the disease is determined in family or population studies. The determination of linkage between parental haplotype and the disease transmitted to offspring (especially in families with many children and more than two generations) is extremely useful, although sometimes the disease may not manifest itself due to incomplete penetration of the gene. The determination of the interrelationship between inherited gene products and susceptibility to various diseases became possible

Received: 1999.03.08
Accepted: 1999.12.10

Correspondence address: Anna Kędzierska PhD, Independent Laboratory of Clinical Immunology, Faculty of Medicine, Collegium Medicum, Jagiellonian University, ul. Grzegórzecka 16, 31-531 Cracow, Poland

due to the phenomenon of non-accidental allele linkage, i.e. linkage disequilibrium. The consequence of this phenomenon is that the occurrence of HLA antigens and haplotypes is repeatedly more frequent than one might expect it to be in a random selection. The phenomenon may be explained by a selective pressure of the environment in the propagation or elimination of specific haplotypes. Genetic recombinations may decrease the role of the phenomenon. Sometimes not only two, but many alleles of adjacent genes are involved. The role of specific haplotype in the determination of susceptibility to the disease was explained hypothetically by many various mechanisms [2,3,6,7].

In this study, the analysis of haplotype frequency was performed in the families of children suffering from primary gluten intolerance, their healthy siblings and parents. The absence of studies published on the association between HLA class I haplotypes with coeliac disease inclined the authors to present this work.

MATERIAL AND METHODS

Genetic analysis was applied to haplotypes present in 46 investigated families consisting in total of 91 parents and 118 children, including 69 patients with clinically and histologically confirmed primary gluten intolerance and their 49 healthy siblings. Affected children were treated in General Paediatric Department of Silesian Medical University in Zabrze and in 2nd Department of Paediatric Diseases of Polish-American Paediatric Institute at the Faculty of Medicine of Jagiellonian University in Cracow.

HLA class I histocompatibility complex antigens, in range of generally occurring specificities, were typed in routine way with the use of two-phase microcytotoxic assay according to Terasaki and McClelland [8] in NIH modification [9]. The typing was applied to 14 specificities determined by HLA-A locus, 20 specificities determined by HLA-B locus and 7 locus C antigens.

Table 1. HLA-AB haplotype frequency in affected children and healthy siblings.

Haplotypes	Haplotype frequency ($f \times 10^4$)		X^2	p	Haplotype frequency ($f \times 10^4$)	X^2	p
	Affected children	Control population			Healthy siblings		
A1-B8	3116	609	99.10	<0.0000...1	1837	21.05	<0.0000...1
A1-B35	72	81	0.16		102	0.13	
A2-B7	290	308	0.02		-	-	
A2-B8	217	16	8.83	<0.0030	102	0.38	
A2-B13	145	114	0.01		306	1.36	
A2-B27	217	284	0.03		284	0.56	
A2-B35	-	114	-		102	0.15	
A2-B38	-	89	-		102	0.18	
A2-B44	290	560	2.30	<0.0510	510	0.00	
A2-B60	-	138	-		306	0.78	
A3-B7	652	568	0.04		510	0.00	
A3-B8	290	24	12.38	<0.0004	204	3.77	<0.0523
A3-B35	145	381	1.39		204	0.38	
A11-B8	145	32	1.48		-	-	
A24-B7	145	106	0.00		102	0.23	
A24-B13	145	89	0.03		204	0.33	
A24-B35	145	179	0.00		102	0.02	
A24-Bx	72	24	0.03		102	0.15	
A25-B8	145	24	2.20		102	0.15	
A25-Bx	145	32	1.48		510	24.13	<0.0000...1
A29-B44	72	65	0.20		204	0.86	
A68-B35	-	97	-		102	0.24	
Ax-B35	145	8	5.29	<0.0214	102	0.91	
Ax-B51	-	8	-		102	0.91	
other	6594	3953			6020		

Anti-HLA sera used originated from Serum Bank of the National Institutes of Health in Bethesda, from commercial companies (Behringwerke-Hoechst, Fresenius, Biotest) and from the collection of Independent Laboratory of Clinical Immunology at the Faculty of Medicine of Jagiellonian University in Cracow [10].

Haplotype frequencies in examined groups were compared to the second control group including 616 unrelated people, men and women in 308 families, in which 1232 haplotypes were determined [11,12] on the basis of segregation in families. The frequency was calculated per 10.000 cases (f x10^4) according to the following equation:

f x10^4 = frequency obtained / the number of haplotypes determined

Statistical analysis

The significance of differences between analysed groups was evaluated on the basis of chi-squared test with Yates' modification.

RESULTS

HLA antigens typing in families of children with primary gluten intolerance enabled the determination of haplotypes and their frequencies in affected children, healthy siblings and parents. Table 1 and 2 present the most frequent HLA-A and HLA-B haplotypes (f x10^4) in these groups as well as the comparison of their frequencies in unrelated marriages in Polish population.

Among 138 haplotypes determined in children with coeliac disease the following were of highly significant frequency ($p<0.0000...1–0.0030$): A1-B8 ($chi^2 = 99.10$), A3-B8 ($chi^2=12.38$), A2-B8 ($chi^2=8.83$). On the other hand, the frequency of Ax-B35 haplotype was different at the level of significance $p<0.0214$. A tendency for increased frequency was observed for A25-B8 haplotype ($f=145\times10^4$), although without statistical significance of differences. Haplotypes including HLA-B8 antigen occurred with the frequency of 3986×10^4 in affected children when compared to control group ($f=763\times10^4$), and their frequency differed significantly ($chi^2=132.95$; $p<0.0000...1$). Fifty-five HLA-B8 haplotypes with the following antigens determined by locus A were obtained: A1 – 43 times, A3 – 4 times, A2 – twice, A11, A25 – twice and A24 and determined by locus C:Cw7 (Table 1).

Table 2. HLA-AB haplotype frequency in parents of children with coeliac disease.

Haplotypes	Haplotype frequency (f x10^4)		X^2	p
	Parenteral population	Control population		
A1-B8	1868	609	35.35	<0.0000...1
A1-B35	110	81	0.00	
A2-B7	110	308	1.61	
A2-B8	55	16	0.04	
A2-B13	385	114	6.21	<0.0127
A2-B27	220	284	0.06	
A2-B35	110	114	0.11	
A2-B38	165	89	0.31	
A2-B44	55	560	7.56	<0.0060
A2-B60	110	138	0.00	
A3-B7	440	568	0.29	
A3-B8	165	24	4.46	<0.0348
A3-B35	165	381	1.59	
A11-B8	55	32	0.04	
A24-B7	165	106	0.11	
A24-B13	110	89	0.02	
A24-B35	110	179	0.13	
A24-Bx	275	24	13.50	<0.0002
A25-B8	55	24	0.00	
A25-Bx	165	32	3.27	
A29-B44	110	65	0.04	
A68-B35	110	97	0.06	
Ax-B35	220	8	14.60	<0.0001
Ax-B51	275	8	20.74	<0.0000...1
other	5604	3953		

As far as healthy siblings and probands' parents are concerned, haplotype HLA-A1-B8 occurred significantly more frequently ($p<0.0000...1$), as expected. It was present in 18 cases with the frequency of 1837×10^4, whereas in parents it was found in 34 cases with the frequency of 1868×10^4. In both groups, the frequency of HLA-A1-B8 haplotype was twice as low as in affected children. It was also found that the following haplotypes were highly significantly more frequent in the population of parents ($p<0.0000...1–0.0001$): Ax-B51, Ax-B35, A24-Bx, while haplotypes A2-B13 ($chi^2=6.21$; $p<0.0127$) and A3-B8 ($chi^2=4.46$; $p<0.0348$) were significantly more frequent also in healthy siblings ($chi^2=3.77$; $p<0.0523$). A2-B44 haplotype was statistically significantly less frequent. Its frequency in probands' parents was 55×10^4 while it was 560×10^4 in control group. The differences between the frequencies were highly statistically significant ($p<0.006$) (Table 2).

DISCUSSION

Familial studies allowed for the determination of the frequency of class I haplotypes in children with primary gluten intolerance as well as healthy siblings and probands' parents. In 46 families examined, the haplotypes including HLA-B8 antigen were highly significantly more frequent when compared to their frequency in the population of control families. Among these, HLA-A1-B8 deserves particular attention. It was 5 times more frequent in affected children ($f=3116\times10^4$) than in controls ($f=609\times10^4$). It was 3 times more frequent in healthy siblings ($f=1837\times10^4$) and in probands' parents ($f=1868\times10^4$). Among the children with coeliac disease, HLA-A1-B8 and HLA-A3-B8 haplotypes accounted for 34% of all haplotypes observed. It was found in examined families of children with primary gluten intolerance that HLA-A1-B8 haplotype was linked to HLA-Cw7 antigen. Estimated frequency of HLA-A1-B8-Cw7 haplotype demonstrated in earlier studies [13] was highly statistically significant in comparison to control population ($chi^2=121.4$; $p<0.0000...1$). Our studies showed that except for haplotypes containing HLA-B8 antigen, there were other significantly more frequent haplotypes in comparison to control population: HLA-A24-Bx, A25-Bx, Ax-B35, Ax-B51.

According to literature on the subject, patients with coeliac disease typically display HLA-A1-B8-Cw7-DR3-DQ2 haplotype. Alper et al. [14] estimated the frequency of HLA-ABC haplotypes in patients with coeliac disease assuming that these are the pathological haplotypes. The authors demonstrated that in patients with gluten malabsorption syndrome two preferential haplotypes are present: HLA-B8-DR3 and HLA-B44-DR7. According to the authors, the increase in the frequency of HLA-B8, HLA-DR3 and HLA-DR7 antigens in patients with primary gluten intolerance is secondary, determined by the increase in the frequency of two haplotypes specific for coeliac disease. According to Alper et al. [14] genes responsible for the incidence of coeliac disease are situated primarily on these chromosomes. This hypothesis is supported by *in vitro* studies in which varying reactivity of lymphocytes for gliadin was obtained depending on the set of antigen traits in haplotypes. Alper et al. [14] demonstrated in the analysed group of patients that unlike subjects with insulin dependent diabetes mellitus, HLA-B8-DR3 does not contain HLA-A1 antigen, but a number of different specificities determined by locus A.

In Polish literature on coeliac disease, the frequency of HLA haplotypes on the basis of familial studies was not analysed comprehensively, except for the data published in 1992 by Siekiera [15]. The author analysed the determinations of HLA-ABC and HLA-DR haplotypes in 51 probands' families from Upper Silesian region. She indicated significantly higher frequency of HLA-A1-B8-Cw7-DR3 haplotype, whose frequency in affected children, healthy siblings and parents equalled 3942×10^4, 1758×10^4 and 2009×10^4, respectively. She did not observe differences in the frequency of other HLA-ABC haplotypes.

Analysing the frequencies of haplotypes in the families of children with classical type of congenital adrenal hyperplasia (CAH), Turowska-Heydel et al. [16] demonstrated that the frequency of HLA-A1-B8-Cw7-DR3 haplotype in the population of affected children's parents was similar to control population. However, it was lower in the group of affected children and equalled 277×10^4 vs. 645×10^4 in controls ($chi^2= 1.5579$). According to Dupont et al. [17] and Pollack [18], HLA-A1-B8-Cw7-DR3 haplotype may prevent the disease manifestation.

According to Bodmer et al. [19] and Cepellini et al. [20], the studies on haplotypes in the aspect of their population and ethnic prevalence contributed to the determination of widespread, white-race haplotype of HLA-A1-B8-Cw7-DR3. On the basis of the studies conducted on 13 European populations, the highest frequency of HLA-A1-B8-DR3 haplotype was found in Scandinavia and among Celts, while it was relatively frequent in remaining groups, too [19]. This suggests strong and selective pressure to retain this haplotype in the white race populations. According to Baisch and Capra [21], the example for such coexistence is the combination of alleles encoding HLA-A1, -B8 and DR3 particles. It is estimated that the frequency of HLA-A*0101 allele for the heavy chain of HLA-A1 and HLA-B*0801 allele for the heavy chain of HLA-B8 in haplotypes equals around 1.4%. Meanwhile, the frequency of their co-occurrence reaches 8.8%, just as in the case of a pair of alleles encoding HLA-B8 and DR3 particles. According to the authors, other allelic combinations found most frequently in white race people include HLA-A2-B15-DR4 and HLA-A3-B7-DRB1*0501 haplotypes. Individuals with HLA-A1-B8-DR3 particles encoded by genes remaining in linkage disequilibrium tend to suffer from autoimmune diseases (such as insulin dependent diabetes mellitus, myasthenia

gravis and Graves-Basedow disease) more often. According to Candore et al. [22] who in 1995 conducted *in vitro* studies on the activation of T-lymphocytes from people with HLA-B8-DR3 haplotype, the genes predisposing to the development of these ailments remained linked to this specific haplotype.

The association of positive correlation between coeliac disease and HLA-A1 and HLA-B8 antigens present in haplotype still attracts researchers' attention, despite the fact that the correlation between coeliac disease and the presence of certain antigens determined by HLA-DR and HLA-DQ loci was shown [23,24]. The most important correlations refer to DR3-DQ2 or DR5/7-DQ2 haplotypes. The α/βheterodimeric HLA-DQ2 particle is encoded by DQA1*0501 and DQB1*0201 alleles, whilst HLA-DR3 is encoded by DRA and DRB1*0301 alleles. The studies on HLA system haplotypes demonstrated that DR7 allele was linked to DQA1*0201 and DQB1*0201 alleles whilst DR5 allele was linked to DQA1*0501 and DQB1*0301. Thus, people with heterozygotic combination of DR5/DR7 possess the same DQ alleles combination (DQA1*0501, DQB1*0201) as people with HLA-DR3 in their phenotype. According to Tighe et al. [25], the correlation between coeliac disease with such a combination of alleles is recorded in 98% of people coming from Northern Europe and in 92% from southern part of the continent. The authors claim that additional associations of coeliac disease with HLA-DPB1 alleles cannot be excluded. The studies on the population of people from Northern Europe suffering from coeliac disease showed the presence of correlation with DP1 allele, found in HLA-B8-DR3-DQ2-DP1 haplotype [26]. Although the association of coeliac disease with some of the loci HLA-DQ alleles is the most significant relationship within HLA system antigens, not all affected people display DQ2 particle. Additional associations (although in much lower percentage of patients) were observed for DR4-DQ8 haplotype (DQA1*0301, DQB1*0302). According to Tighe et al. [25], this may indicate the effect of environmental or genetic factors.

CONCLUSION

The determination of highly significantly more frequent occurrence of HLA-A1-B8 haplotype in probands' families may indicate the presence of correlation between inherited genetic products and increased risk for coeliac disease.

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