HLA types in Turkish children with celiac disease

Zarife Kuloğlu[1], Tümay Doğancı[2], Aydan Kansu[1], Fulya Demirçeken[1], Murat Duman[3], Hüseyin Tutkak[3], Arzu Ensari[4], Nurten Girgin[1]

[2]Department of Pediatric Gastroenterology, Dışkapı Social Security Hospital, and Departments of [1]Pediatric Gastroenterology, [3]Immunology and [4]Pathology, Ankara University Faculty of Medicine, Ankara, Turkey

SUMMARY: Kuloğlu Z, Doğancı T, Kansu A, Demirçeken F, Duman M, Tutkak H, Ensari A, Girgin N. HLA types in Turkish children with celiac disease. Turk J Pediatr 2008; 50: 515-520.

The aim of this study was to assess the distribution of human leukocyte antigen (HLA) groups in Turkish children with celiac disease (CD) and to investigate the association of HLA types and clinical manifestations of CD.

Seventy-five children with CD were evaluated in two groups: Group I consisted of 45 classical celiac patients (15 males, 6.7±3.8 years); Group II consisted of 30 atypical celiac patients (9 males, 9.3±4.3 years). The control group consisted of 100 healthy renal transplantation donors. HLA typing was made serologically using standard lymphocytotoxicity techniques.

HLA A29, B51, CW5, DR14, DR16, and DQ1 were the most common antigens in the control group. Frequency of HLA B13, CW7, B8, DR7, DR17 and DQ2 was higher in CD patients than in the control group ($p<0.005$, <0.05, <0.001, <0.001 and <0.001, respectively). The relative risks for HLA DQ2, B8, DR17 and B13 were 14.9, 13.6, 7.1 and 3.6, respectively. Frequency of HLA B35, DR11 and DQ7 was higher in classical CD than atypical CD, while a positive association was found between HLA B8 and atypical CD.

A positive association was found between HLA B13, CW7 and DR17 in Turkish celiac patients in addition to HLA B8, DR7 and DQ2. This study also suggested that a correlation may exist between genotype and clinical manifestations.

Key words: celiac disease, HLA antigens.

Celiac disease (CD) or gluten sensitive enteropathy is a disease of the proximal small intestine, and is characterized by abnormal small intestinal mucosae and associated with permanent intolerance to dietary gluten[1]. Genetic predisposition is an essential factor in the development of CD. Gluten sensitive enteropathy has often been used to refer to the condition of every individual with gluten sensitivity regardless of clinical findings or degree of mucosal involvement. CD can present at any age and in different clinical forms such as classical, atypical, silent, or latent CD[2,3]. Classical CD is characterized by chronic diarrhea, abdominal distention, vomiting, muscle wasting and failure to thrive. The timing of presentation of CD may be dependent on the amount and timing of gluten introduction to the diet[4]. Within weeks to months of starting to ingest gluten, stool becomes larger, softer, paler and more frequent than usual, and abdominal distention develops and growth is impaired. Secondary to impaired absorption, anemia, hypoalbuminemia, hypocalcemia, hypomagnesemia, hypoprothrombinemia and zinc deficiency may occur. Atypical CD presents with extraintestinal manifestations such as unexplained iron deficiency anemia, short stature, osteoporosis, pubertal delay, dental enamel defects, and abnormalities in liver function tests[4,5]. The symptoms in patients are variable. At present, there is not yet an explanation as to why some patients with CD are symptomatic while others remain asymptomatic.

Genetic, environmental and immunological factors may play important roles in the pathogenesis of the disease[3,5]. A significant association between CD and human leukocyte antigen (HLA) types was shown both in family

and population studies. CD was first found to be associated with HLA B8. Later, it was shown that the disease is associated with expression of HLA DQ2 and HLA DQ8[6-8]. The frequency of HLA groups varies significantly in different populations. Although association of HLA group and CD has been reported in numerous studies, the influence of HLA type on the clinical manifestations of CD has not yet been investigated. The aim of this study was to assess the distribution of HLA groups in Turkish children with CD and to investigate the association of HLA type and clinical manifestations of CD.

Material and Methods

Study Subjects

Seventy-five children (51 girls, 24 boys) diagnosed as CD according to the European Society of Pediatric Gastroenterology and Nutrition criteria were included in our study. The mean age of the study population was 7.3±4.09 years (range: 1 to 16 years). They were divided into two groups according to clinical manifestations at diagnosis: Group I consisted of 45 patients with classical CD (15 boys, mean age: 6.7±3.8 years, range: 1-14 years); Group II consisted of 30 patients with atypical CD (9 boys, mean age: 9.3±4.3 years, range: 2-16 years). One hundred (54 M, 46 F) randomly selected individuals from among renal transplant donors served as healthy control subjects.

HLA Typing

Human leukocyte antigen class I and II typing was studied serologically by the standard microlymphocytotoxicity method[6].

Statistical Analysis

The distribution of HLA types in the groups of patients was compared using χ^2 test. When frequency of HLA types was below 2%, Fisher's exact test was used. $P<0.05$ was considered significant. In order to estimate relative risk, the odds ratio method was also used.

Results

The distribution of HLA groups/types in CD patients and controls is shown in Tables I and II. In the control group, HLA A29 (8%), B51 (22%), CW5 (10%), DR1 (17%), DR14 (15%), DR16 (11%), and DQ1 (38%) were the most common HLA types observed in HLA groups A, B, DR and DQ, respectively. In CD patients, HLA B8 (41.9%), B13 (14.9%), CW7 (48.6%) ($p<0.05$), DR7 (43.1%), DR17 (17.7%) and DQ2 (84.7%) ($p<0.001$) were the most common HLA types observed in HLA groups A, B, C, DR and DQ, respectively.

In odds ratio test, HLA DQ2, B8, DR17 and B13 were demonstrated to have higher risk for development of CD (14.9, 13.6, 7.1 and 3.6, respectively) (Table III).

Classical CD was significantly associated with HLA B35 (24.4%) ($p<0.05$), DR11 (36.7%) ($p<0.01$) and DQ7 (31.8%) ($p<0.05$). In atypical CD, HLA B8 (58.6%) was the most common HLA type in HLA groups ($p<0.05$) (Tables IV, V).

Discussion

The observations that CD may occur in first-degree relatives of children with CD and association between some HLA groups and CD indicate the influence of genetic factors in the disease predisposition. It is demonstrated in both patients and family studies that the genetic feature of CD is multifactorial[3-5].

Exposure to gluten in genetically susceptible individuals is the major factor of development of CD. A gluten molecule contains two fractions: gliadin and glutenin. Gliadin fraction is known to be the main cause of CD. The possible interactions of gliadin (and/or its peptide derivates) with intestinal epithelia and the mechanism through which it crosses the epithelial barrier to reach the submucosa are not yet completely explained. After passage of gliadin into the subepithelial compartment, toxic gliadin derivates are deaminated by tissue transglutaminase. Deaminated gliadin epitopes are taken up by antigen presenting cells and are presented by MHC class II molecules HLA DQ2 and/or DQ8 to CD4+ helper T lymphocytes. Activation of T cells results in release of proinflammatory mediators causing damage of enterocytes resulting in villous atrophy[9-11]. It is thought that class II HLAs are efficient to present peptides produced from digestion of gliadin in the lamina propria of the small bowel mucosa to T cells[11,12]. It is known that most celiac patients (>90%) carry a particular DQ heterodimer encoded by

Table I. The Frequency of HLA-A, B and C in Celiac Disease Patients and Control Group

HLA-A	CD (%)	Control (%)	P	HLA-B	CD (%)	Control (%)	p	HLA-C	CD (%)	Control (%)	p
A1	29.7	24	>0.05	B8	41.9	5	<0.01	CW	4.1	0	>0.05
A2	41.9	45	>0.05	B7	5.4	12	>0.05	CW1	6.8	12	>0.05
A3	20.3	24	>0.05	B13	14.9	5	<0.05	**CW2**	9.5	10	>0.05
A9	0	1	>0.05	B14	1.4	3	>0.05	CW3	10.8	9	>0.05
A11	14.9	12	>0.05	B15	0	1	>0.05	CW4	21.6	26	>0.05
A23	8.1	5	>0.05	B17	1	0	>0.05	CW5	0	10	<0.05
A24	20.3	21	>0.05	B18	5.4	1	>0.05	CW6	24.3	21	>0.05
A26	13.5	14	>0.05	B27	5.4	6	>0.05	CW7	48.6	33	<0.05
A29	0	8	<0.05	B35	16.4	26	>0.05	CW8	0	2	>0.05
A28	5.4	4	>0.05	B38	8.1	8	>0.05				
A30	0.5	8	>0.05	B37	0	2	>0.05				
A31	1.4	3	>0.05	B39	2.7	6	>0.05				
A32	5.4	5	>0.05	B40	0	3	>0.05				
A33	0	2	>0.05	B41	4.1	4	>0.05				
A34	1.4	0	>0.05	B44	12.2	15	>0.05				
A35	0	0	>0.05	B48	0	2	>0.05				
A36	1.4	1	>0.05	B49	8.1	10	>0.05				
A66	1.4	3	>0.05	B50	9.5	6	>0.05				
A68	2.7	3	>0.05	B51	9.5	22	<0.05				
A69	2.7	1	>0.05	B52	4.1	7	>0.05				
A74	1.4	1	>0.05	B53	0	1	>0.05				
				B54	2.7	1	>0.05				
				B55	2.7	12	<0.05				
				B56	1.4	1	>0.05				
				B57	4.1	4	>0.05				
				B58	1.4	4	>0.05				
				B62	2.7	3	>0.05				
				B63	1.4	0	>0.05				
				B64	0	1	>0.05				
				B65	0	2	>0.05				
				B71	1.4	0	>0.05				
				B72	1.4	0	>0.05				
				B73	0	1	>0.05				
				B75	1.4	1	>0.05				
				B78	0	1	>0.05				
				BW	1.4	0	>0.05				

Table II. The Frequency of HLA-DR and DQ in Celiac Disease Patients and Control Group

HLA DR	CD (%)	Control (%)	P	HLA DQ	CD (%)	Control (%)	P
D DR1	1	17	<0.01	DQ1	11.1	38	<0.01
DR4	22.2	32	>0.05	DQ2	84.7	27	<0.01
DR7	43.1	17	<0.01	DQ3	6.9	0	>0.05
DR8	2.8	3	>0.05	DQ4	1.4	3	>0.05
DR9	2.8	1	>0.05	DQ5(DQ1)	6.9	19	<0.05
DR10	2.8	0	>0.05	DQ6(DQ1)	4.2	15	<0.05
DR11	25	34	>0.05	DQ7(DQ3)	23.6	43	>0.05
DR12	2.8	3	>0.05	DQ8(DQ3)	15.3	19	>0.05
DR13	9.9	16	>0.05	DQ9(DQ3)	1.4	4	>0.05
DR14	2.8	15	<0.05				
DR15	8.3	22	<0.05				
DR16	1.4	11	<0.05				
DR17	57.7	16	<0.01				
DR51	2.8	30	<0.01				
DR52	51.4	71	<0.01				
DR53	47.2	50	>0.05				

Table III. HLA Types Associated with Celiac Disease

HLA	CD (n)	Control (n)	p	Odds ratio	Confidence interval
B8	31	8	<0.001	13.6	4.9-37.6
B13	11	5	<0.05	3.3	1.09-1.0
CW7	36	33	<0.05	1.9	1.03-3.5
DR7	31	17	<0.001	3.6	1.8-7.4
DR17	41	16	<0.001	7.17	3.5-14.6
DQ2	61	27	<0.001	14.9	6.8-32.6
A29	–	8	<0.005	0.073	0.004-1.28
B51	7	22	<0.05	0.37	0.14-0.9
B55	2	12	<0.05	0.2	0.4-0.9
CW5	–	10	<0.005	0.0059	0.003-1.07
DR14	2	15	<0.001	0.16	0.03-0.7
DR15	6	22	<0.05	0.3	0.12-0.84
DR16	1	11	<0.05	0.1	0.14-0.91
DQ1	8	38	<0.001	0.2	0.88-0.47
DQ5	5	19	<0.05	0.31	0.11-0.89
DQ6	3	15	<0.05	0.24	0.68-0.88

Table IV. The Frequency of HLA-A, B and C in Classical and Atypical CD

HLA-A	Classical CD (%)	Atypical CD (%)	p	HLA-B	Classical CD (%)	Atypical CD (%)	p	HLA-C	Classical CD (%)	Atypical CD (%)	p
A1	31.1	27.6	>0.05	B8	31.1	58.6	<0.05	CW	2.2	6.9	>0.05
A2	42.2	41.4	>0.05	B7	4.4	6.9	>0.05	CW1	8.9	3.6	>0.05
A3	20	20.7	>0.05	B13	20	6.9	>0.05	CW2	11.2	6.9	>0.05
A9	2.2	0	>0.05	B14	0	3.4	>0.05	CW3	2.2	0	>0.05
A11	8.9	24.1	>0.05	B17	0	3.4	>0.05	CW4	15.6	31	>0.05
A23	4.4	13.4	>0.05	B18	9	0	>0.05	CW6	26.7	20.7	>0.05
A24	20	20.7	>0.05	B19	2.2	0	>0.05	CW7	44.4	55.2	>0.05
A26	13.3	13.8	>0.05	B27	8.9	0	>0.05				
A28	4.4	6.9	>0.05	B35	24.4	3.6	<0.05				
A30	11.4	6i9	>0.05	B38	6.7	10.3	>0.05				
A31	2.2	0	>0.05	B39	0	6.9	>0.05				
A32	6.7	3.4	>0.05	B41	4.4	3.4	>0.05				
A34	0	3.4	>0.05	B44	13.3	10.3	>0.05				
A36	0	3.4	>0.05	B49	8.9	6.9	>0.05				
A49	2.2	3.4	>0.05	B50	11.1	6.9	>0.05				
A66	2.2	0	>0.05	B51	6.7	13.8	>0.05				
A68	2.2	3.4	>0.05	B52	2.2	6.9	>0.05				
A69	2.2	3.4	>0.05	B54	4.4	0	>0.05				
A74	2.2	0	>0.05	B55	2.2	3.4	>0.05				
				B56	2.2	0	>0.05				
				B57	4.4	3.4	>0.05				
				B58	0	3.4	>0.05				
				B60	2.2	13.8	>0.05				
				B62	4.4	0	>0.05				
				B63	2.2	0	>0.05				
				B71	0	3.4	>0.05				
				B72	0	3.4	>0.05				
				B75	2.3	0	>0.05				

Table V. The Frequency of HLA-DR and DQ in Classical and Atypical CD

HLA DR	Classical CD (%)	Atypical CD (%)	p	HLA DQ	Classical CD (%)	Atypical CD (%)	p
DR1	0	3.7	>0.05	DQ1	6.8	17.9	>0.05
DR4	20.5	5	>0.05	DQ2	84.1	85.7	>0.05
DR7	45.5	39.3	>0.05	DQ3	9.1	3.6	>0.05
DR8	2.3	3.6	>0.05	DQ4	2.3	0	>0.05
DR9	4.3	0	>0.05	DQ5(DQ1)	6.8	7.1	>0.05
DR10	4.5	0	>0.05	DQ6(DQ1)	4.5	3.6	>0.05
DR11	36.4	7.1	<0.01	DQ7(DQ3)	31.8	10.7	<0.05
DR12	2.3	3.6	>0.05	DQ8(DQ3)	15.9	14.3	>0.05
DR13	9.3	10.7	>0.05	DQ9(DQ3)	0	3.4	>0.05
DR14	4.5	0	>0.05				
DR15	6.8	10.7	>0.05				
DR16	0	3.7	>0.05				
DR17	2.3	0	>0.05				
DR51	2.3	3.6	>0.05				
DR52	52.3	50	>0.05				
DR53	50	42.9	>0.05				

DQ2 (DQA1*0501/DQB 1*0201) alleles[12,13]. Association of HLA DQ alleles with CD can explain the immunological concept of the disease pathogenesis; however, it does not explain the wide range of disease manifestations.

Our results showed that HLA B8, B13, CW7, DR7, DR17 and DQ2 were found in higher frequencies in celiac patients. Firstly, frequency of HLA B8 in celiac patients has been reported as 45-88%[14]. Later, it was observed that frequency of HLA DR3 is also increased and that the association with HLA B8 simply reflected the linkage disequilibrium between the alleles that coded for these antigens[15]. In our study, frequency of HLA B8 in CD was 41.9%, which is similar to other reports. Our study confirmed the strong association of CD and HLA DQ2. However, HLA DQ8, which is known as one of the most common antigens in CD, was not found to be frequent among our celiac patients.

The association between HLA DR3/DR7 and CD is explained by the linkage disequilibrium of these alleles with DQ2 allele[16]. Although in our study, frequency of HLA DR7 in celiac patients was significantly increased, we did not demonstrate an association between DR3 and CD. Difference of HLA distribution may be associated with genetic heterogeneity and geographic region. While in northern Europe, frequency of HLA DR3 is 95%, in southern Europe, HLA DR3 and DR7 are the most common HLAs[17,18]. It was previously reported in Turkish children by Erkan et al.[19] and Altuntaş et al.[20] that HLA A2, A9, B35, B4, DR2, DR4, DR53, CW4 and DQ4 were the most common antigens among celiac patients. According to these data, we may conclude that the HLA groups that are found to be strongly associated with CD are not seen in high frequency in the general Turkish population.

Based on recent epidemiological data, prevalence of CD is similar in Europe and North America, but clinical presentation is different. Early onset classical form is more commonly seen in Europe than in North America. It is unclear why presentation of CD differs from country to country or person to person. It is suggested that the difference in clinical presentations may be due to HLA-related predisposing genes or gluten intake[21]. Genotype-phenotype association was reported by Vogelsang et al.[22], who found that HLA B8 and CW7 might lead to late onset of CD. To our knowledge, association of HLA types and clinical manifestations has not been studied previously. In our study, an association was found between HLA types and different forms of CD. While in classical CD patients with chronic diarrhea, weight loss, abdominal distention and malnutrition, HLA B35, DR11 and DQ7 were the most frequent antigens, HLA B8 was the most frequent antigen in atypical CD patients who presented with extraintestinal manifestations.

In conclusion, this study demonstrated that HLA types are important in the development of CD, and HLA B8, CW7, DR7, DQ2, B13

and DR17 were the most frequent antigens in Turkish children with CD. Our study also showed that a relation may exist between HLA types and clinical presentations.

REFERENCES

1. Branski D, Fasono A, Troncone R. Latest developments in the pathogenesis and treatment of celiac disease. J Pediatr 2006; 149: 295-300.
2. Treem WR. Emerging concepts in celiac disease. Curr Opin Pediatr 2004; 16: 552-559.
3. Madani S, Kamat D. Clinical guidelines for celiac disease in children: what does it mean to the pediatrician/ family practitioner? Clin Pediatr 2006; 45: 213-219.
4. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005; 40: 1-19.
5. Waker-Smith JA. Celiac disease. In: Walker WA, Durie PR, Hamilton JR, Walker-Smith JA, Wetkins JB (eds). Pediatric Gastrointestinal Disease (3rd ed). Ontario: B.C. Decker; 2004: 932-934.
6. Ludvig M, Sollid LM, Thorsby E. HLA susceptibility genes in coeliac disease: genetic mapping and role in pathogenesis. Gastroenterology 1993; 105: 910-922.
7. Falchuck ZM, Katz AJ, Schachman H, Rogetine GN, Strober W. Gluten sensitive enteropathy: genetic analysis and organ culture study in 35 families. Scand J Gastroenterol 1978; 13: 839-843.
8. Tosi R, Vismara D, Tanigaki N. Evidence that celiac disease is primarily associated with a DC locus allelic specificity. Clin Immunol Immunopathol 1983; 28: 395-404.
9. Robbins G, Howdle PD. Advances in celiac disease. Curr Opin Gastroenterol 2004; 21: 152-161.
10. Matysiak-Budnik T, Candalh C, Dugave C, et al. Alterations of the intestinal transport and processing of gliadin peptides in celiac disease. Gastroenterology 2003; 125: 696-707.
11. Molberg O, Kett K, Scott H, Thorsby E, Sollid LM, Lundin KE. Gliadin specific HLA-DQ–restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. Scand J Immunol 1997; 46: 103-109.
12. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for primary association of celiac disease to a particular HLA-DQ α/β heterodimer. J Exp Med 1989; 169: 345-350.
13. Clot F, Babron MC. Genetics of celiac disease. Mol Genet Metab 2000; 71: 76-80.
14. Mackintosh P, Asquitth P. HLA and coeliac disease. Br Med Bull 1978; 34: 291-294.
15. Pena AS, Mann DL, Hague NE. Genetic basis of gluten sensitive enteropathy. Gastroenterology 1978; 75: 230-235.
16. Corazza GR, Tabacchi P, Frisoni M, Prati C, Gasbarrini G. DR and non-DR allotypes are associated with susceptibility to coeliac disease. Gut 1985; 26: 1210-1213.
17. Tighe R, Ciclitera PJ. Molecular biology of coeliac disease. Arch Dis Child 1995; 73: 189-191.
18. Tighe MR, Hall MA, Barbado M, Cardi E, Welsh KI, Ciclitera PJ. HLA class II alleles associated with celiac disease susceptibility in a southern European population. Tissue Antigens 1992; 40: 90-97.
19. Erkan T, Kutlu T, Yılmaz E, Çullu F, Tümay G. Human leukocyte antigens in Turkish pediatric celiac patients. Turk J Pediatr 1999; 41: 181-188.
20. Altuntaş B, Hasanoğlu A, Söylemezoğlu O, Arınsoy T. Pattern of human leukocyte antigen in Turkish children with celiac disease. Pediatr Int 2000; 42: 678-681.
21. Fasano A, Catassi C. Coeliac disease in children. Best Pract Res Clin Gastroenterol 2005; 19: 467-478.
22. Vogelsang H, Panzer S, Mayr WR, et al. Distribution of HLA class I alleles differs in celiac disease patients according to age of onset. Dig Dis Sci 2003; 48: 611-614.