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Original/*Pediatría*

The influence of HLA-DQ2 heterodimers on the clinical features and laboratory of patients with celiac disease

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Abstract

Background and aim: **the essential genetic marker related with celiac disease (CD) is the HLA-DQ2 molecule encoded by the DQA1*0501 and DQB1*0201 genes. The aim of this study is to evaluate effect of these alleles on the clinical, serological and histological features of Turkish children with celiac disease.**

Material and methods: **we divided 36 celiac patients to 4 groups according to their HLA-DQ2 genotype based on the presence or absence of DQA1*0501 and DQB1*0201 alleles. *Group 1*: 4 patients had no HLA-DQ2A1*0501 and DQ2B1*0201 alleles, *Group 2*: 12 patients had at least one of these alleles with heterozygous status, *Group 3*: 12 patients had both alleles with heterozygous status, *Group 4*: 8 patients had both alleles with homozygous status. We compared groups according to the clinical, serological, histological, and biochemical features.**

Results: **there was no statistical significance among the groups for age, body mass index (BMI), weight for height, and onset of symptoms. However, both in groups 3 and 4 compared with groups 1 and 2, minor differences were observed for BMI and anti-gliadin antibody (AGA) without statistical significance. According to the anti-endomysial antibody (EMA), Marsh scores, clinical presentations, hematological and biochemical values, there was no statistical significance among groups without constipation that observed higher rate in the 4th group without statistically significance. Hypothyroidism was detected in one patient (25%) in the lowest genetic load group (Group 1) with statistically significance ($p<0.046$).**

Conclusion: **in this study, small differences found among groups were not elucidated the impact of HLA-DQ2 A1*0501 and DQ2B1*0201 alleles on the clinical, serological and laboratory manifestations of celiac patients. Further studies are needed to assess the effect of**

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LA INFLUENCIA DE LOS HETERODÍMEROS HLA-DQ2 EN LAS CARACTERÍSTICAS CLÍNICAS Y DE LABORATORIO DE LOS PACIENTES CON ENFERMEDAD CELÍACA

Resumen

Antecedentes y objetivo: **el marcador genético esencial relacionado con la enfermedad celíaca (CD) es la molécula HLA-DQ2 codificada por los genes DQA1*0501 y DQB1*0201. El objetivo de este estudio es evaluar el efecto de estos alelos en las características clínicas, serológicas e histológicas de los niños turcos que tienen la enfermedad celíaca.**

Material y métodos: **nosotros hemos dividido los 36 pacientes celíacos en 4 grupos de acuerdo con su genotipo HLA-DQ2 basado en la presencia o la ausencia de los alelos DQA1*0501 y DQB1*0201. *Grupo 1*: 4 pacientes que no tenían los alelos HLA-DQ2A1*0501 y DQ2B1*0201; *Grupo 2*: 12 pacientes que tenían por lo menos uno de estos alelos con un estado heterocigoto; *Grupo 3*: 12 pacientes que tenían ambos alelos con un estado heterocigoto; *Grupo 4*: 8 pacientes que tenían ambos alelos con un estado heterocigoto. Nosotros hemos comparado los grupos de acuerdo con las características clínicas, serológicas, histológicas y bioquímicas.**

Resultados: **no había significación estadística entre los grupos por edad, índice de masa corporal, (IMC), peso por altura y aparición de síntomas. Sin embargo, en los grupos 3 y 4 comparados con los grupos 1 y 2 se observaron unas diferencias menores en IMC y anticuerpos antigliadina (AGA), sin una significación estadística. De acuerdo con los antiendomisios (EMA), la puntuación Marsh, las presentaciones clínicas y los valores hematológicos y bioquímicos, no había una significación estadística entre los grupos sin estreñimiento respecto a los valores más altos observados en el grupo 4 sin significación estadística. Se detectó hipertiroidismo en un paciente (25%) del grupo de carga genética más baja (grupo 1) con significación estadística ($p<0,046$).**

El resultado: **en este estudio, las pequeñas diferencias que se encontraton entre los grupos no dilucidaron el impacto de los alelos HLA-DQ2 A1*0501 y DQ2B1*0201 en las manifectaciones clínicas, serológicas y de laboratorio de los pacientes celíacos. Se necesitan nuevos estudios para evaluar el efecto de los alelos HLA y otros polimor-**

reported HLA alleles and other genetic polymorphisms on CD outcomes in children.

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Key words*: Celiac disease. HLA-DQ2 alleles. Children.*

fismos genéticos en los resultados sobre la enfermedad celíaca en los niños.

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Palabras clave: *Enfermedad celíaca. Alelos HLA-DQ2. Niños.*

Introduction

Celiac disease (CD) is affect about 1-3% of population in worldwide. CD is an enteropathy of the small intestine in genetically predisposed individuals induced by exposure of gluten[1]. Symptoms and signs due to chronic inflammation of the small intestine mucosa such as anemia, chronic diarrhea, abdominal pain and malnutrition seem the characteristic presentation of CD[2,3].The diagnostic criteria of CD identified by European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) based on HLA genotyping (presence of HLA-DQ2 and/or DQ8), gluten-dependent symptoms, anti-tissue transglutaminase type 2, anti-endomysial antibody (EMA), anti-deaminated forms of gliadin, and specific histological changes[4]. The celiac patients have important genetic load with a strong relation with HLA-DQ2 and HLA-DQ8 haplotypes that are liable for 40% of the genetic risk. The HLA-DQ2 molecule are found in more than 90% of CD which encoded by HLA-DQA1*05/HLA-DQB1*02 and the remaining HLA DQ8 heterodimer are encoded by HLA DQA1*0301/HLA DQB1*0302[4]. Although many studies have been reported about the relevant of HLA genotypes and CD[5-7], however, about the effect of HLA genotypes on the clinical appearance of CD has been reported in few studies in recent years[8-10]. The goal of this study is to evaluate relationship between genetic predisposition (carrying HLA-DQ2A1*501 – HLA-DQ2B1*201 genotypes) and clinical, histological, biochemical features of CD and compare the groups with each other.

Patients and methods

Study group

Thirty six patients (24 Female, 12 Male) with a median age of 8.2±4.6 months (range, 6 months - 17 year) were enrolled in this study that followed up at the Department of Pediatric Gastroenterology of Istanbul University Medical Faculty in Istanbul, Turkey between in 1990 and 2000. The diagnosis of CD based on ESPGHAN criteria[11]. In this study patients were divided to 4 groups according to carrying HLA-DQ2 alleles (DQA1*0501 and DQB1*0201). In the first group (Group 1), 4 patients had neither HLA-DQA1*0501 nor HLA-DQB1*0201 alleles. In the second group (Group 2), 12 patients had either HLA-DQA1*0501 or HLA-DQB1*0201 alleles heterozygosity. In the third group (Group 3), 12 patients had both HLA-DQA1*0501 and HLA-DQB1*0201 alleles as heterozigosity. In the last group (Group 4), 8 patients had either HLA-DQA1*0501 or HLA-DQB1*0201 alleles as homozygosity.

HLA typing

DNAs were obtained from peripheral venous blood samples taken to the tubes containing ethylene diaminetetraacetic acid (EDTA). All samples were tested by polymerase chain reaction (PCR) with the sequence specific primers (Olerup, PCR-SSP kits, Stockholm, Sweden).

Serological tests

The values of anti-endomysial antibodies (EMA) were obtained by an indirect immunofluorescence method using distal esophagus monkey cryostat sections (Euroimmun laboratory, Germany). Serum samples were diluted 1/10 in phosphate-buffered saline. The slides were visualized by the specialist of Microbiology Department of Medical Faculty of Istanbul and results were given (+) or (-) manner. Antigliadin antibodies (AGA) IgA / IgG were determined by enzyme linked immunosorbent assay (ELISA) method at the laboratory of the Pediatric Gastroenterology of Medical Faculty of Istanbul. The upper levels of 25 AU (Arbitrary units) were accepted positive (Above 25 AU: 1+, above 100 AU: 2+, above 150 AU: 3+). All kits were used according to the manufacturer's instructions.

Histology

Biopsy samples were obtained from the duodenum and fixed in the Hollande solution. After, they were stained with haematoxylin and eosin stain (H & E) and assessed according to the Marsh criteria's.

Statistical analyses

Data were evaluated with Chi-square test ($\chi 2$) among the groups and a "p" value <0.05 was considered to be statistically significant.

Table I
Groups of celiac patients

PATIENTS	*HLA-DQ2A1*0501*	*HLA-DQ2B1*0201*	*Age (year)*	*Gender (M/F)*	*BMI*	*WFH (%)*	*AOS (Mean-months)*
Group 1							
P1	–	–	12.2	M	14.2	82	39
P2	–	–	16.8	M	17.7	113	
P3	–	–	17.2	M	10.5	58	
P4	–	–	6 mo	F	12.4	73	
Group 2							
P5	–	Heterozygosity	6.6	F	16.8	108	20
P6	–	Heterozygosity	7.2	M	13.2	84.6	
P7	–	Heterozygosity	3.5	F	14.3	94	
P8	–	Heterozygosity	15	F	14.0	70	
P9	–	Heterozygosity	6.1	F	16.5	99	
P10	–	Heterozygosity	15.1	F	16.8	89	
P11	–	Heterozygosity	7.4	F	16.1	105	
P12	–	Heterozygosity	1.9	F	13.1	73	
P13	–	Heterozygosity	16.5	F	19.8	94	
P14	–	Heterozygosity	2.2	F	14.5	88	
P15	–	Heterozygosity	2.6	M	13.6	72	
P16	Heterozygosity	–	8.4	F	15.1	95	
Group 3							
P17	Heterozygosity	Heterozygosity	6.6	M	17.6	109	37
P18	Heterozygosity	Heterozygosity	8.9	F	14.9	88	
P19	Heterozygosity	Heterozygosity	12.3	F	17.0	93	
P20	Heterozygosity	Heterozygosity	4.8	F	20.6	118	
P21	Heterozygosity	Heterozygosity	7.4	F	15.1	98	
P22	Heterozygosity	Heterozygosity	5.5	M	15.0	95	
P23	Heterozygosity	Heterozygosity	11.8	M	18.4	100	
P24	Heterozygosity	Heterozygosity	7.1	F	14.7	89	
P25	Heterozygosity	Heterozygosity	13.6	M	17.1	103	
P26	Heterozygosity	Heterozygosity	4.8	F	14.1	86	
P27	Heterozygosity	Heterozygosity	7.8	M	11.6	74	
P28	Heterozygosity	Heterozygosity	10.8	F	19.7	103	
Group 4							
P29	Heterozygosity	Homozygosity	8.4	F	13.9	91.2	27
P30	Homozygosity	Homozygosity	10.2	F	14.3	91.3	
P31	Heterozygosity	Homozygosity	1.5	M	16.6	96	
P32	Heterozygosity	Homozygosity	1	F	17.2	101	
P33	Homozygosity	Homozygosity	9.5	F	14.1	86	
P34	Homozygosity	Heterozygosity	10.2	F	17.3	103	
P35	Homozygosity	Homozygosity	8.1	M	17.1	106	
P36	Heterozygosity	Homozygosity	5.4	F	16.4	103	

AOS; Age of symptoms, BMI; Body mass index, P; Patient, WFH; Weight for height

Results

The groups of patients were shown in table I according to the HLA-DQ2 alleles. Of the 36 celiac patients, 32 (89%) had at least one allele of the HLA-DQA1*0501 or HLA-DQB1*0201 genes. Homozygosity of the HLA-DQA1*0501 and HLA-DQB1*0201 alleles were observed in 8 (22%) patients and heterozygosity were observed in 24 (67%) patients. Four patients (11%) had no alleles for HLA-DQA1*0501 or HLA-DQB1*0201. There was no statistical significance among the groups for age, gender, BMI, weight for height, and onset of symptoms. However, we found lower values of BMI and delayed age of onset of symptoms in group 1 (not carrying HLA-DQ2 alleles) without statistical significance.

Among data shown in table II we found minor significant increases for AGA and EMA values both in groups 3 and 4 compared with groups 1 and 2 without

Table II
Serological and histopathological scores of patients

	Patients (n)	*MARSH score*	*Anti-EMA*		*Antigliadin Ab*	
			IgG	*IgA*	*IgA*	*IgG*
Group 1	4	3	25% (+)	25% (+)	105	69
Group 2	5	2	50% (+)	75% (+)	208	55
	7	3	50% (+)	75% (+)	138	67
Group 3	4	2	100% (+)	100% (+)	206	184
	8	3	67% (+)	60% (+)	65	147
Group 4	3	2	100% (+)	100% (+)	218	135
	5	3	50% (+)	75% (+)	110	86

Ab; Antibody, EMA; Endomysial antibody, IgA; Immunoglobulin A, IgG; Immunoglobulin G.

Table III
The distributions of clinical manifestations

	Group 1 n (%)	*Group 2 n (%)*	*Group 3 n (%)*	*Group 4 n (%)*
Diarrhea	3 (75)	6 (50)	6 (50)	6 (75)
Abdominal pain	1 (25)	4 (25)	3 (25)	2 (25)
Abdominal distention	1 (25)	4 (25)	6 (50)	2 (25)
Vomiting	0	0	0	1 (12.5)
Failure to thrive	2 (50)	8 (67)	8 (67)	4 (50)
Constipation	0	0	0	2 (25)

Table IV
Hematological values of groups

	Group 1	*Group 2*	*Group 3*	*Group 4*
Hematocrit (%)	35	33	34	32
MCV (μm^3)	81	76	70	70
Serum iron (μg/dL)	10	27	29	32
TIBC (μg/dL)	402	379	378	300
AST (IU/L)	45	39	46	39
ALT (IU/L)	37	41	32	28
Calcium (mg/dL)	8.9	8.8	8.8	8.7

ALT; Alanine Amino transferase, AST; Aspartate Amino transferase, MCV; Mean Corpuscular Volume, TIBC; Total iron binding capacity.

statistically significance. The clinical presentation of patients was shown in the table III. Also, there were no statistical differences among 4 groups according to the clinical presentations of patients without constipation was observed higher rate in the 4th group without statistically significance ($p>0.05$). Finally, we showed hematological parameters of patients for each group in table IV. There was no statistically significance among groups for hematologic parameters and biochemical values (Table IV).

Discussion

In our study, we investigated the association of clinical, laboratory, and histological data with genetic predispositions carrying HLA-DQ2 genes (HLA-DQA1 and HLA-DQB1 alleles) in the celiac patients. The results of the sensitivity and the specificity of HLA-DQ2 heterodimers are varied between 40-84% in Turkey[12-15]. Our results (88%) were consistent with an article (84%) reported by Tuysuz*et al*.[15] in 2001. In

spite of knowledge that the age of diagnosis seems to be associated with a higher genetic load[18]; in our study, early onset of symptoms was not found in Group 4 (carrying HLA-DQ2 alleles as homozygosity), we observed this finding in Group 2 which patients had either HLA-DQA1*0501 or HLA-DQB1*0201 alleles as heterozygosity status without statistical significance. HLA-DQ2 alleles are important at risk of CD but the severity of the illness is depended to other factors[16]. In another study, the celiac patients diagnosed during childhood had lesser genetic load than those diagnosed in adulthood[18] as a reason, "hygiene hypothesis" said to explain this status. It mentions that our immune system development have been changed by the current environmental factors and these might decrease the usual frequency of infections at younger ages and increase the autoimmune diseases whereas the genetic load was less at that age[20-21]. Also we found lower values of BMI in Group 1 (not carrying HLA-DQ2 alleles) without statistical significance. Gene dose effect of HLA-DQ2 was known on the development of CD[17], however many studies could not found any statistical differences among the data of age, gender, the onset time of disease, BMI, weight for height and HLA-DQ2 gene[12,16,19]. More recent studies, Agardh D *et al*.[8] and Delgado JF1 *et al*.[9] reported as our study that, genetic predisposition was not related on age, gender, and clinical manifestations . We also noted that there was no statistical significance among groups according to other criteria listed in table I.

In contrast with a previous report[23] that the celiac patients carrying HLA-DQ2 alleles as homozygosity demonstrated more histological features of disease, we did not found statistically significance among 4 groups for Marsh scores, EMA and AGA levels. Similar to our results, in some previously reported studies, there was no association between the severity of duodenal lesions and HLA-DQ2 gene dose[10, 22]. These contradictions could be explained with size of studied patients and ethnicity.

There was no statistically significance according to clinical symptoms and signs among Groups 1-4 as shown in table III. Similar to our results, Delgado JF *et al*.[9], reported that there was no statistically significance between clinical appearance and HLA-DQ2 status in celiac patients except irritability and weigh loss .In our study, diarrhea and failure to thrive were the most symptoms (58% and 61% respectively) observed in celiac patients that were consisted with medical literature[24,25]. In the presented study, interestingly, we noticed that constipation was observed as more frequency in celiac patients which carrying homozygous HLA-DQ2 alleles (Group 4) compering with the other groups but not with statistically significance. In a more recent published study[8], constipation was reported as a higher frequency by 2 years of age in children with CD but this symptom was not related with HLA-DQ2 alleles and among symptomatic celiac children, constipation is as commonly reported as diarrhea also. We could not explain the relationship between constipation with the presence of HLA-DQ2 homozygous status in this study. As we mentioned before HLA-DQ2 homozygous persons have at least five-fold higher risk of disease development than HLA-DQ2 heterozygous persons and also it has been reported that different HLA gene dosage triggered the development of the disease[26-27], however correlation with diversity of symptoms and HLA-DQ2 homozygous status had not been explained yet.

In our study groups, some associated autoimmune conditions also were investigated which as type 1 diabetes mellitus (3 patients, 8.3%) hypothyroidism (1 patient, 2.8%) determined (data not shown). Hypothyroidism was detected in one patient (25%) in the lowest genetic load group (Group 1) ($p=0.046$). Some of laboratory findings including hematocrit, mean corpuscular volume (MCV) serum iron, iron binding capacity, liver function tests (AST, ALT), corrected calcium studied in 4 groups and we did not find any significant difference among the groups aspects of laboratory findings.

Conclusion

In our study, we wanted to elucidate the effect of HLA-DQA1 and HLA-DQB1 alleles which were homozygosis and/or heterozygosis, on the clinical, serological and histological features of CD patients. Although we could not found any statistically significance between HLA-DQ2 alleles and clinical, serological, and histological features of pediatric CD patients except for constipation was observed higher frequency in the homozygous group (Group 4) without statistically meaningful. Also, hypothyroidism was observed higher frequency in Group 1. The main point that we would like to make in this study is to give an idea and clues to investigators who will study in the future to detect the association of clinical outcomes and HLA-DQ2 alleles. Due to including small number of patients in each groups, our results had inadequate power to conclude on this topic, perhaps this study will provide a resource to investigators that they needfurther studies with large number of participants to take a result.

Conflict of interest

No conflict of interest was declared by the authors.

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