HLA class II high-resolution genotyping in Greek children with celiac disease and impact on disease susceptibility

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BACKGROUND: Celiac disease (CD) has been associated with HLA class II heterodimers. This study aimed at determining the HLA genotypic and allelic distribution in Greek children with CD as compared with the general population.

METHODS: A total of 118 children with CD and 120 healthy individuals serving as controls were included in the study.

RESULTS: Higher frequencies for *HLA-DQB1**02:01 (40.25 vs. 9.58%, $P < 0.001$) and *DQB1**02:02 (20.34 vs. 5.42%, $P < 0.001$) were observed in patients with CD, whereas *HLA-DQB1**03:01 (16.53 vs. 30.42%, $P < 0.001$), *DQB1**05:01 (0.85 vs. 10%, $P < 0.001$), and *DQB1**05:02 (5.51 vs. 17.92%, $P < 0.001$) were significantly lower, as compared with the controls. *DQA1**02:01 (patients with CD vs. controls: 20.76 vs. 6.67%, $P < 0.001$) and *DQA1**05:01 (40.25 vs. 9.58%, $P < 0.001$) were significantly more frequent in patients. The frequencies of *HLA-DQA1** 01:01, *01:02, *01:04, and *05:05 were significantly lower in patients ($P < 0.001$). The haplotype mainly associated with CD was *DRB1**03-*DQB1**02:*01*-*DQA1**05:01; patients with CD vs. controls: 39.83 vs. 9.58%, $P < 0.001$. In total, 84.75% of patients carried DQ2 (vs. 21.67% in controls, $P < 0.001$), whereas 11.02% were DQ8 positive/DQ2 negative.

CONCLUSION: This study confirms the existing data and provides additional evidence supporting a strong genetic predisposition for CD associated with the class II alleles *DQB1**02 and *DQA1**05 encoding the serological specificity DQ2.

Celiac disease (CD) is a chronic intestinal inflammatory disorder resulting in villous atrophy and flattening of the mucosa. It is activated in genetically susceptible individuals by the dietary ingestion of proline- and glutamine-rich proteins that are found in wheat, rye, and barley and are widely termed "gluten" (1). CD can present at any age with a variety of symptoms ranging from the classical CD, characterized by chronic diarrhea, abdominal distention, and failure to thrive within the first couple of years of life, to the atypical, with extraintestinal manifestations such as unexplained iron deficiency anemia, short stature, osteoporosis, and pubertal delay (2–4). Recent epidemiologic studies have revealed that positive serological tests for CD can be found up to 1% of the general population (5).

Although the exact genetic basis of the disease has not been determined, certain individuals appear to be genetically predisposed to develop CD. The markers that have particularly been associated with CD are the HLA-DQ2 and/or DQ8 heterodimers. This association can be explained by the fact that gluten peptides can be presented in HLA-DQ2 and DQ8 molecules on antigen-presenting cells. Gluten-specific CD4+ T cells in the lamina propria respond to these peptides, and this probably enhances cytotoxicity of intraepithelial lymphocytes against the intestinal epithelium (6).

HLA-DQ (DQ) is a cell surface receptor–type protein found on antigen-presenting cells. It is encoded on the HLA region of chromosome 6p21.3, which is historically known as the "D" antigen region. This region encodes the subunits DP, DQ, and DR, which are the major histocompatibility complex class II antigens in humans. DQ is made up of two different subunits to form a αβ-heterodimer. Each subunit is encoded by its own gene. The α subunit is encoded by the *HLA-DQA1* gene and the β subunit is encoded by the *HLA-DQB1* gene. Both loci are characterized by extensive genetic variability in the human population. The DQ loci are in close genetic linkage with *HLA-DR* but are less closely linked to *HLA-DP*, *HLA-A*, *HLA-B*, and *HLA-C*.

The HLA-DQ2 heterodimer is formed by one β_2 chain encoded by the *HLA-DQB1**02 allele (*DQB1**02:01 or 02:02) and one α_5-chain encoded by the *HLA-DQA1**05 allele (*DQA1**05:01 or 05:05). Most patients with CD carry *DQB1**02:01-*DQA1**05:01 on a *DRB1**03 haplotype (*DRB1**03-*DQB1**02:01-*DQA1**05:01). However, the α_5 and β_2 chains of the DQ2 heterodimer may also be encoded in trans with the *DQA1**05:05 allele, usually on *DRB1**11 or 12 or 13 haplotypes, and the *DQB1**02:02 allele, usually on a *DRB1**07 haplotype (i.e., *DRB1**11 or 12 or 13-*DQB1**03:01-*DQA1**05:05/*DRB1**07-*DQB1**02:02-*DQA1**02:01 genotype) (7–11). This HLA-DQ2 heterodimer is present in at least 90% of patients with CD, although a very small number of patients with CD have been reported carrying only one chain of the DQ2 heterodimer (12).

The few patients with CD who are DQ2-negative, as well as a very small portion of those who are HLA-DQ2 positive,

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frequently have the DQ8 heterodimer. The HLA-DQ8 heterodimer is formed by one β chain and one α chain encoded by the *HLA-DQB1**03:02 and *HLA-DQA1**03 alleles, respectively. These two alleles, along with the *DRB1**04 allele, form the DR4-DQ8 haplotype (*DRB1**04-*DQB1**03:02-*DQA1**03:01) (13,14). More than 95% of patients with CD carry HLA-DQ2 and/or HLA-DQ8, and hence the absence of these molecules reduces significantly the likelihood of CD (15).

In this study, we aimed at assessing the HLA-DR/DQ genotypic and allelic distribution in a large cohort of children with CD in Greece, as compared with the general population, as well as evaluating the HLA effect on disease susceptibility.

RESULTS

Allelic Distribution

The overall distribution of HLA DQB1 and HLA DQA1 alleles significantly differed between patients and controls (Pearson's χ^2, both P values <0.001). The frequencies of all HLA DQB1 and HLA DQA1 alleles in our CD group and comparisons with the general population are shown in Tables 1 and 2. With regard to HLA DQB1 alleles, significantly higher prevalence in patients with CD was found for *DQB1**02:01 ($P < 0.001$) and *DQB1**02:02 ($P < 0.001$). In contrast, *HLA-DQB1**03:01 ($P < 0.001$), *DQB1**05:01 ($P < 0.001$), and *DQB1**05:02 ($P < 0.001$) were significantly lower in patients with CD . Among HLA-DQA1 alleles, *DQA1**02:01 ($P < 0.001$) and *DQA1**05:01 ($P < 0.001$) were significantly more frequent in patients, whereas the observed frequencies of *HLA-DQA1** 01:01, 01:02, 01:04, and 05:05 in patients were significantly lower than those in controls ($P < 0.001$). Table 3 shows the HLA-DRB1 allelic distribution. Significantly higher frequencies of *DRB1**03 ($P < 0.001$) and *DRB1**07 ($P < 0.001$) were found in patients with CD as compared with controls, whereas *HLA-DRB1**01 and *DRB1**16 presented much lower frequencies in the patients group ($P < 0.001$ and $P < 0.001$, respectively).

Haplotypic Distribution

In total, the HLA DRB1-DQB1-DQA1 haplotypic distribution was significantly different in patients with CD as compared with

Table 1. HLA-DQB1 allelic distribution in patients with celiac disease and controls

*DQB1** allele	Patients (236 alleles analyzed)		Controls (240 alleles analyzed)		
	n	(%)	*n*	(%)	OR (95% CI)
02:01	95	40.3	23	9.6	6.3 (3.8–10.5)*
02:02	48	20.3	13	5.4	4.4 (2.3–8.5)*
03:01	39	16.5	73	30.4	0.45 (0.29–0.70)*
03:02	19	8.1	13	5.4	NS
03:03	0	0	4	1.7	NS
03:04	0	0	2	0.8	NS
03:05	0	0	1	0.4	NS
04:02	1	0.4	4	1.7	NS
05:01	2	0.9	24	10	0.08 (0.02–0.33)*
05:02	13	5.5	43	17.9	0.27 (0.14–0.51)*
05:03	5	2.1	15	6.3	NS
06:01	2	0.9	3	1.3	NS
06:02	6	2.5	8	3.3	NS
06:03	6	2.5	10	4.2	NS
06:04	0	0	1	0.4	NS
06:09	0	0	3	1.3	NS

CI, confidence interval; NS, not significant; OR, odds ratio.
*$P < 0.001$.

Table 2. HLA-DQA1 allelic distribution in patients with celiac disease and controls

*DQA1** allele	Patients (236 alleles analyzed)		Controls (240 alleles analyzed)		
	n	(%)	*n*	(%)	OR (95% CI)
01:01	1	0.4	15	6.3	0.06 (0.01–0.49)*
01:02	20	8.5	58	24.2	0.29 (0.17–0.50)*
01:03	7	3	11	4.6	NS
01:04	4	1.7	20	8.3	0.19 (0.06–0.56)*
01:05	0	0	2	0.8	NS
02:01	49	20.8	16	6.7	3.6 (2.0–6.6)*
03:01	19	8.1	15	6.3	NS
03:02	2	0.9	4	1.7	NS
04:01	1	0.4	3	1.3	NS
05:01	95	40.3	23	9.6	6.3 (3.8–10.5)*
05:05	38	16.1	72	30	0.45 (0.29–0.70)*
06:01	0	0	1	0.4	NS

CI, confidence interval; NS, not significant; OR, odds ratio.
*$P < 0.001$.

Table 3. HLA-DRB1 allelic distribution in patients with celiac disease and control

*DRB1** allele	Patients (236 alleles analyzed)		Controls (240 alleles analyzed)		
	n	(%)	*n*	(%)	OR (95% CI)
01	1	0.4	15	6.3	0.06 (0.01–0.49)*
03	95	40.3	25	10.4	5.8 (3.5–9.5)*
04	19	8.1	17	7.1	NS
07	48	20.3	16	6.7	3.6 (2.0–6.5)*
08	1	0.4	4	1.7	NS
09	2	0.9	2	0.8	NS
10	1	0.4	6	2.5	NS
11	36	15.3	61	25.4	NS
12	2	0.9	4	1.7	NS
13	7	3	19	7.9	NS
14	3	1.3	15	6.3	NS
15	8	3.4	18	7.5	NS
16	13	5.5	38	15.8	0.31 (0.16–0.60)*

CI, confidence interval; NS, not significant; OR, odds ratio.
*$P < 0.001$.

controls (Pearson's χ^2, *P* value <0.001). Individual comparisons are listed in Table 4. The *DRB1**03-*DQB1**02:01-*DQA1**05:01 and *DRB1**07-*DQB1**02:02-*DQA1**02:01 haplotypes were significantly more common in patients as compared with controls (both *P* values <0.001). However, the observed frequency of *DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05 in patients was lower than that in control subjects ($P < 0.001$). The frequency of the *DRB1**04-*DQB1**03:02-*DQA1**03:01 haplotype in our CD group was not significantly different from controls.

Genotypic Distribution

The genotypic distribution, as defined by the combination of haplotypes, appeared to be significantly divergent between patients and controls (Pearson's χ^2, *P* value <0.001). Detailed comparisons are illustrated in Table 5.

In the whole data set, 95.8% of individuals with CD were DQ2 and/or DQ8 positive (4.2% DQ2 and DQ8 negative), as compared with 32.5% in the general population ($P < 0.001$). In greater detail, 84.8% of patients were DQ2 positive (vs. 21.7% in controls, $P < 0.001$). Among those, the following subclasses were observed: *DRB1**03-*DQB1**02:01-*DQA1**05:01 in homozygosis 13.6% vs. 1.7% ($P = 0.001$), with *DRB1**07-*DQB1**02:02-*DQA1**02:01 17.8% vs. 1.67% ($P < 0.001$), with *DRB1**04-*DQB1**03:02-*DQA1**03:01 3.4% vs. 0% (not significant (NS)), in trans *DRB1**11 or 12 or 13-*DQB1**03:01-*DQA1**05:05/*DRB1**07-*DQB1**02:02-*DQA1**02:01 17.8% vs. 4.2% ($P = 0.001$), with *DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05 9.3% vs. 5.8% (NS), and *DRB1**03-*DQB1**02:01-*DQA1**05:01 with other haplotypes 22.9% vs. 8.3% ($P = 0.002$).

Eleven percent of patients were DQ8 positive/DQ2 negative (vs. 10.83% in controls, NS). In homozygosis, *DRB1**04-*DQB1**03:02-*DQA1**03:01/*DRB1**04-*DQB1**03:02-*DQA1**03:01 1.7% vs. 0% (NS) and with other haplotypes *DRB1**04-*DQB1**03:02-*DQA1**03:01 /x 9.3% vs. 10.8% (NS). After stratification according to DQ2 positivity, DQ8 was found significantly more frequently in CD-DQ2-negative patients as compared with

Table 4. HLA DRB1-DQB1-DQA1 haplotypes in patients with celiac disease and controls

	Patients (236 haplotypes analyzed)		Controls (240 haplotypes analyzed)		
HLA DRB1-DQB1-DQA1 haplotypes	*n*	(%)	*n*	(%)	OR (95% CI)
*DRB1**03-*DQB1**02:01-*DQA1**05:01	95	40.3	23	9.6	6.3 (3.8–10.5)*
*DRB1**07-*DQB1**02:02-*DQA1**02:01	48	20.3	12	5	4.8 (2.5–9.4)*
*DRB1**04-*DQB1**03:02-*DQA1**03:01	19	8.1	13	5.4	NS
*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05	38	16.1	71	29.6	0.46 (0.29–0.71)*
Other (X)	36	15.3	121	50.4	0.18 (0.11–0.27)*

CI, confidence interval; NS, not significant; OR, odds ratio.

*$P < 0.001$.

Table 5. HLA DRB1-DQB1-DQA1 genotypic distribution in patients with celiac disease and controls

	Patients (118 individuals)		Controls (120 individuals)		
HLA DRB1-DQB1-DQA1 genotypic distribution	*n*	(%)	*n*	(%)	OR (95% CI)
*DRB1**03-*DQB1**02:01-*DQA1**05:01/*DRB1**03-*DQB1**02:01-*DQA1**05:01	16	13.6	2	1.7	9.2 (2.1–41.2)*
*DRB1**03-*DQB1**02:01-*DQA1**05:01/*DRB1**07-*DQB1**02:02-*DQA1**02:01	21	17.8	2	1.7	12.8 (2.9–55.8)**
*DRB1**03-*DQB1**02:01-*DQA1**05:01/*DRB1**04-*DQB1**03:02-*DQA1**03:01	4	3.4	0	0	NS
*DRB1**03-*DQB1**0201-*DQA1**0501/*DRB1**11/12/13-*DQB1**0301-*DQA1**0505	11	9.3	7	5.8	NS
*DRB1**03-*DQB1**02:01-*DQA1**05:01/X	27	22.9	10	8.3	3.3 (1.5–7.1)***
*DRB1**07-*DQB1**0202-*DQA1**0201/*DRB1**11/12/13-*DQB1**0301-*DQA1**0505	21	17.8	5	4.2	5.0 (1.8–13.7)*
*DRB1**07-*DQB1**02:02-*DQA1**02:01/*DRB1**04-*DQB1**03:02-*DQA1**03:01	2	1.7	0	0	NS
*DRB1**07-*DQB1**0202-*DQA1**0201/*DRB1**07-*DQB1**0202-*DQA1**0201	1	0.9	0	0	NS
*DRB1**07-*DQB1**02:02-*DQA1**02:01/X	2	1.7	5	4.2	NS
*DRB1**04-*DQB1**03:02-*DQA1**03:01/*DRB1**04-*DQB1**03:02-*DQA1**03:01	2	1.7	0	0	NS
*DRB1**04-*DQB1**03:02-*DQA1**03:01/*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05	4	3.4	2	1.7	NS
*DRB1**04-*DQB1**03:02-*DQA1**03:01/X	5	4.2	11	9.2	NS
*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05/*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05	1	0.9	10	8.3	NS
*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05/X	0	0	38	31.7	0.01 (0.0–0.07)**[a]
X/X	1	0.9	28	23.3	0.03 (0.004–0.21)**

CI, confidence interval; NS, not significant; OR, odds ratio.

[a]Exact logistic regression, median unbiased estimates. *$P = 0.001$; **$P < 0.001$; ***$P = 0.002$.

healthy DQ2-negative controls (72.2% vs. 13.8%, respectively, $P < 0.001$). In DQ2-positive individuals, there was no difference in DQ8 positivity ($P = 0.6$).

Among the five DQ2/DQ8-negative individuals with CD (4.3% in patients vs. 67.5% in controls, $P < 0.001$), four carry half of the DQ2 molecule. Three patients carried *DQB1**02 in the absence of *DQA1**05, which results in carrying the β_2 chain without the α_5 chain (one patient was *DRB1**07-*DQB1**02:02-*DQA1**02:01 homozygous and two patients were *DRB1**07-*DQB1**02:02-*DQA1**02:01 heterozygous), and one subject had *DQA1**05 in the absence of *DQB1**02 (*DRB1**11-*DQB1**03:01-*DQA1**05:05 homozygous), which also results in carrying half the DQ2 molecule (α_5 chain without the β_2 chain). Finally, the fifth DQ2/DQ8-negative patient was negative for both DQ2 chains (α_5 and β_2) and carried the *DRB1**14-*DQB1**05:03-*DQA1**01:04/*DRB1**16-*DQB1**05:02-*DQA1**01:02 genotype. This corresponds to a 1/118 (0.8%) probability of combined DQ2/DQ8/α_5/β_2 negativity in CD patients.

DISCUSSION

CD is a permanent gluten intolerance strongly associated with HLA class II antigens. The overpresentation of particular HLA alleles and haplotypes has been described in several populations (15,16). The strong genetic predisposition was quantified by Bourgey *et al.* (17), who estimated that the overall risk that a sib of a patient with CD will develop the disease is ~10%, ranging from 0.1% to 29% when HLA-DQ information of the proband, parents, and sib was considered. Megiorni *et al.* (18) obtained a risk gradient ranging from 1:7 for DQ2 and DQ8 individuals down to 1:2,518 for subjects lacking all predisposing HLA-DQ factors. In this study, we provide data, for the first time in a large Greek pediatric population from a single center, supporting a strong association between HLA-DQB1, HLA-DQA1, and HLA-DRB1 and CD. Overall, we found that 95.8% of individuals with CD were DQ2 and/or DQ8 positive, as compared with 32.5% of the healthy individuals, a result similar to those in other Caucasian populations (15–16,19). This result is very important in terms of diagnostic policies in Greece, given that DQ2 and/or DQ8 positivity holds a significant role in the latest European Society of Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of CD (20). The majority of DQ2-positive patients were found to be homozygous or heterozygous for the *DRB1**03-*DQB1**02:01-*DQA1**05:01 haplotype (DR3-DQ2). However, the likelihood of DQ2 positivity (84.8%) is clearly lower than the 90–95% reported in a Northern European population (21). This could be attributed to the remarkably lower frequency of the *DQA1**05:05-carrying haplotypes (*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05) in patients as compared with controls. In populations in which the DQ2 positivity is >90%, the distribution of the *DQA1**05:05-carrying haplotypes (*DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05) is similar between patients and controls. This could explain the slightly lower frequency of DQ2-positive individuals with CD in our population, because among the haplotypes that are necessary for a DQ2 heterodimer, only the aforementioned haplotypes were found more frequently in controls in our study. The proportion of patients carrying the *DRB1**11/12/13-*DQB1**03:01-*DQA1**05:05 haplotypes (formerly known as DR5-DQ7) has been shown to vary across Europe, not only in patients but also in the healthy population as well, with frequencies reported from Spain (19) and Italy (11) being higher as compared with northern Europe.

Among the five DQ2- and DQ8-negative patients, three of them carried the haplotype *DRB1**07-*DQB1**02:02-*DQA1**02:01 (DR7-DQ2) without *DQA1**05 (i.e., had the β_2 chain without the α_5 chain) and one patient carried the *DRB1**11-*DQB1**03:01-*DQA1**05:05 (DR5-DQ7) haplotype in homozygosis (i.e., had the α_5 chain without the β_2 chain). The fifth patient lacked all HLA-susceptibility factors and was a girl with short stature, positive serological test, and partial villous atrophy who responded to a gluten-free diet. She carried the *DRB1**14-*DQB1**05:03-*DQA1**01:04/*DRB1**16-*DQB1**05:02-*DQA1**01:02 genotype. In a multicenter study conducted in Europe in 2002, 61 of the 1,008 patients with CD lacked the DQ2 or DQ8 heterodimer (6.0%), 57 of them (5.6%) encoded one half of the DQ2 heterodimer, and 4 (0.4%) were DQ2, DQ8, α_5, β_2 negative (15). Our results are similar to those reported from the other studies. These findings demonstrate that just one of the alleles *DQA1**05 or *DQB1**02, coding for half of the DQ2 heterodimer molecule, would confer a predisposition toward triggering CD (15,22).

In our CD population, we have found a pronounced increase of the *DQB1**02:01, *DQB1**02:02, *DQA1**02:01, *DQA1**05:01, *DRB1**03, and *DRB1**07 alleles, which are those classically associated with CD in other populations as well (23–27). No association was detected with *DRB1**04, *DQB1**03:02, and *DQA1**03:01 alleles, an observation comparable with results reported by others (19). In our series, the observed frequencies of *DQA1**01:01, *DQA1**01:02, *DQA1**01:04, *DQA1**05:05, *DQB1**03:01, *DQB1**05:01, *DQB1**05:02, *DRB1**01, and *DRB1**16 in patients with CD are lower than those in controls. It remains unclear whether this negative association confers a real protective effect against the development of CD.

The DQ2 and/or DQ8 positivity ratio in our study was 95.8%/32.5% (patients with CD vs. controls), a finding comparable to those reported in other Caucasians. It is interesting that despite the lower proportion of DQ2-positive patients, the relatively higher percentage of DQ8 positivity, as compared with other CD populations, allows the final combined DQ2/DQ8 positivity to marginally exceed 95%, a level frequently observed in other studies.

A number of functional studies have recently focused on the role of a single β_2 chain encoded by *DRB1**07-*DQB1**02:02-*DQA1**02:01 in the absence of *DRB1**03-*DQB1**02:01-*DQA1**05:01 in the pathogenesis of CD. Hernandez-Charro *et al.* suggested that the *DRB1**07-*DQA1**02:01-*DQB1**02:02 haplotype does not confer a significantly higher risk of CD, unless it is expressed with *DRB1**03-*DQA1**05-*DQB1**02. This hypothesis is confirmed in our study by the significantly higher relative risk for CD in the presence of *DRB1**07-*DQA1**02:01-*DQB1**02:02/*DRB1**03-*DQA1**05-*DQB1**02 (odds ratio: 12.8, 95% confidence interval: 2.9–55.9) as compared with the relative risk derived from the combination of

*DRB1**07-*DQA1**02:01-*DQB1**02:02 with any other haplotype (odds ratio: 3.1, 95% confidence interval: 1.4–6.8).

It is noteworthy that, although, most patients with CD carry the HLA DQ2 or HLA DQ8 heterodimer, the HLA susceptibility genes account for only about 40% of the whole genetic risk. The challenge now is to explain the remaining 60% (28,29), which could be attributed to additional genetic factors such as *CTLA-4*, a non-HLA gene thought to regulate T-cell immune function (30).

As recommended, HLA types should not be classified only as DQ2/DQ8 positive or negative. Clinicians involved in the diagnosis of CD must also consider the presence of one half of the DQ2 heterodimer determined by genetic analysis (15).

In conclusion, this study confirms and extends evidence for a strong genetic predisposition for CD associated with the class II alleles *DQB1**02-*DQA1**05 encoding the DQ2 molecule in the pediatric Greek population. The main associated haplotype was *DRB1**03-*DQB1**02:01-*DQA1**05:01, followed by *DRB1**07-*DQB1**02:02-*DQA1**02:01. The frequency of the haplotype *DRB1**04-*DQB1**03:02-*DQA1**03:01 was not significantly higher in patients vs. controls. Among DQ2-negative individuals, DQ8 was found significantly more frequently in patients with CD than in controls.

METHODS

Subjects

This study was carried out in the First Department of Pediatrics in the University of Athens, Greece, following approval by the review board of "Aghia Sophia" Children's Hospital. Written, informed consent was obtained from the legal guardians of all participating children. Any child of Greek origin diagnosed with CD according to the criteria proposed by the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition was eligible for inclusion (31). For children diagnosed prior to 1990, the "Interlaken criteria" were applied, whereas for those diagnosed after 1990, the revised European Society of Pediatric Gastroenterology, Hepatology, and Nutrition criteria were adopted. Finally, a total of 118 patients were included; a panel of 120 healthy individuals, blood and bone marrow donors of the same origin, served as controls.

HLA Typing

Samples of peripheral blood were taken from patients and controls and genomic DNA was extracted using an automated system (MagNA Pure Compact, Roche, Basel, Switzerland). HLA class II (DRB1, DQA1, DQB1 loci) genotyping was performed by sequence-specific primer PCR (Life Technologies, Carlsbad, CA; One Lambda, Canoga Park, CA; Olerup International AB, Stockolm, Sweden) and sequence-specific oligonucleotide primed PCR (Life Technologies; One Lambda). All the technical parts of the study were carried out in the Department of Immunology and Histocompatibility of "Aghia Sophia" Children's Hospital. The haplotypes were deduced by means of the known patterns of linkage disequilibrium in the Greek population (32,33).

Statistical Analysis

Allelic, genotypic, and haplotypic frequencies in patients with CD and controls are presented as absolute (n) and relative frequencies (%). For the comparison of the allelic distribution for each genetic locus (DQA1, DQB1, and DRB1) between patients with CD and controls, 2 × 12, 2 × 16, and 2 × 13 tables were constructed, respectively, and Pearson's χ^2 test was applied using 0.05 as level of significance. To identify which allele differed significantly, in each case, between patients and controls, 12, 16, and 13 2 × 2 tables were formed (for DQA1, DQB1, and DRB1, respectively). Each table compared the distribution of a particular allele against all the others in patients and controls, using Fisher's exact test. Due to the large number of comparisons, the level of statistical significance was adjusted accordingly (0.05/12 = 0.004 for DQA1, 0.05/16 = 0.003 for DQB1, and 0.05/13 = 0.0038 for DRB1). All relative risks were quantified by logistic regression analysis and expressed as odds ratios.

A similar approach was adopted for the comparison of haplotypes and genotypes, with the exception of all haplotypes not coding for either the α or β chain of DQ2 and DQ8, which were grouped together. The same classification was applied for the genotypes resulting from these haplotypes. All statistical analyses were performed with Stata 11.0 MP statistical software (Stata Corp, College Station, TX)

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