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HLA Genotyping in Romanian Adult Patients with Celiac Disease, their First-degree Relatives and Healthy Persons

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ABSTRACT

Background & Aims: Celiac disease (CD) is characterized by an inappropriate T-cell-mediated response to gluten in small bowel in genetically predisposed individuals, carriers of the DQ2 and/or DQ8 haplotypes of the human leukocyte antigen (HLA). Our aim was to assess HLA typing in adult patients with CD, in their first-degree relatives and in a healthy control group.
Methods: Our prospective observational study included three cohorts: 117 patients diagnosed with CD, 41 first-degree relatives of CD patients and 57 asymptomatic healthy volunteers. Low resolution HLA typing for DQ alleles was performed in all subjects with DNA extracted from peripheral blood, using SSP HLA-DQB1 kit (InnotrainDiagnostik GmbH, Germany). Next generation sequencing was used only in 18 patients for typing confirmation of DQB1 and DQA1 loci and whole gene sequencing.
Results: Prevalence of HLA-DQ2 was significantly higher in the CD group compared to the healthy subjects group (95.6% vs 29.8%, p<0.001), with no statistically significant differences in HLA-DQ8 and combined HLA-DQ2/DQ8 prevalences.Several HLA DQA1 and DQB1 alleles (HLA-DQA1*05:01, HLA-DQB1*02:01, HLA-DQB1*02:02) and haplotypes (DQA1*02:01-DQB1*02:02,DQA1*05:01-DQB1*02:01) were strongly associated with CD in our group (OR 4.28, 4.28, 4.67 and 5.43 and 4.28 respectively). Screening adherence for first-degree relatives was very low: 16%. Familial screening diagnosed 4 cases of asymptomatic CD. 32 relatives (78%) had HLA-DQ2 haplotype, 5 carried HLA-DQ8, 4 did not carry any risk of a haplotype.
Conclusions: This study demonstrated a higher prevalence of the HLA-DQ2 genotype in patients with CD compared to the healthy population but not of HLA-DQ8 or combined HLA-DQ2/DQ8. Alleles HLA-DQA1* 05:01, HLA-DQB1*02:01, HLA-DQB1*02:02 and haplotypes (DQA1*02:01-DQB1*02:02,DQA1*05:01-DQB1*02:01) were strongly associated with CD in our cohort.

Key words: celiac disease – HLA – genotyping – family screening.

Abbreviations: CD: celiac disease; EATL: enteropathy-associated T cell lymphoma; GFD: gluten free diet; HLA: human leukocyte antigen; NGS: next generation sequencing; RCD: refractory celiac disease; tTG-IgA: tissue transglutaminase immunoglobulin A.

INTRODUCTION

Celiac disease (CD) represents a significant global issue due to the effects of gluten intake on health. A recent meta-analysis reported a global prevalence of 1.4% based on serological tests and 0.7% based on biopsy results (0.8% in Europe). The disease is slightly more prevalent in females (0.6% vs. 0.4%, p<0.001) [1].

Celiac disease is an autoimmune disease, driven by an important hereditary component. The susceptibility to CD is conferred by human leukocyte antigen (HLA) class II alleles in the HLA-DQ region, with certain combinations of alleles offering a higher genetic risk of disease than others [2-4].

Most patients (>90%) have the HLA-DQ2 heterodimer (encoded by the alleles HLA-DQA1*0501 or *0505 and HLA-DQB1*0201or *0202), 5-10% have the HLA-DQ8 heterodimer (encoded by DQA1*03 and DQB1*0302) and <5% of patients are half DQ2 positive which means they have only the alpha chain (HLA-DQA1*0501 or *0505) or only the beta chain (HLA-DQB1*0201 or *0202) [5]. Approximately 5% of patients are positive for both HLA-DQ2 and DQ-8 [6]. Susceptibility

to disease in the case of the HLA-DQ2 genotype is transmitted according to the predisposing parent HLA genotype (either autosomal dominant or recessive), whereas in the case of HLA-DQ8 it is transmitted autosomal dominant [5].

Numerous studies identified familial aggregation in CD relatives. The risk of developing the disease in first-degree relatives of patients with CD varies between 7.5% and 10-15% but can reach up to 40% in the case of certain genetic combinations [7-9]. Studies performed on monozygotic twins demonstrate a disease concordance of up to 75-80% [8].

However, it should be noted that the HLA-DQ2/DQ8 prevalence in the general population varies between 30 and 40%, but only about 3% of carriers develop CD [10-12].

The primary objective of this study was to determine the prevalence of HLA-DQ2/DQ8 in CD patients and their first-degree relatives, compared to a healthy population. In a sub-group of patients, we performed high resolution HLA genotyping and compared the results with the control group. The secondary objective of the study was to assess the clinical, biological, histopathological parameters and the response to the gluten-free diet (GFD). We also evaluated the complications and the presence of autoimmune comorbidities among patients with CD.

METHODS

We conducted an observational study using three cohorts.

A total of 392 patients were evaluated in the Fundeni Clinic Institute Gastroenterology Center with the diagnosis of CD in the past 10 years. We selected 117 patients who had complete data and who agreed to come for HLA-DQ typing. These 117 patients diagnosed with CD were interviewed and their blood samples were collected between January 2018 and December 2020. The inclusion criteria were age over 18 years and the positive diagnosis of CD according to serology [positive tissue transglutaminase immunoglobulin A (tTG-IgA) antibodies] and duodenal biopsy (Marsh 1, 2, 3 according to the modified Marsh classification). 37 patients with inconclusive results in the two diagnostic methods were excluded.The follow-up period of the patient represents the interval between the moment of diagnosis of celiac disease in our clinic and the time of collection of the blood sample for HLA genotyping.

The second group included 41 first-degree relatives of the CD patients from 29 CD cases in the first group. The third group included 57 asymptomatic healthy volunteers, employees of the Fundeni Clinic Institute (healthy subjects control group). These two groups had similar distributions in terms of age and gender.

The variables recorded for the three groups were: age at study inclusion, gender and the presence or absence of the HLA-DQ2, HLA-DQ8 or combined HLA-DQ2/DQ8 genotypes. Additionally, for the CD group the following were included: age at diagnosis, latency, follow-up period, initial symptoms, biological parameters (serum tTG-IgA levels, sideremia, albuminemia, liver enzymes) and histological (Marsh classification at diagnosis and in evolution) data, GFD compliance, complications and the presence of autoimmune comorbidities.

Low resolution HLA typing for DQ alleles was performed with DNA extracted from peripheral blood, using SSP HLA-DQB1 kit (InnotrainDiagnostik GmbH, Germany).

The study was approved by the Ethics Committee of the Fundeni Clinic Institute. An informed consent was obtained from all subjects included in the study.

For high resolution HLA genotyping, genomic DNA was isolated from the peripheral blood using the QIAamp® DNA Blood Mini Kit (Qiagen), according to the manufacturer's instructions. Genotyping was performed with low-resolution sequence specific primers (SSP) PCR (Innotrain Ready Gene, Germany). Confirmation of DQB1 locus typing was performed with Next Generation Sequencing (NGS) MIAFORA, Illumina platform USA using a MiniSeq sequencer. NGS was used only in 18 patients for typing confirmation. The MIAFORA NGS MFlex HLA typing protocol uses long-range PCR to capture the clinically relevant class II HLA genes. We have typed DQB1 and DQA1 loci and sequenced in their entire genes. For the interpretation of HLA allele assignment, we have used MIAFORA NGS FLEX software which delivers HLA typing information for DQB1 and DQA1 genes.

Data collection and statistical analysis were performed using the Statistical Program for Social Sciences for Windows (SPSS, Version 27). In relation to the parametric or non-parametric distribution (according to the Shapiro-Wilk test), the descriptive data were presented as median (minimum÷maximum) (min÷max) or as percentages. The comparison of the proportions was made by the Chi-Square Test. The value of p considered to indicate statistical significance was <0.05.

Cross-tabulation (using χ^2 test) or the Fisher's test was used to calculate the differences in HLA-DQA1 and -DQB1 allele, genotype, and haplotype percentages between patients with CD and controls in SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). A *p* value less than 0.05 was considered statistically significant. All the obtained significant probability values were corrected for multiple testing using the Bonferroni correction formula to avoid the risk of false-positive allele, genotype or haplotype association identifications.

Odds ratios with 95% confidence intervals (95% CI) were also calculated to establish the strength of each studied allele, genotype and haplotype risk association with CD.

RESULTS

HLA-DQ2/DQ8 Prevalence

Prevalence of HLA-DQ2 was significantly higher in the CD group compared to the healthy subjects' group (95.6% vs 29.8%, p <0.001), with no statistically significant differences in HLA-DQ8 prevalence and combined HLA-DQ2/DQ8 prevalence (8.8% vs 10.5%, p=0.749, respectively 4.4% vs 1.8%, p=0.402) (Table I).

Screening adherence for first-degree relatives was very low: only 16%, most of them offsprings of CD patients (children aged 6-14 years old). Four cases of asymptomatic CD were diagnosed by screening. 32 relatives (78%) had HLA-DQ2 haplotype, 5 carried HLA-DQ8, 4 did not carry any risk haplotype.

Table I. Prevalence of HLA DQ-2/DQ-8 in celiac disease patients compared to healthy subjects

Variable	CD patients (n=117)	Healthy controls (n=57)	p
HLA-DQ2 (%)	95.6	29.8	<0.001
HLA-DQ8 (%)	8.8	10.5	0.770
Combined HLA-DQ2 and DQ8 (%)	4.4	1.8	0.625

HLA Typing-SSP PCR with High-resolution Typing

The confirmed typings by NGS for our patients showed that the most relevant alleles were HLA-DQA1*05:01:01:02 and DQA1*02:01:01:01 compared to the control group.

Table II presents the HLA-DQA1 and -DQB1 alleles in patients with CD compared with the control. Eight alleles were identified from loci DQA1 and DQB1, respectively.

After applying the Bonferroni correction, we found that only HLA-DQA1* 05:01 (OR=4.28, p=0.0016, pc=0.0128), HLA-DQB1*02:01 (OR=4.28, p=0.0016, pc= 0.0128) and HLA-DQB1*02:02 (OR=4.667, p=0.0053, pc=0.0424) were associated with CD in our group of patients (Table II).

We also studied the possible association of HLA-DQA1 and DQB1 alleles heterozygosity or homozygosity with the disease. Our study did not reveal any allele combination that remained significantly and positively associated with CD after applying the Bonferroni correction (Table III).

In Table IV we mentioned all the haplotypes that were common identified in both patients and controls. After Bonferroni correction, only DQA1*02:01-DQB1*02:02 (equivalent with DQ2.2, OR=5.429, p=0.0045, pc=0.0405) and DQA1*05:01-DQB1*02:01 (equivalent with DQ2.5, OR=4.28, p=0.0016, pc=0.0144) remained significantly positively associated with CD appearance in our patients (Table IV).

Characteristics of CD Patients

Celiac disease subjects were predominantly female (79.5%) with a median age at inclusion in the study of 45 years and with a median of 37 years (2÷73 years) at disease onset. Delayed diagnosis in CD is a common problem in our patients, as the median latency to diagnosis (the time between onset of symptoms and diagnosis) was 10 months (0÷180). The median follow-up period was 6 years (1÷29 years). Symptoms at disease onset were dominated by diarrhea (69.2%), abdominal pain (66.7%), weight loss (61.5%) and in lower percentages by abdominal bloating (33.3%) and dyspepsia (12%).

From the biological point of view, 53% of patients presented with anemia, 50.4% with hyposideremia, 40.2% had elevated liver enzymes and 23.1% had hypoalbuminemia. Regarding the tTG-IgA level, 73.1% had a value higher than 200 IU/ml, while the rest had a median value of 85.5 IU/ml.

Histological examination indicated in most cases Marsh type 3c modifications (57.5%), followed by 3b (33.3%) and in a much lower percentage grade 3a (4.6%) and grade 1 (4.6%).

After diagnosis, 70.9% of patients were compliant to the GFD. Normalization of the antibody titer occurred in 40% of subjects. Histological remission was assessed in 44.4% of patients, of whom 57.5% achieved complete remission. Of the 117 subjects included in the analysis, 9.4% developed complications: refractory CD (RCD) (4.27%), enteropathy-associated T cell lymphoma (EATL) (3.42%), intestinal adenocarcinoma (1.71%). The death rate was 6.8% during the median follow-up of 6 years.

Table II. Analysis of HLA-DQA1 and -DQB1 alleles in patients with celiac disease compared with the control

Allele	Cases		Controls		Odds ratio	95% Confidence interval	p	pc
	n	%	n	%				
DQAI* alleles								
DQA1*01:02	4	11.11	33	27.50	0.3295	0.1183 to 0.9830	0.0712	0.5696
DQA1*01:03	1	2.78	7	5.83	0.4612	0.03990 to 2.738	0.6825	5.4600
DQA1*01:04	1	2.78	6	5.00	0.5429	0.04608 to 3.548	>0.9999	>8.0000
DQA1*02:01	8	22.22	8	6.67	4	1.312 to 12.10	0.0171	0.1368
DQA1*03:01	2	5.56	11	9.17	0.5829	0.1242 to 2.661	0.7338	5.8704
DQA1*03:03	1	2.78	2	1.67	1.686	0.1133 to 14.77	0.5475	4.3800
DQA1*05:01	13	36.11	14	11.67	4.28	1.800 to 9.664	0.0016	0.0128
DQA1*05:05	6	16.67	27	22.50	0.6889	0.2659 to 1.771	0.2694	2.1552
DQBI* alleles								
DQB1*02:01	13	36.11	14	11.67	4.28	1.800 to 9.664	0.0016	0.0128
DQB1*02:02	9	25.00	8	6.67	4.667	1.679 to 13.69	0.0053	0.0424
DQB1*03:01	6	16.67	29	24.17	0.6571	0.2546 to 1.679	0.4725	3.7800
DQB1*03:02	2	5.56	6	5.00	1.118	0.2210 to 4.702	>0.9999	>8.0000
DQB1*05:02	3	8.33	23	19.17	0.3834	0.1157 to 1.270	0.2004	1.6032
DQB1*05:03	1	2.78	7	5.83	0.4612	0.03990 to 2.738	0.6825	5.4600
DQB1*06:02	1	2.78	5	4.17	0.6571	0.05435 to 5.121	>0.9999	>8.0000
DQB1*06:03	1	2.78	6	5.00	0.5429	0.04608 to 3.548	>0.9999	>8.0000

n: number of cases/controls; pc: p value after Bonferroni correction.

Table III. Combined HLA alleles in both celiac disease patients and controls

Allele	Cases		Controls		Odds ratio	95% Confidence interval	p	pc
	n	%	n	%				
DQA1 genotypes								
01:02-03:01	1	5.56	5	8.33	0.6471	0.05214 to 5.556	>0.9999	>9.0000
01:02-05:01	3	16.67	6	10.00	1.8	0.4476 to 6.893	0.4234	3.8106
01:03-05:01	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.0772
01:04-03:03	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.0772
02:01-05:01	4	22.22	1	1.67	16.86	2.335 to 208.5	**0.0091**	0.0819
02:01-05:05	4	22.22	1	1.67	16.86	2.335 to 208.5	**0.0091**	0.0819
03:01-05:05	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.0772
05:01-05:01	2	11.11	1	1.67	8.429	0.8953 to 123.6	0.1314	1.1826
05:01-05:05	1	5.56	3	5.00	1.118	0.08168 to 7.907	>0.9999	>9.0000
DQB1 genotypes								
02:01-02:01	2	11.11	1	1.67	8.429	0.8953 to 123.6	0.1314	1.3140
02:01-02:02	4	22.22	1	1.67	16.86	2.335 to 208.5	0.0091	0.0910
02:01-03:01	1	5.56	1	1.67	3.353	0.1673 to 64.59	0.4106	4.1060
02:01-05:02	2	11.11	2	3.33	3.625	0.5251 to 24.02	0.2263	2.2630
02:01-06:02	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.3080
02:01-06:03	1	5.56	1	1.67	3.353	0.1673 to 64.59	0.4106	4.1060
02:02-03:01	4	22.22	1	1.67	16.86	2.335 to 208.5	**0.0091**	**0.091**
02:02-05:03	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.3080
03:01-03:02	1	5.56	0	0.00	+infinity	0.3509 to +infinity	0.2308	2.3080
03:02-05:02	1	5.56	3	5.00	1.118	0.08168 to 7.907	>0.9999	>9.000

n: number of cases/controls; pc: p value after Bonferroni correction.

In our study, 23.1% of patients reported other autoimmune diseases, the most common being autoimmune thyroiditis (11.96%). Other autoimmune disorders were type I diabetes (0.85%), autoimmune pancreatitis (1.71%), autoimmune hepatitis (2.56%), primary biliary cholangitis (0.85%), Crohn's disease (0.85%), ulcerative colitis (0.85%), microscopic colitis (1.71%), idiopathic thrombocytopenic purpura (0.85%) and Sjogren's syndrome (0.85%).

Table IV. HLA specificity combinations between DQA1 and DQB1 loci

DQA1-DQB1 haplotypes	Cases		Controls		Odds ratio	95% Confidence interval	p	pc
	n	%	n	%				
DQA1*01:02-DQB1*05:02	3	8.33	22	18.33	0.405	0.1219 to 1.353	0.1988	1.9880
DQA1*01:02-DQB1*06:02	1	2.78	5	4.17	0.6571	0.05435 to 5.121	>0.9999	>8.000
DQA1*01:03-DQB1*06:03	1	2.78	3	2.50	1.114	0.08361 to 7.665	>0.9999	>8.000
DQA1*01:04-DQB1*05:03	1	2.78	6	5.00	0.5429	0.04608 to 3.548	>0.9999	>8.000
DQA1*02:01-DQB1*02:02	8	22.22	6	5.00	5.429	1.614 to 17.54	0.0045	0.0405
DQA1*03:01-DQB1*03:02	2	5.56	6	5.00	1.118	0.2210 to 4.702	>0.9999	>8.000
DQA1*03:03-DQB1*02:02	1	2.78	2	1.67	1.686	0.1133 to 14.77	0.5475	5.4750
DQA1*05:01-DQB1*02:01	13	36.11	14	11.67	4.28	1.800 to 9.664	0.0016	0.0144
DQA1*05:05-DQB1*03:01	6	16.67	25	20.83	1.9	0.6318 to 5.228	0.7555	7.5550

n: number of cases/controls; pc: p value after Bonferroni correction.

DISCUSSION

This is the first study in Romania that describes the results of HLA-DQ genotyping in 3 population types: CD patients, their first-degree relatives, and healthy individuals.

In this Romanian population, data similar to those reported in the literature were recorded: a prevalence of over 90% of HLA-DQ2 and about 10% of HLA-DQ8 among CD patients, while in the healthy population the percentages were around 30% and 10%, respectively [5, 10]. We also proved that the prevalence of HLA-DQ2 was significantly higher in subjects with CD than in healthy ones, a difference that was not encountered in the case of HLA-DQ8.

There are studies coming from other populations, that have analyzed the expression of the two genotypes in CD as well as in first-degree relatives and healthy populations.

In the European region the prevalence of HLA-DQ2 varied between 79 and 86% in CD patients and between 24 and 32% in the general population, while for HLA-DQ8 the values were 10-21%, respectively 15-17% [14-16]. In the Asian continent, HLA-DQ2 was registered in 80-83% and HLA-DQ8 in approximately 25% of CD patients. Regarding Asian general population, the presence of HLA-DQ2 was observed in 22-35% and HLA-DQ8 in 3-22% of subjects. The presence of HLA-DQ2 in first-degree relatives of patients with CD was 51% [17-20].

Unfortunately, our study was partially conducted during the period when the restrictions imposed by the COVID19 pandemic made it very difficult for patients to access hospitals. The 117 patients with CD had a number of 256 first-degree relatives alive eligible for screening. Of these, only 41 (16%) came to our outpatient department to perform the necessary investigations for screening: HLA genotyping and tTG Ig A antibodies. Even in this very small number of individuals we positively diagnosed a number of 4 asymptomatic cases (children under 16 years), and 37 found that they had a genetic predisposition (HLA-DQ2/DQ8 positive). For this reason, it would be necessary to perform a periodic screening test.

There are some data regarding the prevalence of HLA genes in the Romanian population; however, there are no data on frequency in patients with CD [21]. This is the first study in Romania that reports HLA typing information for DQB1 and DQA1 genes in patients with CD and in healthy persons. Regarding the DQA1 alleles, we met with the highest frequency DQA1*05: 01 (36%), followed by DQA1*02: 01 (22%) and DQA1*05: 05 (17%), results similar with those published by Zubillaga et al. [22] and by Balakrishnan et al. [23]. The most common DQB1 alleles were DQB1*02: 01 (36%), followed by DQB1*02: 02 (25%) and DQB1*03: 01 (17%), consistent with the literature data. Because of the low sample size (only 18 CD), we did not confirm a possible association of HLA-DQA1 and DQB1 alleles (heterozygote or homozygote) with the disease. Regarding the haplotype, our results showed that DQA1*05:01-DQB1*02:01 (equivalent with DQ2.5) and DQA1*02:01-DQB1*02:02 (equivalent with DQ2.2) were strongly associated with CD in our Romanian population, which is similar with data reported for other populations [22, 23].

The general characteristics of the studied CD population had multiple similarities with the data reported in the current guidelines and in other similar studies.

The age and gender of our CD patients were similar to those seen in literature, including those from another Romanian cohort. Celiac disease can be diagnosed at any age (with a third decade peak in adults) and has a higher incidence in women than in men (17.4% vs. 7.8% according to a systematic review and a recently published meta-analysis) [24-26].

The cardinal symptom in CD has long been dominated by diarrhea, but currently three types of presentations are described: typical with symptoms such as diarrhea, weight loss, atypical with symptoms such as constipation, abdominal pain, flatulence, neurological symptoms, fatigue, metabolic bone disorders, infertility and asymptomatic form detected by screening [27]. Patients in this study predominantly presented with typical symptoms (approximately two-thirds).

Iron deficiency anemia is a common element in CD [31, 32]. In our study, anemia was present in almost half of the patients (40%), a similar percentage to other literature data [33]. Elevated transaminases are described in approximately 50% of CD patients on a gluten-containing diet, the percentage being congruent with the data obtained in this study [34]. Hypoalbuminemia was described in one-fifth of our group. Although not a common biological finding in CD, it has been documented and reported in other publications as an atypical presentation of the disease [35].

Histological examination indicated that most of the patients had Marsh type modifications 3c which is consistent with the results of Rostami-Nejad et al. [36] from a multicentre study. Histological remission was observed in approximately half of those who underwent control endoscopy with biopsy, a result similar to that of Silva et al. [37] (approximately 40%).

Of great importance in CD is the follow-up in order to detect the occurrence of complications. Existing data indicate a low incidence of RCD (between 0.04 and 1.5%) [38, 39]. However, our study indicated a higher percentage of RCD. This could be due to non-compliance to GFD, or the coexistence of other diseases. Regarding the risk of cancer, the association with lymphoproliferative diseases, EATL and to a lesser extent B cell non-Hodgkin lymphomas is well known [40]. In our group there were four patients diagnosed with EATL. Intestinal adenocarcinoma, which was diagnosed in two patients in this study, is a rare type of cancer in the general population but which, among CD, seems to be more common [standardized incidence ratio of 4.29 (2.83-6.24) in a Finnish study] [41].

Regarding the risk of death, a Swedish study of approximately 50,000 patients followed for 12.5 years found that CD was associated with a statistically significantly higher risk of overall mortality than the general population (HR=1.21, 95%CI: 1.17-1.25) [42]. The death rate in this analysis was 6.8% during the median follow-up of 6 years.

We reported in 23.1% of CD patients the coexistence of other autoimmune diseases, a similar percentage to other cohorts [43, 44]. Autoimmune endocrine dysfunctions were the most common comorbidities reported in our study. One study found that up to 30% of patients with CD have positive thyroid antibodies and/or type 1 diabetes. Also, 5-7% of those with autoimmune thyroid disease, type 2 diabetes and/or polyglandular autoimmunity have positive serology for gluten enteropathy [45]. Liver diseases in CD have also been documented: autoimmune hepatitis, primary

sclerosing cholangitis, primary biliary cholangitis [46, 47]. The present analysis described three patients with autoimmune hepatitis and one with primary biliary cholangitis. In terms of association between CD and inflammatory bowel disease, the risk has been reported to be approximately 10 times higher in patients with CD to develop IBD and approximately 4 times higher conversely [48, 49]. In the present study, two patients associated IBD.

One of the strengths of this study is that it is the only one providing data on HLA genotyping in the Romanian CD patients, while presenting fairly broad descriptive information about CD adult patients. It also emphasizes the importance of screening for CD in first-degree relatives, which has not been done so far in Romania. This is one of the first studies in Romania to report preliminary data regarding HLA-DQA1 and HLA-DQB1 genes identified by NGS, and our results are similar to those reported by the Iasi team [50].

A limit of our studies is represented by the small number of included patients.

CONCLUSIONS

Our data indicate that several HLA DQA1 and DQB1 alleles (HLA-DQA1*05:01, HLA-DQB1*02:01, HLA-DQB1*02:02) and haplotypes (DQA1*02:01-DQB1*02:02,DQA1*05:01-DQB1*02:01) were strongly associated with CD in this Romanian cohort. Low resolution HLA genotyping demonstrated a higher prevalence of the HLA-DQ2 genotype in patients with CD compared to the healthy population but not of HLA-DQ8 or combined HLA-DQ2/DQ8.

Conflicts of interest: None to declare.

Authors' contribution: I.M., C.M.P. and I.C. designed the study. I.S., M.M., M.D., A.T., L.T., T.M., T.S., A.C.A., C.T. collected the data. D.I. performed the statistical analyses. A.E.C drafted the manuscript. I.M., C.M.P., I.S. and I.C. critically revised the paper. All the authors approved the final version of the manuscript.

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