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ORIGINAL ARTICLE

High-risk genotypes HLA-DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR4-DQ8 in co-occurrence of type 1 diabetes and celiac disease

Darja Smigoc Schweiger[1], Andrijana Mendez[2], Sabina Kunilo Jamnik[2], Nina Bratanic[1], Natasa Bratina[1], Tadej Battelino[1,3], Jernej Brecelj[3,4], and Blanka Vidan-Jeras[2]

[1]*Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, UMC – University Children's Hospital, Ljubljana, Slovenia,* [2]*Blood Transfusion Center of Slovenia, Tissue Typing Centre, Ljubljana, Slovenia,* [3]*Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, and* [4]*Department of Gastroenterology, Hepatology and Nutrition, UMC – University Children's Hospital, Ljubljana, Slovenia*

Abstract

Shared susceptibility alleles in the HLA region contribute to the co-existence of type 1 diabetes (T1D) and celiac disease (CD). The aim of our study was to identify HLA genotype variations that influence co-occurrence of T1D and CD (T1D + CD) and the order of their onset. Totally 244 patients, 67 with T1D, 68 with CD and 69 with T1D + CD, (split into "T1D first" and "CD first"), were analyzed. Control group consisted of 130 healthy unrelated individuals. Two-tailed Fisher's exact test was used for statistical analysis. The genetic background of Slovenian CD patients resembled more northern than southern European populations with DR3-DQ2/DR3-DQ2 (odds ratio [OR] = 19.68) conferring the highest risk. The T1D + CD was associated with DR3-DQ2/DR3-DQ2 (OR = 45.53) and even more with DR3-DQ2/DR4-DQ8 (OR = 93.76). DR3-DQ2/DR7-DQ2 played a neutral role in susceptibility for T1D + CD. The order of the onset of T1D or CD in patients with co-occurring diseases was not influenced by HLA risk genotype profile. DR3-DQ2/DR3-DQ2 was associated with an increased risk for developing CD in patients with T1D, whereas patients with CD carrying DR3-DQ2/DR4-DQ8 were at higher risk for developing T1D. In addition to other genetic factors including HLA class I alleles present on DR3-DQ2 extended haplotype, the second extended haplotype may moderate the risk for T1D + CD conferred by DR3-DQ2. Our results suggested that individuals carrying high-risk genotypes DR3-DQ2/DR3-DQ2 or DR3-DQ2/DR4-DQ8 would more likely develop both T1D and CD than either disease alone.

Keywords

Autoimmune diseases, genetic susceptibility, major histocompatibility complex, order of onset, Slovenian population

History

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Introduction

Certain individuals carry a higher burden of autoimmune diseases. Reasons for concordance of various autoimmune diseases have not been entirely established [1,2]. Type 1 diabetes (T1D) is a chronic autoimmune disorder resulting from immune-mediated destruction of insulin producing beta cells within the pancreatic islets requiring daily insulin treatment [3]. Autoantibodies directed toward insulin (IAA) and beta cell-specific antigens, glutamic acid decarboxylase 65 (GAD65), insulinoma-associated antigen 2 (IA-2) and zinc transporter 8 (ZnT8) are considered diagnostic at disease onset, as well as during the preclinical phase of the disease [4]. Celiac disease (CD) is a systemic disease caused by an inappropriate immune response to gluten with a variable combination of clinical manifestations and further characterized by the presence of enteropathy and serum antibodies directed against tissue transglutaminase (tTG) and gluten. The only treatment is a strict lifelong gluten-free diet [5]. The association between T1D and CD is intensely studied. The prevalence of CD ranges from 1% to 10% in patients with T1D [6] compared with 1% in the general population [7]. In most subjects the onset of T1D precedes the diagnosis of CD or the two diseases are diagnosed simultaneously [8,9]. However, it is shown that CD–associated antibodies often develop earlier than or at the same age as T1D-associated autoantibodies in children with a genetic susceptibility for both diseases, hence further studies are needed to identify the natural history of autoimmunity in patients with T1D [10]. Although ~11–25% of individuals with both diseases first receive a diagnosis of celiac disease [11,12], most studies focused on the risk of CD in individuals with a prior diagnosis of T1D. Only one population-based cohort study reports two- to three-fold increased risk of subsequent T1D in patients with CD [13].

Shared susceptibility genes in the HLA region contribute to the coexistence of T1D and CD (T1D + CD), with HLA-DR3-DQ2 and HLA-DR4-DQ8 haplotypes being considered the strongest genetic risk factors for T1D and CD. The DR3-DQ2 is found in about 90% of CD and 55% of T1D patients, whereas DR4-DQ8 is found in about 10% of CD and 70% of

Correspondence: Dr. Blanka Vidan-Jeras, Blood Transfusion Centre of Slovenia, Tissue Typing Center, 1000 Ljubljana, Slovenia. Tel: +386 1 5438224. Fax: +386 1 4327018. E-mail: Blanka.Vidan-Jeras@ztm.si

T1D patients [14,15]. HLA class II molecules HLA-DR and HLA-DQ are heterodimeric trans-membrane glycoproteins composed of α and β chains expressed mainly on professional antigen presenting cells and act as receptors of processed self- and non-self peptides, which are presented to CD4 + T lymphocytes. HLA-DQ2 and DQ8 are encoded by the alleles HLA-DQA1*05:01, DQB1*02:01 (DQ2) and DQB1*03:02, DQA1*03:01(DQ8), respectively [16]. The main mechanism of HLA-DR and HLA-DQ molecules involvement in T1D and CD pathogenesis may be related to their ability to bind peptides derived from insulin-secreting cells of the pancreas or sets of gluten-derived peptides, respectively [17,18]. Susceptibility and resistance to both diseases has been linked to particular polymorphic peptide-binding pockets of the DQ and DR molecules. HLA class II molecules bind peptides from beta cell-related autoantigens in T1D and present them to CD4+ T cells both in thymus and peripheral lymphoid tissues. Predisposing HLA molecules may lead to less than ideal presentation of antigens and promote the escape from thymic negative selection, leading to impaired tolerance [19]. In CD the HLA-DQ2 and HLA-DQ8 molecules present different repertoires of gluten peptides to CD4+ T lymphocytes in lamina propria. These activated CD4+ T lymphocytes produce high amounts of pro-inflammatory cytokines that act on intestinal epithelial cells and promote the activation of intraepithelial cytotoxic T lymphocytes, resulting in epithelial cell destruction and villous atrophy [20]. The process of epitope spreading is an important contributor to the extensiveness of the immune response and has also been implicated in both T1D and CD [21].

The extent of T1D risk is determined by the number, type and specific combinations of DRB1, DQA1 and DQB1 alleles, forming a hierarchy of haplotypes ranging from highly predisposing to highly protective [22–24]. The individuals carrying DR3-DQ2/DR4-DQ8 heterozygous genotype are at the highest risk, higher than that of homozygotes for either of the haplotypes. This may be owing to the ability to form DQ heterodimers that are encoded in trans in the heterozygous genotype [25]. Meta-analysis from 14 studies including the one in Slovenian population [26] showed study and population-specific estimates placing Slovenian patients (odds ratio [OR] = 18.13) very close to the overall mean odds ratio of 16.59 [27]. The influence of HLA on the CD susceptibility shows a dose effect. It is described that individuals homozygous for the disease-associated DQA1*05:01-DQB1*02:01 haplotype or individuals possessing DQA1*05:01-DQB1*02:01/DQA1*02:01-DQB1*02:02 genotype have the highest risk for developing CD [28,29]. The influence of the HLA region in T1D and CD is not limited to the factors encoded by DRB1, DQB1 and DQA1. Differential contribution of the DR3-DQ2 haplotypes implicates that additional factors may independently determine the extent of disease risk [30–32]. In our previous study the A*01-B*08-DRB1*03-DQB1*02:01-MICA*008 haplotype is significantly over-represented in individuals with both T1D + CD compared with the control group or individuals with T1D alone. Interestingly, B*08 is the most significant risk factor comprised in this haplotype [33]. When HLA typing is extended to locus C, C*07 through interaction with killer immunoglobulin-like receptors (KIRs), rather than B*08, can have an impact on the innate immunity route of this susceptibility [34].

The aim of this study was to identify HLA class II variations that contribute to the development of T1D + CD in the Slovenian population, analyzed in the genotype context. As most previous studies have focused on the risk of celiac disease in individuals with a prior diagnosis of T1D, we also investigated a group of patients with CD as the first diagnose.

Material and methods

Patients and the control group

Serological screening for CD has been performed in all children with T1D at the disease onset and annually thereafter since 1995 by endomysial immunoglobulin A (IgA) autoantibodies (EMA) measured by indirect immunofluorescence until 2000, and subsequently by anti-tissue transglutaminase IgA antibodies (tTG) measured by enzyme-linked immunosorbent assay [35]. In IgA-deficient individuals with CD, the same type of antibodies in IgG class were determined. All patients positive for anti-tTG and/or EMA underwent endoscopy for histological confirmation. From 1995 to 2009, 862 children from 0 to 17.99 years of age at diagnosis were referred to the University Children's Hospital Ljubljana, Slovenia at the onset of T1D, and 69 were diagnosed with co-occurring CD (male to female ratio 0.9), giving a crude prevalence of 8%. Ten (14.5%) had already been diagnosed with CD prior to the T1D onset, mean age at onset of T1D was 8.5 ± 4.3 years and the median lag between diagnoses was 4.4 ± 4.5 years. The diagnosis of CD in all other cases was reached through the annual screening. Twelve (17.4%) had positive tTG antibodies and/or EMA at the onset of T1D, mean age at T1D onset was 9.9 ± 4.2 years. Forty-seven (68.1%) developed antibodies during the follow-up, mean age at the onset of T1D was 7.5 ± 4.7 years and the mean lag between diagnoses was 3.7 ± 3.6 years. Sixty-seven individuals with T1D, with mean age at the onset of T1D 7.6 ± 4.3 years, who did not develop CD after a minimum of 10 years of follow-up, were included in the T1D group. In 68 children with CD only diagnosed and followed up in the University Children's Hospital Ljubljana, Slovenia, the mean age at diagnosis of CD was 6.0 ± 4.4 years and the mean duration of CD was 7.5 (± 4.5) years. All were diagnosed according to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria [36]. In addition, a group of 130 healthy unrelated Slovenian individuals recruited for a population study [37] at Blood Transfusion Center of Slovenia served as the control group. The study protocol was approved by the National Committee for Medical Ethics (No. 98/09/12) and written informed consent was obtained from all participating individuals and/or their parents, when appropriate, prior to the enrollment. To study an influence of the risk haplotypes on the onset of the T1D and CD, individuals with coexisting T1D and CD were split into two groups according to the order of the onset of the diseases. The group of 47 individuals who developed T1D first and CD later were designated as ''T1D first'' and those who developed CD first as ''CD first''. As individuals with CD diagnosed at the onset of T1D were considered to have had asymptomatic CD before the onset of T1D, they were

included in the same group as those diagnosed with CD before the onset of T1D, composing a group of 22 individuals.

HLA typing

The DRB1, DQB1 and DQA1 specificities in individuals with T1D were determined using PCR–sequence-specific primer (PCR–SSP) typing (Olerup SSP, Stockholm, Sweden). Individuals with T1D + CD and CD were typed for DRB1, DQB1 and DQA1 using PCR–reverse sequence-specific oligonucleotide probe (PCR–rSSO) typing assay (LabType SSO, One Lambda, Kanoga Park, CA) and Luminex technology on a flow cytometer-like instrument (LABSCAN TM 100, Luminex corporation, Austin, TX) with Luminex 100™ IS Software. In cases of ambiguous results, especially for DQA1, PCR–SSP was used for more detailed determination. In the control group PCR–SSP and PCR sequencing-based typing (PCR–SBT) (Conexio's SBT Resolver™, Conexio-Genomics, Fremantle, Western Australia) were used for HLA-DRB1and DQB1 and PCR-rSSO for DQA1 typing.

Statistical analysis

Alleles were deduced into three loci haplotypes (DRB1-DQB1-DQA1) based on known DR-DQ linkage disequilibrium in individuals of European origin and by means of the known patterns of linkage disequilibrium in the Slovenian population [38,39] DRB1*04:01-DQA1*03:01-DQB1*03:02, DRB1*04:02-DQA1*03:01-DQB1*03:02, DRB1*04:04-DQA1* 03:01-DQB1*03:02, DRB1*04:05-DQA1*03:01-DQB1*03:02 and DRB1*04:08-DQA1*03:01-DQB1*03:04 haplotypes were considered as DR4-DQ8 and DRB1*03:01-DQA1*05:01-DQB1*02:01 haplotype as DR3-DQ2. We also determined DR7-DQ2 haplotype as being encoded by DRB1*07:01–DQA1*02:01-DQB1*02:02 and DR5-DQ7 as DRB1*11/12–DQA1*05:05–DQB1*03:01. We took into account that DQA1*05:01 and DQA1*05:05 do not differ in functionally relevant exon 2 [40]. Other haplotypes were defined as X. In order to establish the effect of these haplotypes on the disease association at the genotype level different deduced DRB1-DQA1-DQB1 haplotype combinations were studied. DRB1-DQA1-DQB1 haplotypes of interest are shown in Table 1. Frequencies of particular DRB1, DQA1, DQB1 alleles, deduced haplotypes and deduced genotypes were calculated by direct phenotype counting. Two-tailed Fisher's exact test was used for comparisons of the frequencies of analyzed alleles, deduced haplotypes and genotypes between the control group and groups of patients, respectively. Additionally, groups of patients with T1D and CD only were compared with T1D + CD group in the same manner. p-value of ≤ 0.05 was considered significant. Odds ratios were determined using a cross-product and confidence intervals (CIs) were calculated.

Table 1. HLA class II deduced and abbreviated haplotypes.

HLA-deduced haplotype	Abbreviation
DRB1*04-DQA1*03-DQB1*03:02	DR4-DQ8[a]
DRB1*03-DQA1*05:01-DQB1*02:01	DR3-DQ2
DRB1*07–DQA1*02:01–DQB1*0202	DR7-DQ2
DRB1*11–DQA1*0505–DQB1*0301	DR5-DQ7

[a]Alleles considered as the DR4-DQ8 haplotype included DRB1*04:01, DRB1*04:02, DRB1*04:04, DRB1*04:05, DRB1*04:08, DQA1*03:01, DQA1*03:03, DQB1*03:02 and DQB1*03:04.

Results

HLA class II haplotypes and genotypes in Slovenian patients with CD

Alleles DQB1*02:01 ($f = 83.82$, OR = 20, $p < 0.001$), DQA1*05:01 ($f = 83.82$, OR = 9.43, $p < 0.001$), DQA1*05:05 ($f = 23.53$, OR = 3.03, $p = 0.009$), DRB1*03:01 ($f = 83.82$, OR = 20, $p < 0.001$) and DRB1*12:01 ($f = 4.41$, OR = 14.28, $p = 0.04$) were significantly associated with risk for developing CD (Table 2). HLA-DRB1-DQA1-DQB1 haplotypes are listed in Table 3. Fifty-seven patients (83.82%) carried DRB1*03-DQA1*05:01-DQB1*02:01 haplotype, the only significantly over-represented haplotype in CD group (OR = 19.77, $p < 0.001$). Distribution of deduced genotypes in patients and controls is illustrated in Table 4. Taking into account DQB1*02:01-DQA1*05:01 molecules encoded in cis or trans, 61 (89.7%) carried the DQ2 heterodimer. Four patients (5.88%) carried DR7-DQ2/DR5-DQ7 and four (5.88%) carried DR4-DQ8 without DR3-DQ2. The frequency of DQ2 and/or DQ8 in our population reached 95.6% (65 patients). Two individuals carried one copy of DR5-DQ7 and one was DR7-DQ2 homozygous. We observed the dose effect of the DQ2 on the risk for CD. The DR3-DQ2/DR3-DQ2 (OR = 19.68, $p < 0.001$) and the DR3-DQ2/DR7-DQ2 (OR = 12.35, $p < 0.001$) were both associated with significantly higher relative risk for CD compared to the DR3-DQ2/X (OR = 3.63, $p < 0.001$). Although nine patients (13.23%) carried DR3-DQ2/DR3-DQ2, 11 (16.18%) carried DR3-DQ2/DR7-DQ2. DR7-DQ2 was associated with increased risk only in the presence of DR3-DQ2. Statistically significant association with CD was also observed for DR3-DQ2/DR4-DQ8 (OR = 13.95, $p = 0.04$).

Distribution of DRB1-DQA1-DQB1/DRB1-DQA1-DQB1 deduced genotypes in patients with coexisting T1D and CD according to the order of onset

Table 5 lists the frequencies for DRB1-DQA1-DQB1/DRB1-DQA1-DQB1 deduced genotypes in individuals with T1D, CD and coexisting T1D + CD according to the order of their onset (''T1D first'' and ''CD first'' groups), and presents comparisons of patient groups with the control group. When individuals in ''T1D first'' group were compared with those with T1D only, genotype DR3-DQ2/DR3-DQ2 (OR = 7.31, $p = 0.001$) conferred an increased risk for CD. Genotypes DR4-DQ8/DR4-DQ8 (OR = 0.08, $p = 0.04$) and DR4-DQ8/X (OR = 0.18, $p < 0.001$) were negatively associated. Comparisons of patients in ''CD first'' group with those in CD only group revealed that DR3-DQ2/DR4-DQ8 genotype was associated with an increased risk for developing T1D (OR = 8.12, $p = 0.006$).

There were no significant differences observed in the distribution of genotypes between the groups with different order of onset of T1D or CD. Both high-risk genotypes DR3-

Table 2. HLA-DRB1, -DQB1 and -DQA1 allelic distribution in patients with celiac disease and controls.

	C	CD	CD vs C	
Allele	$N=130$ f (%)	$N=68$ f (%)	p Value	OR (95% CI)
DQB1*02:01	21.54	83.82	<0.001	20 (8.75–40.73)
DQB1*05:01	29.23	8.82	0.001	0.23 (0.09–0.59)
DQB1*05:02	14.62	2.94	0.01	0.18 (0.04–0.78)
DQB1*06:03	13.85	2.94	0.01	0.19 (0.04–0.84)
DQA1*01:01	28.46	7.35	<0.001	0.20 (0.07–0.53)
DQA1*01:02	43.08	14.71	<0.001	0.23 (0.11–0.48)
DQA1*05:01	35.38	83.82	<0.001	9.43 (4.52–19.81)
DQA1*05:05	9.23	23.53	0.009	3.03 (1.34–6.84)
DRB1*01:01	28.46	5.88	<0.001	0.16 (0.05–0.46)
DRB1*03:01	20.77	83.82	<0.001	20 (9.13–42.78)
DRB1*12:01	0.00	4.41	0.04	14.28 (0.71–274.05)
DRB1*13:01	12.31	2.94	0.04	0.22 (0.05–1.00)
DRB1*13:02	8.46	0.00	0.02	0.07 (0.004–1.31)
DRB1*16:01	11.54	1.47	0.01	0.11 (0.01–0.88)

CD, celiac disease; C, control group.

Table 3. HLA-DRB1-DQA1-DQB1 deduced haplotypes in patients with CD and controls.

	C	CD	CD vs C	
Haplotype	$N=130$ f (%)	$N=68$ f (%)	p Value	OR (95% CI)
DRB1*03:01-DQA1*05:01-DQB1*02:01	20.77	83.82	<0.001	19.77 (9.13–42.78)
DRB1*04-DQA1*03:01-DQB1*03:02	11.54	10.29	1.00	0.88 (0.34–2.27)
DRB1*07-DQA1*02:01-DQB1*02:02	16.15	17.65	0.84	1.11 (0.51–2.42)
DRB1*11/12/13-DQA1*05:05-DQB1*03:01	27.69	20.59	0.30	0.68 (0.33–1.37)

CD, celiac disease; C, control group.

DQ2/DR3-DQ2 and DR3-DQ2/DR4-DQ8 were present in similar frequencies in both groups. Based on this finding ''T1D first'' and ''CD first'' groups were pooled together and compared with the control group. Both high-risk genotypes DR3-DQ2/DR4-DQ8 (OR = 93.76, $p<0.001$) and DR3-DQ2/DR3-DQ2 (OR = 45.53, $p<0.001$) were associated with an increased risk for developing the combination of both diseases, with higher odds ratio for DR3-DQ2/DR4-DQ8. Low-risk genotypes for one disease were neutral for co-occurrence of both diseases. Furthermore, the summarized percentages of the high risk DR3-DQ2/DR4-DQ8 and DR3-DQ2/DR3-DQ2 were significantly increased in individuals with coexisting diseases ($f=56.53\%$) compared with T1D ($f=31.34\%$) or CD alone ($f=33.82\%$) (data not shown). In addition, unlike in CD where DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2 contributed to the increased disease risk, only DQ2 homozygotes composed of two DR3-DQ2 haplotypes were at a greater risk for co-occurrence of T1D + CD. DR3-DQ2/DR3-DQ2 genotype also represented higher risk for obtaining both diseases together (OR = 45.53, $p<0.001$) compared with CD alone (OR = 19.68, $p<0.001$) (Table 4).

Discussion

The frequency of HLA risk alleles in CD differs in populations worldwide [41]. The frequency of DQ2 or DQ8 in our population reached 95.6%, which is an important new information in terms of diagnostic policies, given that the presence of DQ2 or DQ8 holds a significant role in the latest ESPGHAN guidelines for the diagnosis of CD [36]. Eighty-three percent of individuals with DQ2 carried the DRB1*03:01-DQA1*05:01-DQB1*02:01 and 5.88% carried DQ2 encoded in trans as DRB1*07-DQA1*02:01-DQB1*02:02/DRB1*11/12-DQA1*05:05-DQB1*03:01. It has been reported that the proportion of DR7-DQ2/DR5-DQ7 patients increases from north to south of Europe with distinct north–south gradient [42,43]. Frequency of DR7-DQ2/DR5-DQ7 in Slovenian CD patients (5.88%) resembled more closely those from northern European populations (4%) then southern (37%) where the frequency of DR7-DQ2/DR5-DQ7 combination is higher [42]. Interestingly, the frequencies of DR3-DQ2 and DR7-DQ2/DR5-DQ7 in Slovenian patients with CD differed from those found in neighbouring region of Trieste in Italy, where the frequencies of DR3-DQ2 and DR7-DQ2/DR5-DQ7 were 74.7% and 14.9%, respectively [43]. However, they were similar to those found in Serbia [44], where DR3-DQ2 was present in 87.7% and DR7-DQ2/DR5-DQ7 in 6.8%, although the frequency of DQ8 positive patients was lower (2.7%) compared with our cohort (10.29%). In Northern European countries DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2 genotypes occur in similar frequencies with no differences in susceptibility risk between DRB1*03:01-DQA*05:01-DQB1*02:01 homozygotes and DRB1*03:01-DQA*05:01-DQB1*02:01/DRB1*07-DQA1*02:01-DQB1*02:02 heterozygotes [28,29]. In Southern European countries the DR3-DQ2/DR7-DQ2 is more common, whereas the DR3-DQ2/DR3-DQ2 is more rare [45]. In our population the highest risk for CD was conferred by DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2 genotypes resembling Northern European pattern.

Table 4. Distribution of HLA-DRB1-DQA1-DQB1 deduced genotypes in patients with CD and controls.

	C	CD	CD vs C	
HLA- DRB1-DQA1-DQB1 genotypes	$N = 130$ f (%)	$N = 68$ f (%)	p Value	OR (95% CI)
DR3-DQ2/DR3-DQ2	0.77	13.23	<0.001	19.68 (2.44–158.91)
DR3-DQ2/DR7-DQ2	1.54	16.18	<0.001	12.35 (2.65–57.53)
DR3-DQ2/DR5-DQ7	2.31	8.82	0.06	4.10 (0.99–16.93)
DR7-DQ2/DR5-DQ7	3.85	5.88	0.50	1.56 (0.40–6.02)
DR3-DQ2/DR4-DQ8	0.00	4.41	0.04	13.95 (0.71–274.05)
DR3-DQ2/X[a]	16.15	41.18	<0.001	3.63 (1.85–7.11)
DR4-DQ8/DR4-DQ8	0.00	1.47	0.34	5.80 (0.23–144.31)
DR4-DQ8/DR5-DQ7	1.54	2.94	0.61	1.94 (0.27–14.08)
DR4-DQ8/DR7-DQ2	0.00	0.00	1.00	1.90 (0.04–97.07)
DR4-DQ8/X	10.00	1.47	0.04	0.13 (1.02–1.05)
DR5-DQ7/X	16.15	2.94	0.005	0.16 (0.03–0.69)
DR5-DQ7/DR5-DQ7	3.85	0.00	0.17	0.17 (0.009–3.06)
DR7-DQ2/X	9.23	0.00	0.009	0.07 (0.004–1.19)
DR7-DQ2/DR7-DQ2	1.54	1.47	1.00	0.95 (0.08–10.73)

CD, celiac disease; C, control group.
[a]X includes non-DR3-DQ2, non-DR4-DQ8, non-DR7-DQ2 and non-DR5-DQ7 haplotypes.

Most studies have focused on the risk of CD in individuals with a prior diagnosis of T1D [46–49]. As it has been described that CD is also associated with an increased risk of subsequent T1D [13], we assessed the HLA-related risk for the development of T1D in patients with CD. The risk for developing T1D in individuals with CD was conferred only by T1D high-risk DR3-DQ2/DR4-DQ8 genotype, but not with DR4-DQ8/DR4-DQ8 or low-risk DR4-DQ8/X. In accordance with previous studies [46–49], the risk of CD in individuals with T1D was conferred only by the high-risk DR3-DQ2/DR3-DQ2 genotype. Other high-risk genotype for CD DR3-DQ2/DR7-DQ2 was not associated with higher risk for obtaining CD after T1D, and had similar influence as the low-risk DR3-DQ2/X. Additionally, DR4-DQ8/DR4-DQ8 and DR4-DQ8/X were protective for developing CD in T1D patients. Considering that optimal screening frequency for CD in T1D is not yet determined and that contemporary guidelines provide variable clinical guidance, HLA risk gradient could help to improve detection of the disease. Annual screening for CD-related antibodies in DR3-DQ2/DR4-DQ8 and DR3-DQ2/DR3-DQ2 patients with a higher predisposition for CD may not be necessary in patient with a low risk related to DR4-DQ8/DR4-DQ8 or DR4-DQ8/X genotype.

High risk genotypes DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR4-DQ8 did not affect the order of onset of T1D or CD in patients with coexisting diseases. When the risk for co-occurrence of both diseases was applied to the general population, it was not only associated with the high-risk genotype DR3-DQ2/DR3-DQ2, but even more with DR3-DQ2/DR4-DQ8, which is in concordance with a Hungarian study where the risk of developing both diseases together was increased when DQ2/DQ8 genotype was present [50]. In our children with T1D + CD DR3-DQ2/DR3DQ2 or DR3-DQ2/DR4-DQ8 showed high odds ratios. The confidence intervals were broad because of the low number of identified subjects positive for both genotypes in the control group. A previous study of the general European ancestry population shows that DR3-DQ2/DR3-DQ2 is found in only 1.34% and DR3-DQ2/DR4-DQ8 in 2.34% of subjects [51]. Even when taking into account the estimated frequencies of these genotypes in the general population, only high risk genotypes DR3-DQ2/DR4-DQ8 and DR3-DQ2/DR3-DQ2 conferred increased risk with high odds ratios for developing both diseases together.

Most patients with CD share DQ2 encoded by the DQA1*05:01 and DQB1*02:01 alleles [15]. On the contrary, DQ2 molecule encoded by DQB1*02:02-DQA1*02:01 does not predispose to the CD, although DQB1*02:01 and DQB1*02:02 differ only in one amino acid located outside peptide binding groove, and are to the current knowledge functionally identical [52]. The study of Vader et al. provides an explanation for the strong disease association with DQB1*02:01-DQA1*05:01 molecule by its ability to present a large repertoire of gluten peptides, whereas the non-disease-associated HLA-DQB1*02:02-DQA1*02:01 molecule can present only a subset of those [53]. In agreement with the present and previous studies [29,45,53], in our CD patients DR3-DQ2 haplotype was associated with celiac disease and DR7-DQ2 was not. In addition, DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2 contributed similarly to the disease risk which supported the hypothesis suggested by Donat et al. [54] that in case of DR3-DQ2/DR7-DQ2 genotype, DQB1*02:01 as well as DQB1*02:02 form heterodimer with DQA1*05:01, which is encoded in trans with DQB1*02:02, confers the risk. Unlike in CD, we have not observed the association of the DR3-DQ2/DR7-DQ2 genotype with the co-occurrence of both T1D + CD. Only DQ2 homozygotes composed of two DR3-DQ2 haplotypes were at greater risk for development of both T1D + CD. It seemed that DR7-DQ2 in combination with DR3-DQ2 rather decreased than increased the risk for T1D + CD. DR3-DQ2/DR3-DQ2 genotype represented higher risk (OR = 45.53) for obtaining both diseases together than for CD alone (OR = 19.68). Similarly, DR3-DQ2/DR4-DQ8 genotype also represented much higher risk (OR = 93.76) for obtaining both diseases together than CD alone (OR = 13.95) or T1D alone (OR = 18.13), reported in another study [27]. This suggests that the presence of high-risk genotypes DR3-DQ2/DR3-DQ2

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Table 5. Comparison in HLA-DRB1-DQA1-DQB1 genotype distribution between patients with co-existing T1D and CD according to the order of their onset and with the patients with T1D, CD and controls.

	C $N=130$ f (%)	T1D $N=67$ f (%)	CD $N=68$ f (%)	T1D first $N=47$ f (%)	CD first $N=22$ F (%)	T1D first vs T1D		CD first vs CD		T1D first vs CD first		T1D + CD (T1D first + CD first) vs C	
						p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)
DR3-DQ2/DR3-DQ2	0.77	4.48	13.23	25.53	27.27	0.001	7.31 (1.93–27.67)	0.18	2.46 (0.76–7.93)	1.00	0.91 (0.29–2.87)	<0.001	45.53 (5.92–350.02)
DR3-DQ2/DR7-DQ2	1.54	0.00	16.18	6.82	0.00	0.07	10.62 (0.53–210.58)	0.06	0.11 (0.006–1.96)	0.55	3.54 (0.17–71.54)	0.34	2.86 (0.47–17.57)
DR3-DQ2/DR4-DQ8	0.00	26.86	4.41	25.53	27.27	1.00	0.93 (0.40–2.18)	0.006	8.12 (1.83–36.05)	1.00	0.91 (0.29–2.87)	<0.001	93.76 (5.55–1584.83)
DR4-DQ8/DR4-DQ8	0.00	10.45	1.47	0.00	0.00	0.04	0.08 (0.005–1.52)	1.00	1.00 (0.04–25.43)	1.00	0.47 (0.009–24.65)	1.00	1.88 (0.04–95.66)
DR3-DQ2/X[a]	21.54	16.42	55.88	31.91	31.82	0.07	2.39 (0.98–5.82)	0.08	0.37 (0.13–1.02)	1.00	1.00 (0.34–2.98)	0.12	1.70 (0.88–3.29)
DR4-DQ8/X	11.54	40.30	4.41	10.64	13.64	<0.001	0.18 (0.06–0.50)	0.15	3.42 (0.64–18.35)	0.7	0.75 (0.16–3.48)	1.00	1.00 (0.40–2.50)

[a]DR3-DQ2/X includes DR3-DQ2 in conjugation with non-DQ2 and non-DQ8 haplotypes and DQB1*02:02 in conjugation with DQA1*05:05 in trans.
T1D, type 1 diabetes; CD, celiac disease; T1D first, patients who first developed T1D and later CD; CD first, patients who first developed CD and later T1D; T1D + CD, co-existing T1D and CD; C, control group.

or DR3-DQ2/DR4-DQ8 leads to the development of the second autoimmune disease rather than predispose for only one of them.

DQ2 homozygosity is usually associated with an increased risk and more aggressive forms of CD due to the better gluten presentation, known as gene dosage effect [55]. On the other hand, in T1D the risk conferred by the DR3-DQ2/DR4-DQ8 genotype is greater than the additive risk of DR3-DQ2/DR3-DQ2 or DR4-DQ8/DR4-DQ8 genotypes. This may be due to the increased functional diversity produced by the formation of four different DQ heterodimers (in cis and in trans) in individuals carrying heterozygous genotype [56]. The variety of α/β heterodimers probably enhances presentation of different autoimmune peptides known to play a role in T1D. Therefore, functional diversity rather than gene dosage effect may enable progression to overt T1D mediated by epitope spreading to an array of beta cell antigens [57]. Indeed in T1D the overt disease is associated with the production of multiple auto-antibodies, compared with CD where repertoire to gluten and self-antigens is more limited. However, the associations of particular DR-DQ genotypes (DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR4-DQ8) with both autoimmune diseases indicate that the mechanism of autoimmune susceptibility may partly be shared. On the other hand the shared disease associations could not be explained by the binding of the same peptides to DR and DQ molecules [58]. An additional explanation for the frequent simultaneous occurrence of T1D and CD irrespective of the presented self-peptides in individuals carrying either DR3-DQ2/DR3DQ2 or DR3-DQ2/DR4-DQ8 could be in genetic factors present on DR3-DQ2 and DR4-DQ8 haplotypes [31,32,49]. Recently, the TEDDY study showed that DPB1*04:01 on DR3-DQ2 haplotype protects genetically susceptible children from CD [59]. In patients with T1D the analysis of the single-nucleotide polymorphism (SNP) and protein–protein interactions encoded by genes within the HLA region identified proteins specific for DRB1*03:01 carriers involved in stress response and inflammation, whereas in DRB1*04:01 carriers the proteins were involved in antigen presentation [60]. Moreover, our previous data imply that alleles from C*07 allele family [34] present on DR3-DQ2 haplotype contributed to the co-occurrence of the T1D and CD through an interaction with KIRs and in this way through the innate immunity pathway.

In conclusion, co-occurrence of T1D and CD was associated only with high-risk genotypes DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR4-DQ8. We did not observe the association of the DR3-DQ2/DR7-DQ2 genotype with the co-occurrence of T1D and CD. Only DQ2 homozygotes composed of two DR3-DQ2 haplotypes were at greater risk for development of both T1D and CD. The order of the onset of T1D or CD in patients with co-occurring diseases was not influenced by HLA risk genotype profile. DR3-DQ2/DR3-DQ2 was associated with an increased risk for developing CD in patients with T1D, whereas the patients with CD carrying DR3-DQ2/DR4-DQ8 were at the higher risk for developing T1D. Less frequent screening for the second disease-related antibodies could be recommended in patients with the first disease and low-risk genotype for obtaining the second one. In addition to other genetic factors, including HLA class I alleles

present on DR3-DQ2 extended haplotype, the second extended haplotype may moderate the risk for co-occurrence of T1D and CD conferred by the DR3-DQ2 haplotype. We suggested that individuals carrying high-risk genotypes DR3-DQ2/DR3-DQ2 or DR3-DQ2/DR4-DQ8 would more likely develop both autoimmune diseases together than T1D or CD alone, however this needs confirmation in a larger study.

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Declaration of interest

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