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Association of HLA-DQ2 and HLA-DQ8 risk alleles to intestinal injury and serology in patients with celiac disease

Associação dos alelos de risco HLA-DQ2 e HLA-DQ8 à lesão intestinal e sorologia em pacientes com doença celíaca

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ABSTRACT

Objective
This study aims to describe the distribution of main celiac disease risk alleles related to human leucocyte antigen complex and their association to the severity of intestinal injury, serological test results, CD-associated diseases and symptomatology.

Methods
The DNA of 140 celiac disease patients was analyzed, and the distribution of the three most important risk alleles to the celiac disease development was identified (DQA1*05:01, DQB1*02:01 and DRB1*04, the last one as a DQB1*03:02/DQA1*03/DRB1*04 haplotype marker). Data on serological tests, biopsy results, symptomatology and incidence of CD-associated diseases were collected by using a previously validated questionnaire.

Results
It was found that 98% of patients presented at least one copy of the studied alleles. Patients carrying simultaneously both HLA-DQ2 risk alleles were highly prevalent (75%), and 20.7% patients carried the HLA-DQ8 haplotype. Positive patients for both HLA-DQ2 risk alleles presented positive association to anti-gliadin (p=0.037), anti-endomysium (p=0.001) and anti-transglutaminase (p=0.032) serological tests, and a higher prevalence of osteoporosis and hypothyroidism. Patients carrying one or none of those alleles often presented negative

serological results. In addition, it was found an association between intestinal injury severity and genetic profile (p<0.001).

Conclusion
Results suggest that the HLA-DQ genotyping are associated to serological tests and the severity of intestinal damage in celiac disease patients.

Keywords: Autoimmune enteropathy. Genetic markers. Gluten.

RESUMO

Objetivo
Este estudo tem como objetivo descrever a distribuição dos principais alelos de risco da doença celíaca relacionados ao complexo antígeno leucocitário humano e sua associação com a gravidade da lesão intestinal, resultados de testes sorológicos, doenças associadas à doença celíaca e sintomatologia.

Métodos
*Foi analisado o DNA de 140 pacientes com doença celíaca e identificada a distribuição dos três alelos de risco mais importantes para o desenvolvimento de doença celíaca (DQA1*05:01, DQB1*02:01 e DRB1*04, sendo este o último um marcador para o aplótipo de risco DQB1*03:02/DQA1*03/DRB1*04). Os dados de exames sorológicos, resultados de biópsia, sintomatologia e incidência de doenças associadas à doença celíaca foram coletados por meio de questionário previamente validado.*

Resultados
Verificou-se que 98% dos pacientes apresentavam pelo menos uma cópia dos alelos estudados. Pacientes portadores simultaneamente de ambos os alelos de risco HLA-DQ2 foram altamente prevalentes (75.0%), e 20,7% dos pacientes carregavam o haplótipo HLA-DQ8. Pacientes positivos para ambos os alelos de risco HLA-DQ2 apresentaram associação positiva aos testes sorológicos anti-gliadina (p=0,037), anti-endomísio (p=0,001) e anti-transglutaminase (p=0,032), e maior prevalência de osteoporose e hipotireoidismo. Pacientes portadores de um ou nenhum desses alelos frequentemente apresentavam resultados sorológicos negativos. Além disso, foi encontrada associação entre gravidade da lesão intestinal e perfil genético (p<0,001).

Conclusão
Os resultados sugerem que a genotipagem do HLA-DQ está associada aos testes sorológicos e à gravidade do dano intestinal em pacientes com doença celíaca.

Palavras-chave: *Enteropatia autoimmune. Marcadores genéticos. Glúten.*

INTRODUCTION

Celiac Disease (CD) is an immune-mediated systemic pathology triggered by the ingestion of prolamins present in gluten and resulting in injuries in the intestinal mucosa [1,2]. Although CD primarily affects the small intestine resulting in several typical symptoms such as chronic diarrhea, bloating and pain, it is also characterized by extra-intestinal symptoms such as osteopenia and infertility, thus reducing the quality of life of CD patients [1,3]. Such diversity of symptoms, which are often characteristic of other intestinal diseases, and even their absence can make the CD diagnosis difficult [3,4].

The diagnosis of CD in clinical practice is performed in stages, starting with a detailed anamnesis, followed by serological tests (anti-endomysium, anti-gliadin and anti-tissue transglutaminase antibodies) [5,6] and finally intestinal biopsy for patients with positive serology [6]. However, one of the main limitations of the serological test is the relatively high rate of false negative results, as up to 28% of CD patients may have negative serology [7]. False-negative serological results are supposed to be associated to the degree of intestinal damage, where higher degrees of intestinal damage are correlated to a higher likelihood of positive serology [8].

Environmental and genetic factors contribute to the onset and development of CD. Among the genetic factors, the most quoted in the literature are related to the Human Leukocyte Antigen (HLA) complex. As an alternative for the CD screening or as an exclusion criterion, the genotyping of risk alleles for DQ2 and DQ8 heterodimers genes encoding has been suggested [6,8]. The prevalence of these risk alleles in CD patients is over 95%, although it is only 30% in the general population [6,9,10]. Therefore, the genotyping of the risk alleles associated to CD and other diseases can become a powerful, rapid and cost-effective tool for clinical diagnosis [10,11].

However, only few studies have attempted to associate CD risk alleles to the symptom presentation, severity of the disease or possible interactions with diagnostic factors. Therefore, the purpose of the present investigation was to assess the association of HLA risk alleles (DQA1*05:01, DQB1*02:01 and DRB1*04) with symptoms related to the CD of CD-related comorbidities, severity of intestinal injuries, and serological results in a cohort of CD patients in southern Brazil.

METHODS

Target Population

This cross-sectional study was carried out in the Southern Brazilian region, specifically in the states of Santa Catarina and Rio Grande do Sul. Subjects of any age were included in the study whenever they had a confirmed diagnosis of CD through intestinal biopsy (n=140). The research project was approved by the Research Ethics Committee of the Faculty of Medicine of the Universidade Federal de Pelotas (UFPel, Federal University of Pelotas) with the report number 1,842,808. All participants signed a consent form.

Material Collection

The participants who signed the informed consent form underwent buccal cell collection as part of the experiment. To minimize the presence of bacteria and other contaminants in the oral cavity, participants were first instructed to rinse their mouths with water. A sterile swab was then used to gently scrape the inner cheek mucosa for 30 seconds, and the collected sample was stored in a sterile tube. The sample was preserved by refrigeration and sent to the Nutrifisiogenomics and Metabology Laboratory at the Faculty of Nutrition – UFPel, where genomic DNA extraction was performed. Following the sample collection, participants were invited to complete a validated questionnaire adapted from Cassol et al. [12] to assess the clinical profile of CD patients including: The questionnaire collected demographic and health data from participants, including information such as name (or initials), date of birth, gender, race, and ethnic origin. It also inquired about the presence of family members with celiac disease and the age at diagnosis. The questionnaire addressed symptoms experienced before diagnosis, such as mouth sores, diarrhea, abdominal pain, among others, and associated conditions like diabetes and hypothyroidism. Additionally, it asked about the performance of tests such as bone densitometry, intestine biopsy, and antibody tests, as well as any medical advice for vitamin and mineral supplementation. Finally, it inquired about the presence of related conditions, such as cow's milk protein intolerance and osteoporosis. The results of the serologies extracted come from different serological techniques varying from one laboratory to another, as well as from more recent and older diagnoses. Regarding antigliadin, diagnoses obtained by IgA, IgG, and deamidated antigliadin were grouped. Buccal cells were collected for the DNA analyzes.

Extraction of Genomic DNA and Quality Control

An adapted protocol for genomic DNA purification from Miller et al. [13] was used. DNA quality was confirmed by a PCR-test using primers that recognize a constitutive gene (angiotensin-converting enzyme) as control. The primers used were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTA GA-3' and the PCR product was analyzed in 1% agarose gel after electrophoreses by the observation of a 490pb product [14].

Genotyping of CD Related HLA-DQ Risk Alleles

The amplification of the studied alleles was performed by PCR. Alleles of interest were DQA1*05:01 and DQB1*02:01 (relative to the DQ2.5 complex and the haplotypes DR3-DQ2, DR5-DQ7 and DR7-DQ2) and DRB1*04 (relative to the DQ8 complex identifying the haplotype DR4-DQ8). Reactions were prepared in 20µL final volume using the GoTaq® Green Master Mix (PROMEGA, USA) with 2µL genomic DNA and specific primers adapted from the protocol described by Sacchetti et al. [15]. Primers to identify the DQ2 alpha-1 risk alleles (5'-AGC AGT TCT ACG TGG ACC TGG GG-3' and 5'-GGT AGA GTT GGA GCG TTT AAT CAG A-3') generated a PCR product of 144bp (this reaction identifies both alleles DQA1*05:01 and DQA1*05:05 in cross reaction), while primers identifying the DQ2 beta-1 risk alleles (5'-CGC GTG CGT CTT GTG AGC AGA AG-3'and 5'-GGC GGC AGG CAG CCC CAG CA-3') generated a 110bp amplicon. (this reaction identifies both alleles DQB1*02:02 and DQB1*02:01 in cross reaction). Together, these two reactions identify the alleles coding to the heterodimers DQ2.2, DQ2.5 and DQ7.5 [16,17]. To simplify, we refer further only the alleles DQA1*05:01 and DQB1*02:01 in the text and tables). The primers identifying the DQ8 risk allele (5'-GGT TAA ACA TGA GTG TCA TTT CTT AAA C-3' and 5'-GTT GTG TCT GCA GTA GGT3') amplified a 217bp product (this reaction identifies the allele DRB1*04 that is in the haplotype named DR4-DQ8 that also carries the DQB1*03:02 and DQA1*03alleles and identifies the alleles coding DQ8 heterodimer). To better understanding, see the figure 3 in Abadie et al. [16] and figure 1 in Espino et al. [17]. Reactions were performed in a thermocycler (AMPLITHERM®) using the following protocol: 95ºC for 5 minutes; 35 cycles of 95°C for 20 seconds, 56°C for 10 seconds and 72°C for 50 seconds; 72°C for 5 minutes. Fragments sizes were analyzed after 3% agarose gel electrophoresis stained with SYBR Safe (Invitrogen®-Thermo Fisher Scientific, USA) by photo documentation.

Genetic Profile and Grouping of Data for Analyses

In the present study, the most important CD-related alleles were analyzed, two of which belonging to the DQ2.5 and DQ2.2 heterodimers representing the alpha and beta subunits, and one which is a marker for the haplotype (DRB1*04) encoding DQ8 heterodimer. This PCR protocol is able to identify the presence or absence of those alleles, but not the number of copies in homologue chromosomes.

To better understand the relationship between genotypes, the degree of intestinal injury and serological tests, the data analysis was performed combining all alleles and considering all combinations of those alleles in CD patients (n=140). It was also analyzed the distributions and associations, considering only the DQ2 alleles (DQA1*05:01 and DQB1*02:01) or only the allele related to DQ8 (DRB1*04; Table 1).

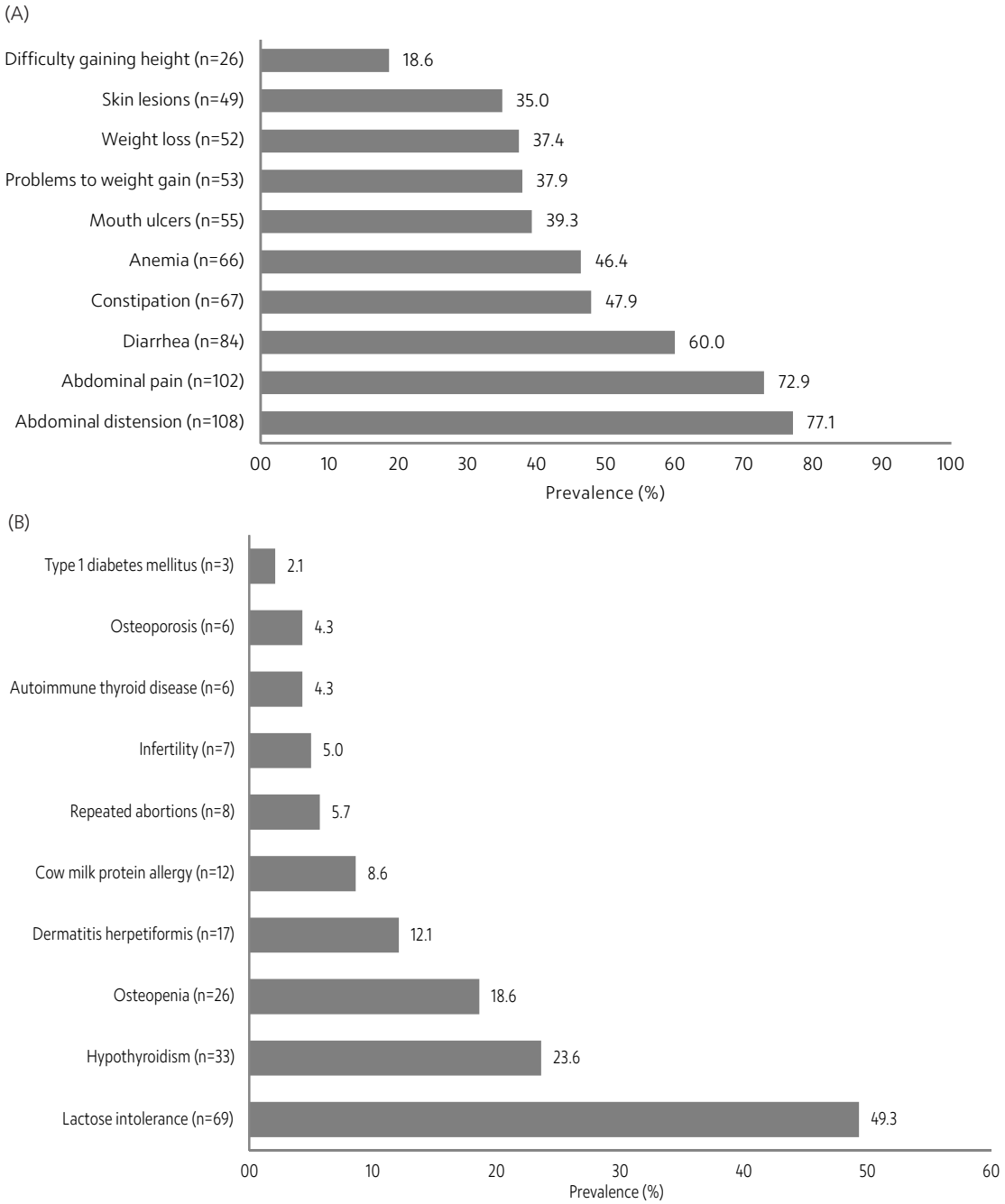

Figure 1 – Prevalence of CD related symptoms (A), CD associated co-morbidities and non-associated pathologies (B) in a Southern Brazilian cohort of CD patients (n=140).
Note: Participants of the study were asked to state disease symptoms at the time of diagnosis.

Table 1 – Distribution of celiac disease related symptoms and their association to Human leukocyte antigen-DQ risk alleles in patients with celiac disease from Southern Brazil (n=140). Data presented as n (%).

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Genetic Profiles	Prevalence of genetic profile	Anemia	Mouth ulcers	Problems to gain weight	Weight loss	Skin injuries	Difficulty gaining height	Abdominal distension	Abdominal pain	Diarrhea	Constipation
Combined analysis											
Negative for all studied alleles	3 (2.1)	1 (33.31)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	1 (33.3)	2 (66.7)	3 (100.0)	2 (66.7)	2 (66.7)
DQA1*05:01	5 (3.6)	1 (20.0)	5 (100.0)	3 (60.0)	5 (100.0)	2 (40.0)	1 (20.0)	5 (100.0)	5 (100.0)	4 (80.0)	3 (60.0)
DQB1*02:01	11 (7.9)	7 (63.4)	3 (27.3)	2 (18.2)	4 (36.4)	5 (45.5)	2 (18.2)	10 (90.9)	7 (63.3)	6 (54.6)	5 (45.5)
DQA1*05:01 + DQB1*02:01	92 (65.7)	40 (43.5)	34 (37.0)	36 (39.1)	31 (34.1)	27 (29.4)	15 (16.3)	69 (75.0)	65 (70.7)	57 (62.0)	42 (45.6)

Table 1 – Distribution of celiac disease related symptoms and their association to Human leukocyte antigen-DQ risk alleles in patients with celiac disease from Southern Brazil (n=140). Data presented as n (%).

Genetic Profiles	Prevalence of genetic profile	Anemia	Mouth ulcers	Problems to gain weight	Weight loss	Skin injuries	Difficulty gaining height	Abdominal distension	Abdominal pain	Diarrhea	Constipation
Combined analysis											
DRB1*04	11 (7.9)	3 (27.3)	4 (36.4)	4 (36.4)	5 (45.6)	7 (63.6)	2 (18.2)	8 (72.7)	9 (81.8)	7 (63.6)	5 (45.6)
DQA1*05:01 + DRB1*04	1 (0.7)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0(0.0)	1 (100.0)	1 (100.0)	0 (0.0)
DQB1*02:01 + DRB1*04	4 (2.9)	3 (75.0)	2 (50.0)	2 (50.0)	2 (50.0)	2 (50.0)	3 (75.0)	4 (100.0)	3 (75.0)	1 (25.0)	3 (75.0)
DQA1*05:01 + DQB1*02:01 + DRB1*04	13 (9.3)	9 (69.2)	6 (46.2)	4 (30.8)	4 (37.7)	4 (30.8)	2 (15.4)	10 (76.9)	9 (69.2)	6 (46.2)	7 (53.9)
Fisher's exact *p*-value		0.168	0.167	0.657	0.057	0.247	0.226	0.455	0.833	0.715	0.915
DQ2 isolated analysis											
Negative for DQA1*05:01 + DQB1*02:01	14 (10.0)	4 (28.6)	5 (35.7)	5 (35.7)	5 (35.7)	8 (57.1)	3 (21.4)	10 (71.4)	12 (85.7)	9 (64.3)	7 (50.0)
DQA1*05:01	6 (4.3)	2 (33.3)	5 (83.3)	4 (66.7)	6 (100.0)[#]	3 (50.0)	1 (16.7)	5 (83.3)	6 (100.0)	5 (83.3)	3 (50.0)
DQB1*02:01	15 (10.7)	10 (66.7)	5 (33.3)	4 (26.7)	6 (40.0)	7 (46.7)	5 (33.3)	14 (93.3)	10 (66.8)	7 (46.7)	8 (53.3)
DQA1*05:01 + DQB1*02:01	105 (75.0)	49 (46.7)	40 (38.1)	40 (38.1)	35 (33.7)	31 (29.5)	17 (16.2)	79 (75.2)	74(70.5)	63(60.0)	49 (46.7)
Fisher's exact *p*-value		0.274	0.226	0.532	0.021	0.165	0.288	0.305	0.886	0.682	0.949
DQ8 isolated analysis											
Negative for DRB1*04	111 (79.3)	49 (46.4)	43 (38.7)	42 (37.8)	40 (36.4)	35 (31.5)	19 (17.2)	86 (77.5)	80 (72.1)	69 (62.2)	52 (46.9)
DRB1*04	29 (20.7)	16 (55.2)	12 (41.4)	11 (37.9)	12 (41.4)	14 (48.3)	7 (24.1)	22 (75.9)	22 (75.9)	15 (51.7)	15 (51.7)
Fisher's exact *p*-value		0.195	0.556	0.464	0.512	0.130	0.339	0.589	0.476	0.191	0.554
Total prevalence	140 (100.0)	65 (46.4)	55 (39.3)	53 (37.9)	52 (37.4)	49 (35.0)	26 (18.6)	108 (77.1)	102 (72.9)	84 (60.0)	67 (47.9)

Note: DQA1*05:01, DQB1*02:01, DRB1*04: names of the main risk alleles for celiac disease.

Statistical Analysis

Data were analyzed by using the software STATA® version 12.0. Means and standard deviations were performed to describe continuous variables. The Shapiro-Wilk normality test and "ladder of powers" command in STATA were used to find the best fit method for each data. The Fisher's exact test was used to compare the genetic profile and other variables by comparing the distributions. Fisher's exact test was also used to analyze each genotype against the rest of the sampling to localize the significant associations points between variables. *P* values <0.05 were considered significant.

RESULTS

Prevalence of HLA-DQ Risk Alleles in CD Patients in Southern Brazil

The cohort of the present study encompassed 140 patients with CD from the states of Santa Catarina (n=72) and Rio Grande do Sul (n=68), in the Brazilian southern. The mean age was 35±16 years, and 87% of patients were female.

It was found that 97.9% of patients presented at least one of the three risk alleles assessed. Moreover, 75% of patients were positive for both alleles encoding DQ2.5 heterodimer, and 9.2% were positive for all three studied alleles. 90% of patients carry at least one of the DQ2 risk allele (DQA1*05:01 and/or DQB1*02:01) making this the most frequent genotype in the cohort. On the other hand, 20.7% of the cohort carried the DQ8 related allele in combination or not with the DQ2 related alleles (Table 2).

Table 2 – Prevalence and distribution of three Human leukocyte antigen-DQ alleles in Southern Brazilian patients with celiac disease.

Genetic profiles	Prevalence n (%)
Combined analysis	
Negative for all studied alleles	3 (2.1)
DQA1*05:01	5 (3.6)
DQB1*02:01	11 (7.9)
DQA1*05:01 + DQB1*02:01	92 (65.7)
DRB1*04	11 (7.9)
DQA1*05:01 + DRB1*04	1 (0.7)
DQB1*02:01 + DRB1*04	4 (2.9)
DQA1*05:01 + DQB1*02:01 + DRB1*04	13 (9.3)
Total positives	137 (97.9)
DQ2 isolated analysis	
Negative for DQA1*05:01 + DQB1*02:01	14 (10.0)
DQA1*05:01	6 (4.3)
DQB1*02:01	15 (10.7)
DQA1*05:01 + DQB1*02:01	105 (75.0)
Total positives	126 (90.0)
DQ8 isolated analysis	
Negative for DRB1*04	111 (79.3)
DRB1*04	29 (20.7)

Note: DQA1*05:01, DQB1*02:01, DRB1*04: names of the main risk alleles for celiac disease.

Association of Genetic Profiles with the Disease Symptoms and CD Related Co-Morbidities

Participants were requested to report the commonly presented symptoms at the time of the CD diagnosis, when gluten was still a component of the daily diet. Among the extra intestinal symptoms, anemia was the most frequent related to a frequency of 46.4% in the patients, followed by mouth ulcer (39.3%) and difficulty to gain weight (37.9%). Regarding the intestinal symptoms, 77.1% of participants reported abdominal distension, 72.9% abdominal pain, 60.0% diarrhea and 47.9% constipation (Figure 1).

Regarding the CD related symptomatology association to the genotyping, it was found an increased frequency of weight loss in positive patients exclusively for DQA1*05:01 allele (100% of CD patients exclusively positive for this allele, p=0.013). Such association was not observed in other combinations with or without this allele (Table 1).

Regarding the occurrence of CD-related co-morbidities associated to the genotyping, 71% of CD patients reported having at least one associated disease. The most commonly reported pathologies were lactose intolerance (49.3%), followed by hypothyroidism (23.6%) and osteopenia (18.6%). Although Williams, Turner and Down syndromes were related to CD, none of these syndromes were reported in the present cohort. No significant associations were found between the genetic profiles and the presence of other pathologies (Table 3).

Table 3 – Frequency of celiac disease related pathologies and their association with genetic profiles in patients with celiac disease from southern Brazil (n=140).

Genetic profiles	Prevalence of genetic profile	Lactose intolerance	Hypothyroidism	Osteopenia	Dermatitis herpetiformis	Allergy to cow's milk protein	Repetition abortions	Infertility	Autoimmune thyroid disease	Osteoporosis	Type 1 diabetes *Mellitus*
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Combined analysis											
Negative for all studied alleles	3 (2.1)	3 (100.0)	1 (33.3)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DQA1*05:01	5 (3.6)	3 (60.0)	2 (40.0)	2 (40.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DQB1*02:01	11 (7.9)	9 (81.8)	3 (27.3)	2 (18.2)	2 (18.2)	2 (18.2)	2 (18.2)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)
DQA1*05:01 + DQB1*02:01	92 (65.7)	43 (46.7)	23 (25.0)	17 (18.5)	11 (12.0)	5 (5.4)	4 (4.4)	4 (4.4)	5 (5.4)	4 (4.4)	2 (2.2)
DRB1*04	11 (7.9)	4 (36.4)	1 (9.1)	2 (18.2)	2 (18.2)	2 (18.2)	1 (9.1)	1 (9.1)	0 (0.0)	1 (9.1)	1 (9.1)
DQA1*05:01 + DRB1*04	1 (0.7)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DQB1*02:01 + DRB1*04	4 (2.9)	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
DQA1*05:01 + DQB1*02:01 + DRB1*04	13 (9.3)	4 (30.8)	2 (15.4)	2 (15.4)	0 (0.0)	1 (7.7)	1 (7.7)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)
Fisher's exact *p*-value		0.085	0.822	0.891	0.106	0.147	0.422	0.527	0.856	0.412	0.598
DQ2 isolated analysis											
Negative for DQA1*05:01 + DQB1*02:01	14 (10.0)	7 (50.0)	2 (14.3)	2 (14.3)	3 (21.4)	2 (14.3)	1 (7.1)	1 (7.1)	0 (0.0)	1 (7.1)	1 (7.1)
DQA1*05:01	6 (4.3)	4 (66.7)	2 (33.3)	2 (33.3)	0 (0.0))	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DQB1*02:01	15 (10.7)	11 (73.3)	4 (26.7)	3 (20.0)	3 (20.0)	2 (13.3)	2 (13.3)	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)
DQA1*05:01 + DQB1*02:01	105 (75.0)	47 (44.8)	25 (23.8)	19 (18.1)	11 (10.5)	6 (5.7)	5 (4.8)	6 (5.7)	5 (4.8)	4 (3.8)	2 (1.9)
Fisher's exact *p*-value		0.223	0.959	0.536	0.620	0.610	0.338	0.747	0.838	0.564	0.402
DQ8 isolated analysis											
Negative for DRB1*04	111 (79.3)	58 (52.3)	29 (26.1)	21 (19.2)	14 (12.6)	9 (8.1)	6 (5.4)	4 (3.6)	6 (5.4)	4 (3.6)	2 (1.8)
DRB1*04	29 (27.7)	11 (37.9)	4 (13.8)	5 (17.2)	3 (10.3)	3 (10.3)	2 (6.9)	3 (10.3)	0 (0.0)	2 (6.9)	1 (3.6)
Fisher's exact *p*-value		0.235	0.342	0.798	0.764	0.522	0.563	0.182	0.338	0.614	0.531
Total prevalence		69 (49.3)	33 (23.6)	26 (18.6)	17 (12.1)	12 (8.6)	8 (5.7)	7 (5.0)	6 (4.3)	6 (4.3)	3 (2.1)

Note: DQA1*05:01, DQB1*02:01, DRB1*04: names of the main risk alleles for celiac disease.

Association Between the Severity of the Intestinal Injury and HLA-DQ Risk Allele Profile

In order to find a possible association between the main risk alleles for CD and intestinal injury in patients with CD, the degree of intestinal injury set by Marsh [18] described in the intestinal biopsy report of patients was investigated. Only 43 patients had complete biopsy reports including the grade of the injury. It was found an association of the genotype distribution to the intestinal injury (p=0.018). Such association was apparently triggered by the large amount of grade 3 injuries in positive patients both for DQA1*05:01 and DQB1*02:01 alleles exclusively (85.2%). The frequency of grade 3 injuries in all other genotype combinations together was 68.7%. However, post hoc statistical analysis did not confirm this apparent association. On the other hand, an association between grade

4 intestinal injury with negative patients for all three studied alleles, as it was also found positive patients only for DQ8 haplotype, although the number of patients with these genotypes are very low, thus reducing the power of the analyses (p=0.003 for both analyses).

Association of the Genetic Profiles with Results of Serological Tests

It was investigated the association between genetic profiles and the results of the serological tests. Only some patients presented serological results for all three types of CD-related antibodies, in detail: 65 CD patients were tested to the presence of anti-AGA; 70 to anti-EMA, and 81 to anti-TG2 antibodies. It was found an association of the genotypes with all three antibodies in the combined genetic analysis (anti-AGA p=0.048; anti-EMA p=0.002; anti-TG2 p=0.002), as well as in the DQ2 exclusive analysis (anti-AGA p=0.01; anti-EMA p=0.001; anti-TG2 p=0.001). In the DQ8 exclusive analysis It was found only an association with the anti-EMA test (p=0.016; Table 4).

Table 4 – Analysis of celiac disease related Human leukocyte antigen risk alleles and serological test results.

Genetic Profiles	Positive serological test results					
	Anti-gliadin (n=65)		Anti-endomysium (n=70)		Anti-transglutaminase (n=81)	
	n (%)	Fisher's exact p-value	n (%)	Fisher's exact p-value	n (%)	Fisher's exact p-value
Combined analysis						
Negative for all studied alleles	1 (100.0)	1	1 (100.0)	1	1 (100.0)	1
DQA1*05:01	0 (0.0)	0.058	0 (0.0)	0.043	1 (33.3)	0.056
DQB1*02:01	3 (42.9)	0.056	3 (50.0)	0.108	4 (80.0)	0.561
DQA1*05:01 + DQB1*02:01	34 (85.0)	0.037	44 (91.7)	<0.001	53 (91.4)	0.032
DRB1*04	4 (80.0)	1	1 (33.3)	0.114	1 (25.0)	0.009
DQA1*05:01 + DRB1*04	0 (0.0)	–	0 (0.0)	–	0 (0.0)	–
DQB1*02:01 + DRB1*04	2 (66.7)	1	1 (50.0)	0.385	2 (100.0)	1
DQA1*05:01 + DQB1*02:01 + DRB1*04	5 (71.4)	1	5 (62.5)	0.355	7 (87.5)	1
Fisher's exact p-value	0.030		<0.001		0.006	
DQ2 isolated analysis						
Negative for DQA1*05:01 + DQB1*02:01	5 (83.3)	1	2 (50.0)	0.199	2 (40.0)	0.022
DQA1*05:01	0 (0.00)	0.058	0 (0.0)	0.043	1 (33.3)	0.056
DQB1*02:01	5 (50.0)	0.103	4 (50.0)	0.058	6 (85.7)	1
DQA1*05:01 + DQB1*02:01	39 (83.0)	0.05	49 (87.5)	0.001	60 (90.9)	0.007
Fisher's exact p-value	0.018		<0.001		0.001	
DQ8 isolated analysis						
Negative for DRB1*04	38 (76.0)	0.514	48 (84.2)	0.026	59 (88.1)	0.240
DRB1*04	11 (73.3)	0.514	7 (53.9)	0.026	10 (71.4)	0.240
Fisher's exact p-value	0.514		0.013		0.240	
Total	49 (75.4)		55 (78.6)		29 (85.2)	

Note: DQA1*05:01, DQB1*02:01, DRB1*04 = names of the main risk alleles for celiac disease.

By analyzing each specific genotype in the combined analysis, a positive association of patients carrying both DQA1*05:01 and DQB1*02:01 alleles and negative for DRB1*04 was found in all serological tests. This association persisted in the DQ2 exclusively analysis. Patients carrying both DQA1*05:01 and DQB1*02:01 alleles had positive results between 83 to 91.7% in each serologic test (Table 4). On the other hand, it was evident that DQA1*05:01 allele carriers alone presented more often negative results in the serological tests. The frequency of positive results in the serologic tests were 0 to 33.3% in patients with this genotype.

DISCUSSION

Patients with CD may present both intestinal and systemic symptoms. However, those signs may vary and be non-specific. Some patients do not manifest any typical symptoms. Therefore, science is looking for more effective methods to clearly identify carriers the affected subjects. In this sense, the genotyping of the three most important risk alleles of the HLA-DQ system was performed in patients with confirmed CD in the southern Brazilian region describing associations of the genetic profiles with intestinal injury severity and serological results.

The use of serological parameters can reach a sensitivity and specificity over 90% for CD [19]. However, in this study patients had 25% negative serology for anti-AGA, 21% for anti-EMA, and 15% for anti-TG2. Those results indicate a high number of negative results in patients with confirmed CD diagnosis of CD, agreeing with the literature that describes that about 3-28% of patients with CD and presenting negative serology [7,20], reinforcing the importance of the knowledge on the prevalence of false-negative results in the CD serology in each specific population.

The higher percentage of negative serological results in patients carrying only one of the alpha and beta DQ2 risk alleles compared to the higher percentage of positive serological results in patients carrying both alleles reinforce the clinical relevance of the HLA-DQ risk allele genotyping for the diagnosis of CD, but recently published guidelines include the HLA-DQ genotyping as a tool to be used only to exclude the CD diagnosis in some cases [5,6,21].

The higher prevalence of grade 3 injuries observed in carriers of both DQ2 risk alleles studied suggests that this genotype is associated to the injury severity. Interestingly, all individuals negative for DQ2-related alleles showed the maximum degree of injury (4 on the Marsh-Oberhuber scale), but the low number (n=2) of those individuals in the sampling suggests caution in any conclusion. However, we speculate that this result may be consequence of another genetic risk factor non-studied in the present paper. The association between the genetic profile and the degree of intestinal injury is not yet defined in the literature [2,22,23]. Therefore, the results contribute to this area of knowledge.

The high prevalence of positive patients for at least one of the DQ2 risk alleles confirms data from the European and North American studies [11,22,24]. In general, patients carrying both the alpha and beta alleles have the highest predisposition to CD and have been reported to be prevalent between 75.3 and 94.5% in patients with CD, depending on the ethnicity [10,11]. A minority of patients with CD presents the DQ8 risk haplotype with frequencies between 2.7 and 24.6% in those studies. Our findings reveal a high prevalence of the DQ8 allele (20.7%), similar to another study with CD patients in the northern Brazilian region [10].

As to the prevalence of symptoms associated to CD, they seem vary depending on the dietary habits and ethnicity of the populations [25,26]. Therefore, we believe that the present study provides important information on the regional differences of the symptom manifestation, which over time can be used to specify the diagnostic protocols.

The association of weight loss to alpha allele was very high (100% of patients carrying only this risk allele for DQ2 reported weight loss). However, further work is required to test this association in order to attain a definitive conclusion.

The pathologies associated to CD most reported by patients were lactose intolerance, hypothyroidism and osteopenia. Lactose intolerance can occur secondary to CD, caused by a damage to the intestinal mucosa [27]. However, individuals affected reported persistently here lactose intolerance, even on a gluten-free diet showing that there may be an association between these

pathologies not limited to injuries of the gastrointestinal tract. On the other hand, considering the high prevalence of lactose intolerance in the general population, both osteopenia and lactose intolerance need to be reviewed regarding their classification as comorbidities in patients with celiac disease.

One of the possible mechanisms associating CD to autoimmune hypothyroidism is the cytotoxic T lymphocytes activation by the human transglutaminase reaction in the thyroid [28]. Furthermore, both pathologies were linked to specific HLA genotypes, whose presence increases the risk to develop at least one of them [29]. Although it was not found in our study significant associations between the alleles studied and hypothyroidism, it was found a slightly increased occurrence in this cohort (23.6%). The third most prevalent disease found in our cohort was osteopenia. The causes for such symptom may be due to the reduction of calcium absorption associated to a vitamin D deficiency, as well as the inflammatory state of the patient and the consequent release of cytokines affecting the bone resorption [3,8]. It was found an increased prevalence (18.6%) of osteopenia in CD patients from the southern Brazilian region when compared to the estimates by the American Gastroenterology Association [8], describing that 1 to 3.4% of CD patients have osteopenia.

A limitation for the interpretation of this study is certainly the sample size, due to the difficulty to access this specific population. Furthermore, a large number of patients is still diagnosed with no complete serological tests and biopsy analysis, further to a reduced amount and power of the correlated analyses.

CONCLUSION

Taken together, our results indicate that CD patients in the southern Brazilian region have a high prevalence of DQA1*05:01 and DQB1*02:01 and DRB1*04 risk alleles, with only 2% of patients negative for either of the three alleles studied, encouraging the use of these genetic tests in risk analysis for CD. Furthermore, it was observed in our study that specific genetic profiles are associated to the severity of intestinal damage, evidencing a genetic predisposition not only for the disease susceptibility, but for the symptom severity as well. Added to this, it was found a positive association between the DQA1*05:01 and DQB1*02:01 genotype with the results of the classic serological tests for CD, and a description of a higher incidence of osteoporosis and hypothyroidism in the population studied. These data contribute to the knowledge in this area.

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