IL-15 Gene Polymorphism in Celiac Disease Patients and Their Siblings

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ABSTRACT

Background: *Celiac disease (CD) is an immune-mediated enteropathy characterized by lifelong gluten intolerance. Interleukin-15 (IL-15) is a proinflammatory cytokine that is considered a key component in the immune reaction triggered by gluten. Our aim of this study was to evaluate the influence of IL-15 gene polymorphisms on CD development and clinical presentation.*

Methods: *The study was enrolled-with 90 CD patients (49 female/41 male, median years of age 11), their 38 siblings (20 female/18 male, median years of age 8), and 99 healthy controls (66 female/33 male, median years of age 13). Their demographic findings, symptoms, and signs histopathological grade, Human Leukocyte Antigen (HLA) types were recorded. IL-15 gene polymorphisms rs2857261, rs10519613, and rs1057972 were analyzed through PCR.*

Results: *There was a significantly higher frequency of GG genotype in rs2857972 polymorphisms and TT genotype in rs1057972 polymorphisms in celiac families compared to controls [41% vs. 23% (P = .0008), 36% vs. 11% (P = .001), respectively]. Without considering their HLA status, there was not any difference between celiacs and healthy siblings. However, when stratified according to their HLADQ2 status, rs2857972 GG polymorphism was 1.5 times prominent in celiacs than siblings at homozygous state, whereas rs1057972 TT genotype was found to be 2.5 times prominent in celiac siblings at heterozygous state. There was no association between these polymorphisms and clinical presentation.*

Conclusion: *rs2857972 GG and rs1057972 TT variants of IL 15 are more prominent in celiac families than controls. However, the impact of IL-15 gene polymorphism on CD development is dependent on HLADQ2 status.*

Keywords: *Celiac disease, IL-15, gene polymorphism*

INTRODUCTION

Celiac disease (CD) is recognized as the most prevalent autoimmune enteropathy in children. It is triggered by gluten in genetically susceptible individuals. The genetic basis of CD is clearly defined. The primary gene is the MHC gene coding class II HLA DQ2/DQ8.[1] However, although 95% of celiac patients are HLA-positive, there is a remarkable discordance between HLA positivity and CD prevalence in certain populations. HLA-DQ2/DQ8 positivity is present in approximately 35-40% of the population. Yet only about 1% of these individuals develop CD, suggesting effects of other possible genetic factors. More recently, human genome studies have identified many non-HLA gene loci and specific single-nucleotide polymorphisms (SNP) related to the cytokines involved in CD.[2,3] One of these cytokines is interleukin (IL)-15, which has a pivotal role in the immunopathogenesis of CD, as shown by significant positive correlations between IL-15 levels and histopathological severity of the disease. In healthy subjects, it is expressed only on villous enterocytes and almost absent in the lamina propria. In contrast, in inflammatory conditions, it is overexpressed by enterocytes and lamina propria mononuclear cells.[4] The inflammation in CD is characterized by a dual immune reaction taking place in the lamina propria and the epithelium of the intestine. It includes both the adaptive and innate immune system. After ingestion and modification by tissue transglutaminases, gluten-derived peptides bind to disease predisposing HLA DQ2 and DQ8 molecules on antigen-presenting cells, triggering a CD4+ T cell response in the lamina propria—a primary hallmark of inflammation in CD. This response leads to the secretion of many proinflammatory cytokines, including interferon-gamma, IL-10, and IL-15. In addition to this adaptive T cell

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response, an innate response mainly driven by IL-15 and results in intraepithelial lymphocyte activation occurs. T cell activation drives B cell recruitment and antibody response to deamidated gluten and tissue transglutaminase. This reaction ends with basal membrane antigen–antibody complex deposition, thereby leading to CD8+ intraepithelial lymphocyte infiltration. These inflammatory activities in the lamina propria and intraepithelium are associated with IL-15. As a result of these functions and the IL-15-induced differentiation of natural killer cells, the second hallmark of CD, epithelial cell damage, and villous atrophy, occurs.[5]

Due to its broad expression, IL-15 is also involved in the immunopathogenesis of other autoimmune diseases; for instance, variants in the IL-15 gene have already been associated with psoriasis, type 1 diabetes, ulcerative colitis, and rheumatoid arthritis.[6,7]

Based on these findings, we hypothesized that variations in the IL-15 gene may contribute to the genetic susceptibility to CD and could explain the reason why some children with the same HLA genetic background develop CD, while others do not. Thus, we evaluated IL-15 gene polymorphisms in patients with CD, their healthy siblings, and healthy controls with the primary objective of evaluating the effect of these IL-15 gene polymorphisms on CD occurrence and presentation. The secondary aim was to evaluate the effects of concomitant HLA positivity and IL-15 gene polymorphisms on CD development.

MATERIALS AND METHODS

This prospective cohort study was conducted with histopathologically proven CD cases diagnosed in the Pediatric Gastroenterology Department of our institute between August 2007 and July 2015. The healthy siblings of these patients and healthy controls were also included. CD was confirmed with a positive serological test for anti-tissue transglutaminases and/or antiendomysium and consistent histopathological findings. Pathologic grading was performed according to the Marsh criteria.[8]

Demographic findings, family history of CD, past medical history, physical examination findings, HLA status, celiac screening tests, bone mineral density (BMD) values, and the endoscopic and histopathological findings of patients with CD were analyzed from their electronic health records. Since sibling screening is strongly recommended in our clinic, data of non-celiac siblings were also available and were obtained from the same electronic health records. All of the siblings were screened with the aforementioned antibodies and HLA tests. The control group consisted of non-CD patients who had undergone upper gastrointestinal endoscopy due to any other gastrointestinal symptom and had been defined to have normal duodenal histopathology.

Four milliliters of blood was collected from all of the participants for IL-15 gene polymorphism analysis. All samples were stored at -20°C until measurements were performed. Exclusion criteria were as follows: acute or chronic gastrointestinal disease (gastroenteritis, gastritis, inflammatory bowel disease, hepatitis, upper and lower respiratory tract infection, and cirrhosis) or extra gastrointestinal diseases, such as juvenile rheumatoid arthritis, renal failure, cerebral palsy, and allergic conditions. Previously defined IL-15 gene polymorphisms, rs2857261, rs10519613, and rs1057972, were studied via PCR.[9]

The study was approved by the institutional ethics committee (date: July 2, 2013, number: 80558721/08). A written informed consent form was obtained from the parents of all patients and also from participants that were older than 14 years of age.

Patients with malabsorption symptoms such as abdominal pain, diarrhea, and abdominal distention were classified as "typical CD." Those presenting with extra-intestinal symptoms were classified as "atypical CD." Children presenting with intestinal symptoms other than those listed and extra-intestinal symptoms were defined as "atypical celiacs." Malnutrition was defined according to World Health Organization guidelines; the measurements of patients were plotted on national growth charts with cut-off points determined according to standard deviations: –2 SD weight-to-height or body mass index (representing acute malnutrition) and –2 SD height-to-age (representing chronic malnutrition). Patients diagnosed younger than 2 years of age were defined as "early celiacs." BMD measurements were performed in patients older than 5 years. We defined osteopenia in those with BMD Z-scores between –2 and –2.5, while osteoporosis was defined in those with Z-scores lower than –2.5.

DNA Isolation and Cytokine Genotyping

Genomic DNA was isolated from whole blood by using the "MagNA pure Compact Nucleic Acid Isolatıon Kit" and extraction roboting system. IL-15 gene rs2857261, rs10519613, rs10557972 polymorphisms were studied according to the study by Xue-Jun

Zhang et al., by using *Mbo*II, *Dra*I, and *Dde*I restriction enzymes.[9] The rs2857261 polymorphism was amplified with forward 5'-TCTTCAATACTTAAGGATTTAC-3′ and reverse 5'-AAGAAGAGCCTATCAAGATG-3'. The rs10519613 polymorphism was amplified with forward 5'-AGTGTTTCTGTTATTAACAAAC-3' and reverse 5'-CATTATTCCACAAATATGTAC-3'. Finally, the rs1057972 polymorphism was amplified with forward 5'-AGTTGCACTGATATTTTACCT-3' and reverse 5'-CAGTAGTCAGTGGTTCCACTC-3'. HLA typing was performed by the Sequence-Specific Oligonucleotide (SSO) method, and the LAB Type™ SSO Class II DQA1/DQB1 Typing Test (One Lambda; Thermo Fisher Scientific, Canoga Park, CA, USA) was used as the test kit. Test analysis was performed using the Luminex Lab Scan 100™ Xmap instrument (Luminex Corp., Austin, TX, USA), and data analysis were performed using the same HLA Fusion V.356 computer program.

Statistical Analysis

Statistical analyses were performed with the IBM SPSS Statistics v20.0 (IBM Corp., Armonk, NY, USA) software. The normality of the distribution of data was checked with the Kolmogorov–Smirnov test. Except for age, variables with normal distribution were provided as arithmetic mean and SD. Data regarding age were provided as median (minimum-maximum). Categorical variables were compared with chi-squared tests and the Fisher Exact test when necessary. The mean of 2 non-normally distributed variables was compared with the Mann–Whitney *U* test. To compare the means of nonparametric variables in more than 2 groups, the Kruskal–Wallis one-way analysis was performed. Logistic regression analysis was applied to data showing significant differences between the groups and the odds ratio (OR) and 95% CI. *P* values less than .05 were considered to be statistically significant. Genotype distribution of IL-15 polymorphisms in the control group was examined to determine whether it conformed to the Hardy–Weinberg equilibrium (HWE). Also, linkage disequilibrium between IL-15 polymorphisms was also detected to estimate their relationship with disease incidence.

RESULTS

Ninety patients with CD (49 female/41 male, median age: 11 (3-18) years), 38 healthy siblings of these patients (20 female/18 male, median age: 8 (1-17) years), and 99 healthy controls (66 female/33 male, median age: 13 (5-18) years) were eligible for the study. No statistically significant differences were observed between the 3 groups in terms of age and sex distribution ($P = .06$ and $P = .08$, respectively).

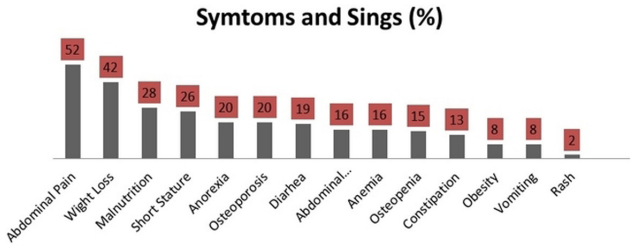

Figure 1. Celiac patients symptoms and signs.

There were only 4 early celiac patients. Sixteen (17%) of the patients were younger than 5 years of age. Among all CD cases, 65 (72.2%) were atypical, and 25 (28%) were typical celiac. The most common symptom at diagnosis was abdominal pain (Figure 1). Short stature and protein-energy malnutrition were more prevalent in the celiac group. Only 6 of the 90 celiac patients (6.7%) had Marsh grade 1 lesion, while the remaining 84 patients (%93.3) had Marsh grade 3 lesions.

IL-15 gene rs2857261, rs10519613, and rs10557972 polymorphisms and their different genotypes were studied in all participants and compared between groups. There were statistically significant differences in AA and GG genotype for the rs2857261 polymorphism, in the CA genotype for rs10519613 polymorphism, and in the TT genotype for rs1057972 polymorphism (Table 1). Differing from controls, celiac families had GG genotype in the rs2857261 polymorphism and TT genotype in the rs1057972 polymorphism.

Both the GG genotype with rs2857261 polymorphism and the TT genotype with rs1057972 polymorphism stratified similar in celiacs and their healthy siblings (CD vs. HS: 37 [41%] vs. 11 [28%], $P = .2$ and 19 [21%] vs. 14 [36%], $P = .1$, respectively).

Comparing healthy siblings and control groups in terms of these 3 genotypes revealed a difference only in the TT genotype with rs1057972 polymorphism (Table 2).

There was a significantly higher frequency of GG genotype in rs2857261 polymorphisms and TT genotype in rs1057972 polymorphism in celiac patients compared to control groups (Table 3). Conversely, AA genotype in rs2857261 polymorphism was more prevalent among controls than celiac patients (Table 3).

Table 1. IL-15 Gene Polymorphisms of the Whole Study Group

Polymorphism	Genotype	Celiac Patients, *n* (%)	Healthy Siblings, *n* (%)	Controls, *n* (%)	χ^2	*P*
rs2857261 (MboII)	AA	43 (47)	21 (55)	68 (68)	0.22	**.013**
	GG	37 (41)	11 (28)	23 (23)	3.75	**.028**
	GA	10 (11)	6 (15)	8 (8)	6.98	.5
rs10519613 (DraI)	CC	29 (32)	13 (34)	22 (22)	5.42	.7
	AA	53 (58)	25 (65)	63 (63)	9.13	.2
	CA	8 (9)	0 (0)	14 (14)	7.82	**.04**
Rs1057972 (DdeI)	AA	66 (73)	23 (60)	77 (77)	4.31	.1
	TT	19 (21)	14 (36)	11 (11)	0.44	**.03**
	AT	5 (5.6)	1 (2.6)	11 (11)	35.64	.1

P values in bold indicate the presence of statistical significance.

GG genotype with rs2857261 polymorphism and TT genotype with rs1057972 polymorphism were much more prevalent among celiac patients than controls rather than healthy siblings. GG genotype at rs2857261 polymorphism was 2.3 times higher in celiac patients than controls [OR: 2.3 (1.2-4.3), (*P* = .008)]. Likewise, TT genotype with rs1057972 polymorphism was 1.4 times more frequent in celiac patients than control [OR: 1.4 (1.1-1.9), (*P* = .04)]. Finally, the AA genotype with rs2857261 polymorphism was 1.5 times higher in control groups than celiac patients [OR: 1.5 (1.1-2.1) (*P* = .004)].

Seventy-five (83%) of the patients with CD and 23 (61%) of the siblings were HLADQ2 positive. Among these, 23 (25%) of those with CD and 8 (21%) of the siblings were HLA DQ2 homozygous (*P* = .9). None of the subjects carried the HLA DQ8 haplotype.

Stratifying these 3 polymorphisms between HLA-positive celiacs and their healthy siblings revealed a difference only in the TT genotype with rs1057972 polymorphism (Table 4).

In the HLADQ2 positive state (either homozygous or heterozygous), TT genotype with rs1057972 polymorphism was much more prevalent in healthy siblings than celiac patients [OR: 2.4 (1.2-4.7), *P* = .02]. At homozygous state, rs2857972 polymorphism GG genotype was found to be present only in celiac patients but not in their siblings [7 (30%) vs. 0 (0%), *P* = .028]. In contrast, this difference was not significant in the heterozygous state [22 (42%) vs. 6 (40), *P* = .8]. Although rs1057972 TT genotype was more prominent in celiac siblings at heterozygote state [10 (19.2%) in patients vs. 7 (46.7%) in siblings, *P* = .04, OR: 2.5 (1.1-1.6)] this difference was not observed in the homozygous state [6 (26.1%) in patients vs. 4 (50%) in siblings, *P* = .2]. Therefore, it seems that the GG genotype with rs2857972 may increase disease probability only if the patient is also homozygously HLA DQ2 positive. And TT genotype with rs1057972 polymorphism may be protective only in the heterozygous HLA DQ2 state.

We further analyzed the impact of these 2 polymorphisms on CD symptoms and histopathological grade. Ten out of 25 (40%) typical celiac patients and 27 out of 65 (41.5%) atypical celiac patients had rs2857261 GG genotype polymorphism (*P* = .8). Six (24%) typical celiac patients and 3 (20%) atypical celiac patients had TT genotype with rs1057972 polymorphism (*P* = .8). Except for abdominal pain, none of the clinical findings were influenced by the presence of these polymorphisms and genotypes. In the

Table 2. IL-15 Gene Polymorphisms in Healthy Siblings and Controls

Polymorphism	Genotype	Healthy Siblings, *n* (%)	Controls, *n* (%)	OR (95% CI)	*P*
rs2857261 (MboII)	AA	21 (55)	68 (68)	0.56 (0.26-1.2)	.1
	GG	11 (28)	23 (23)	1.3 (0.58-3.1)	.6
rs1057972	TT	14 (36)	11 (11)	4.6 (1.8-11.5)	**.001**

P values in bold indicate the presence of statistical significance.
OR, odds ratio.

Table 3. IL-15 Gene Polymorphisms in Celiac Patients and Controls

Polymorphism	Genotype	Celiac Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)	*P*
rs2857261 (MboII)	AA	43 (47)	68 (68)	0.41 (0.23-0.75)	**.004**
	GG	37 (41)	23 (23)	2.3 (1.2-4.3)	**.008**
rs1057972	TT	19 (21)	11 (11)	2.1 (0.9-4.7)	**.04**

P values in bold indicate the presence of statistical significance.
OR, odds ratio.

presence of rs1057972 polymorphism, abdominal pain was more prevalent in TT negative patients (Table 5).

Eighty-four (93.3%) celiacs were determined to be Marsh grade 3. Neither the presence of rs2857261 GG genotype nor the absence of rs1057972 TT genotype had any impact on the histopathological grade of CD (Table 6).

DISCUSSION

Here we studied IL-15 gene polymorphisms to highlight the discordance of CD development between celiacs and their healthy siblings with identical HLA characteristics. Our findings show that rs2857261 GG and rs1057972 TT variants are more prominent in celiac families than healthy controls. Although GG genotype with rs2857261 seems to increase disease development and TT genotype with rs1057972 seems to be protective against disease development, the impact of these polymorphisms on CD is dependent on HLA DQ2 status. GG genotype with rs2857261 polymorphism had a disease-enhancing effect only in the homozygous state, while the TT genotype with rs1057972 could demonstrate its protective effect only in the heterozygous state.

Major genetic predisposition for CD is associated with HLA DQ2 and DQ8. However, the high prevalence of HLA DQ2 does not coincide with celiac prevalence in certain populations. Even in HLA-identical siblings, the disease concordance rate has been reported to be between 30-50% suggesting other possible genetic factors.[10] Also, in our study, 23 (61%) of the siblings were healthy despite having HLA DQ2 expression. The HLA DQ2 prevalence in our country is unknown; however, HLA DQ2 prevalence among Turkish celiac patients has been reported to be 52-97%.[11-13] In the current study, 83% of the celiacs were HLA DQ2 positive and 17% were HLA negative. Valentina and colleagues studied 249 celiac patient siblings and reported that some non-HLA gene regions and some specific polymorphisms were more common in celiac siblings.[14] IL-15 is one of the pivotal cytokines bridging the adaptive and innate immune responses in the pathogenesis of CD, and it has been found to be increased both in the lamina propria and intestinal epithelium of patients.[15,16] Recently, overexpression of IL-15 has been linked with the immunopathology of several other HLA-related autoimmune disorders. Variants of the IL-15 gene and their effects on these disease presentation, activity, serum, and tissue expressions have been well studied. The

Table 4. IL-15 Polymorphisms in HLA-Positive Celiac Disease Patients and Healthy Siblings

Polymorphism	Genotype	HLA (+) Celiac Patients, *n* (%)	HLA (+) Healthy Siblings, *n* (%)	*P*	P^a	P_{HWE}
rs2857261 (MboII)	AA	39 (52)	14 (60.9)	.6	Ref.	.29
	GG	29 (38.7)	6 (26.1)	.3	.63	
	GA	7 (9.3)	3 (13.8)	.6	.12	
rs10519613 (DraI)	CC	42 (56)	14 (60)	.8	Ref.	.27
	AA	26 (34.7)	9 (39.1)	.6	.32	
	CA	7 (9.3)	0 (0)	.1	.24	
rs1057972 (DdeI)	AA	54 (72)	12 (52)	.1	Ref.	.17
	TT	16 (21.3)	11 (47.8)	**.02**	.02	
	AT	5 (6.7)	0 (0)	.2	.13	

P^a value was adjusted by clinical parameters.
P values in bold indicate the presence of statistical significance.
HLA, human leukocyte antigen; Ref, reference category; HWE, Hardy–Weinberg equilibrium.

Table 5. IL-15 Gene Polymorphisms, Symptoms, and Signs in Patients With Celiac Disease

	rs2857261 GG			rs1057972 TT		
Symptoms	GG (+), *n* (%)	GG (–), *n* (%)	*P*	TT (+), *n* (%)	TT (-), *n* (%)	*P*
Abdominal pain	20 (42.6)	27 (57.4)	.7	15 (31.9)	32 (68.1)	**.01**
Weight loss	15 (39.5)	23 (60.5)	.7	9 (23.7)	29 (76.3)	.8
Anorexia	8 (44.4)	10 (55.6)	.9	4 (22.2)	14 (77.8)	.5
Diarrhea	8 (47.1)	9 (52.9)	.7	4 (23.5)	13 (76.5)	.7
Abdominal distention	6 (42.9)	8 (57.1)	.8	5 (35.7)	9 (64.3)	.1
Constipation	4 (33.3)	8 (66.7)	.7	3 (25)	9 (75)	.7
Vomiting	2 (28.6)	5 (71.4)	.6	1 (14.3)	6 (85.7)	.5
Characteristics						
Age at presentation						
≤5	10 (34.5)	19 (65.5)	.5	8 (27.6)	21 (72.4)	.4
	27 (44.3)	34 (55.7)		10 (18)	50 (82)	
Malnutrition	12 (48)	13 (52)	.8	4 (16)	21 (84)	.6
Short stature	9 (39.1)	14 (60.9)	.8	4 (17.4)	19 (82.6)	.7
Osteoporosis	8 (47)	9 (53)	.9	3 (16.7)	14 (83.3)	.7
Anemia	4 (28.6)	10 (71.4)	.4	3 (21.4)	11 (78.6)	.7
Osteopenia	5 (45)	6 (55)	.5	2 (15.4)	9 (84.6)	.6

P values in bold indicate the presence of statistical significance.

celiac disease shares many features with these disorders; in fact, some of these diseases, such as rheumatoid arthritis, psoriasis, and inflammatory bowel diseases, are often concomitant with CD.[9,17-19] With this background in mind, we studied 9 genotypes in 3 different previously defined IL-15 gene polymorphisms. Yamamoto-Frusho et al. reported the IL-15 rs2254514 polymorphism as a possible susceptibility marker in ulcerative colitis.[19] Zhang and colleagues studied rs2857261, rs10519613, rs1057972, and g96516 SNP polymorphisms in 632 psoriatic patients and 485 controls. They demonstrated a possible association between g.96516 A/T and increased psoriasis risk.[9] In another study, Pavkova Goldbergova et al. studied IL-15 gene polymorphisms, IL-15 serum levels, and their affects on rheumatoid arthritis activity. They showed that 14035 A/T and –260 A/G polymorphisms increased IL-15 serum levels and serum RF isotypes and influenced rheumatoid activity.[18] Most of the studies regarding the association between IL-15 and CD investigate the serum level and tissue expression of IL-15. In a trial including 728 celiacs and 816 unselected blood donors, Escudero-Hernández et al. identified 2 regulatory SNP rs4956400 and rs11100722 that might be associated with CD.[7] This association was further supported by the results of expression analysis, where they demonstrated that the identified SNPs were indeed associated with increased protein expression.

Table 6. The Relationships Between IL-15 Gene Polymorphisms and Histopathological Grade of Patients

	rs2857261 GG		rs1057972 TT		
Marsh	GG (+), *n* (%)	GG (–), *n* (%)	TT (+), *n* (%)	TT (–), *n* (%)	*P*
Stage 1	1 (16.7)	5 (83.3)	0 (0)	6 (100)	.3
Stage 3	36 (42.9)	48 (57.1)	19 (2.26)	65 (77.4)	

Our study is the first research that evaluates the combined effects of IL-15 gene polymorphisms and HLA in terms of their effects on CD. Here, we provided evidence for a possible association between GG genotype with rs2857261 and TT genotype with rs1057972 IL-15 gene polymorphisms. Without considering HLADQ' status, we did not observe any major difference in terms of these polymorphisms between celiacs and healthy siblings; however, analyses performed with regard to HLA DQ2' status revealed 1.5-time more frequent GG genotype with rs2857261 in celiacs that were homozygous, and 2.4-time more prevalent TT genotype with rs1057972 in healthy siblings that were heterozygous. This suggests

that rs2857261 GG polymorphism might exhibit a disease-promoting effect in the presence of homozygous HLADQ2 positivity, whereas rs1057972 TT polymorphism could not provide protection due to its relationship with the heterozygous state.

Associations between IL-15 and clinical symptoms have been studied in other autoimmune diseases. Five SNPs (rs7667746, rs7665842, rs2322182, rs6821171, and rs4371699) were correlated with joint rate destruction in rheumatoid arthritis. According to a study performed by Yamamoto-Flusho et al., rs2254514 CC genotype was associated with young age in Ulcerative colitis (UC). Our study is also the first study that provides information about the effects of IL-15 polymorphism and the clinical presentation of CD. In the last 2 decades, the presentation of CD has increasingly shifted toward atypical signs and symptoms.[11,19,20] Similarly, most of our patients presented with atypical symptoms. Evaluation of the effects of rs2857261 GG and rs1057972 TT variants on celiac type revealed insignificant results. Consistent with other trials, the most common intestinal sign observed in our study was abdominal pain.[11,21] Here, in our study, abdominal pain was less frequent in rs1057972 TT variants. We did not observe any relationships with regard to the age of presentation or any other presenting sign.

Increased IL-15 expression has been reported in the duodenal biopsy specimens of active and refractory CD patients.[15,16] Thus, we also assessed the prevalence of rs2857261 GG and rs1057972 TT variants according to Marsh grade and could not demonstrate any significant association. However, we must emphasize that most of our patients had Marsh type 3 lesions, and there were only 6 patients with Marsh type 1 lesions. This situation may have affected statistical analyses.

Despite providing important data regarding the possibility of utilizing IL-15 polymorphisms as a susceptibility marker in conjunction with HLA type, and determining the effects of these polymorphisms on CD presentation and clinical characteristics, this study has certain limitations. Here, we studied previously defined polymorphisms in other autoimmune diseases rather than newly defined polymorphisms specific to CD. While we could provide some evidence for relationships between CD and variants such as rs2857261 GG and rs1057972 TT, the effects of these polymorphisms on tissue expression, activity, and serum IL-15 level were not determined. The possible links between rs2857261 GG and rs1057972 TT variants on tissue expression, serum levels, and their subsequent effects on clinical presentation need to be demonstrated with further trials.

In conclusion, the results of our study suggest that IL-15 gene rs2857261 GG, rs1057972 TT variants are more prevalent in celiac families than healthy controls. However, the impact of these polymorphisms on CD development is dependent on HLADQ2 status, and they seem to have effects only in the homozygous state. Despite these results, we must note that the number of patients included in this limits the generalizability of our findings, and there is a need for larger-scale research to elucidate these relationships accurately. However, this is the first study investigating IL-15 gene polymorphisms in conjunction with HLA status in patients with CD despite the limitations mentioned earlier. It is evident that in order to confirm our findings, larger studies (in which serum and tissue IL-15 levels are also measured) should be conducted.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Eskişehir Osmangazi University (Approval Number: 01.07.2013-250).

Informed Consent: Written informed consent was obtained from parents.

Peer Review: Externally peer-reviewed.

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