İzmir Kâtip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi 2024; 9(3): 365-370
DOI: 10.61399/ikcusbfd.136339

RESEARCH / ARAŞTIRMA

Polymorphisms of CTLA-4 (rs231775) and FOXP3 (rs3761548) Genes with Celiac Disease in Turkish Pediatric Patients

Çölyak Hastalığı Olan Türk Pediatrik Hastalarda CTLA-4 (rs231775) ve FOXP3 (rs3761548) Genlerinin Polimorfizmleri

Abdullah Said YILMAZ[1], Aslı ELDEM[1], Maşallah BARAN[2], Melek PEHLİVAN[3], Tülay KILIÇASLAN AYNA[1], İbrahim PİRİM[1], Mustafa SOYÖZ[1]

[1]*Faculty of Medicine, Department of Medical Biology, Izmir Katip Celebi University, Izmir, Türkiye.*
[2]*Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology and Nutrition Izmir Katip Celebi University, Izmir, Türkiye.*
[3]*Vocational School of Health Services, Izmir Katip Celebi University, Izmir, Türkiye.*

Geliş tarihi/Received: 26.09.2023
Kabul tarihi/Accepted: 04.04.2024

Sorumlu Yazar/Corresponding Author:

Mustafa SOYÖZ, Prof. Dr.
Balatçık Mahallesi Havaalanı Şosesi No:33/2
İzmir Katip Çelebi Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu, 35620 Çiğli İZMİR
E-posta: mustafa.soyoz@ikc.edu.tr
ORCID: 0000-0001-5159-6463

Abdullah Said YILMAZ, Yok
ORCID: 0000-0002-7393-4774

Aslı ELDEM, Dr
ORCID: 0000-0003-3510-6748

Maşallah BARAN, Prof. Dr.
ORCID: 0000-0003-3827-2039

Melek PEHLİVAN, Doç. Dr.
ORCID: 0000-0001-8755-4812

Tülay KILIÇASLAN AYNA, Prof. Dr.
ORCID: 0000-0001-7993-978X

İbrahim PİRİM, Prof. Dr.
ORCID: 0000-0001-8485-3286

Our study was approved and supported by Izmir Katip Celebi University Scientific Research Projects Administration by the Project number of 2020-TYL-SABE-003.

Abstract

Objective: Celiac disease (CD) is one of the most common autoimmune disorders in which gluten damages the small intestine. The *CTLA-4* and *FOXP3* genes play an important role in immune tolerance, so it is hypothesized that polymorphisms of these genes may be related to celiac disease. Our study aimed to investigate the associated with celiac disease and the *CTLA-4* +49 A/G (rs231775) and *FOXP3* -3279 C/A (rs3761548) polymorphisms by comparing celiac disease patients with a healthy control group.

Material and Methods: The single nucleotide polymorphisms (SNP) of +49 A/G in *CTLA-4* (rs231775) gene and -3279 C/A in *FOXP3* (rs3761548) gene were studied by Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) method in 125 pediatric celiac patients and 100 healthy controls.

Results: The A and G alleles of the *CTLA-4* gene were found more frequently in the celiac patient group than in the control group. In addition, the A and C alleles of the *FOXP3* gene were found more frequently in celiac disease patients than in healthy controls. There were no statistically significant results for the two polymorphisms *CTLA-4* +49 A/G and *FOXP3* -3279 C/A based on genotype or allele frequency ($p > 0.05$). When analyzing the risk allele, the *FOXP3* gene polymorphism -3279 C/A proved to be significant in CD patients ($p<0.05$).

Conclusion: This is the first study to evaluate the *CTLA-4* and *FOXP3* polymorphisms in Turkish pediatric celiac patients. The significance of polymorphisms of non-HLA genes may be associated with celiac risk as that of HLA genes, but further studies should be performed.

Keywords: Celiac disease, *CTLA-4*, *FOXP3*, gene polymorphism.

Öz

Amaç: Çölyak hastalığı, glutenin ince bağırsağa zarar verdiği en yaygın otoimmün hastalıklardan biridir. *CTLA-4* ve *FOXP3* genleri, immün toleransta önemli bir rol oynamaktadır, bu nedenle bu genlerin polimorfizmlerinin çölyak hastalığı ile ilişkili olabileceği varsayılmaktadır. Çalışmamız çölyak hastalarını sağlıklı kontrol grubu ile karşılaştırarak çölyak hastalığı ile *CTLA-4* +49 (rs231775) ve *FOXP3* -3279 C/A(rs3761548) polimorfizmleri arasındaki ilişkiyi araştırmayı amaçlamıştır.

Gereç ve Yöntemler: 125 pediatrik çölyak hastasında ve 100 sağlıklı kontrolde *CTLA-4* (rs231775) genindeki +49 A/G ve *FOXP3* (rs3761548) genindeki -3279 C/A'nın tek nükleotid polimorfizmleri (SNP), Polimeraz Zincir Reaksiyonu Restriksiyon Fragment Uzunluk Polimorfizmi (PCR-RFLP) yöntemi ile araştırıldı.

Bulgular: *CTLA-4* geninin A ve G alelleri çölyak hasta grubunda kontrol grubuna göre daha sık bulundu. Ayrıca *FOXP3* geninin A ve C alelleri çölyak hastalarında sağlıklı kontrollere göre daha sık bulundu. *CTLA-4* +49 A/G ve *FOXP3* -3279 C/A için genotip veya alel frekansına dayalı olarak istatistiksel olarak anlamlı bulunmamıştır ($p>0,05$). Risk aleli analiz edildiğinde, *FOXP3* gen polimorfizminin CD hastalarında anlamlı bulunmuştur ($p<0,05$).

Sonuç: Türk pediatrik çölyak hastalarında *CTLA-4* ve *FOXP3* polimorfizmlerini değerlendiren ilk çalışmadır. HLA dışı genlerin polimorfizmlerinin önemi, HLA genlerinde olduğu gibi çölyak riski ile ilişkilendirilebilir, ancak daha ileri çalışmalar yapılmalıdır.

Anahtar Kelimeler: Çölyak hastalığı, *CTLA-4*, *FOXP3*, gen polimorfizmi.

1. Introduction

Celiac disease (CD) is known as gluten-induced immune response-associated enteropathy in genetically susceptible individuals. The aetiology is unknown but genetics and environmental factors are crucial in its progress. It has been shown that CD is closely associated with Human leukocyte antigen (HLA)-DQ2 (DQA1*0201/B1*0202; DQA1*0501/ B1*0201) or HLA-DQ8 (HLA-DQA1*0301/ DQB1*0302) alleles (1). Dendritic cells present gluten-derived peptides through HLA molecules in the lamina propria, and these peptides recognise the HLA epitopes that can activate the individual's immune system. Genome-wide association studies (GWAS) have shown that genes encoding pro-inflammatory cytokines contribute to susceptibility to CD by expanding our genetic predisposition. Furthermore, there has been evidence of a relationship between CD and over 40 non-HLA molecules, including X-linked Forkhead Box P3 (*FOXP3*) and cytotoxic T lymphocyte antigen 4 (*CTLA-4*) (2,3). *CTLA-4* is a molecule that controls immune tolerance and T-cell activity and is encoded by the *CTLA-4* gene, which consists of 4 exons. Numerous polymorphisms have been found in the *CTLA-4* gene's promoter region and exon 1 (4). A single nucleotide polymorphism (SNP) in the 3' untranslated region (UTR) of exon 1, resulting in the exchange of an alanine at position 49 (49A/G; rs231775) for a threonine amino acid, has been associated to disease vulnerability in autoimmune diseases such as type 1 diabetes (T1DM) and rheumatoid arthritis (RA). In addition, *CTLA-4*-deficient animals die from autoimmune disease characterized by tissue infiltration and organ failure due to self-reactive T cells (5).

T lymphocytes, T lymphocyte-related cytokines and B lymphocytes also play a role in the progress of CD. Type 1 T helper (Th1) and Th17 cells secrete pro-inflammatory cytokines, Th2 and regulatory T cells (Tregs) secrete anti-inflammatory cytokines (6). Tregs play an important role in controlling the immune system through various mechanisms such as regulating antigen-presenting cells (APCs) function, inducing tolerance, and expressing inhibitory cytokines. Treg cells keep immunological self-tolerance and homeostasis in control by limiting inflammation.

FOXP3 has been proven in vivo and in vitro investigations to play a serious role in the development and stability of Tregs (7). Many polymorphisms in the *FOXP3* gene are associated with low *FOXP3* expression and Treg cell suppressive activity. Recent studies have indicated that SNPs in the *FOXP3* and *CTLA-4* genes are associated with autoimmune disorders such as allergies, systemic lupus erythematosus, and Graves' disease (6, 8).

There are many controversies for the pathogenesis of CD. We hypothise that non-HLA genetic factors may play a role in this disease. For this purpose, we aimed to investigate the relationship between *CTLA-4* (rs231775) and *FOXP3* (rs3761548) polymorphisms and the pathogenesis of celiac disease in children.

2. Material and Methods

2.1. Study Group

The study included 125 children with CD who were identified with the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) criteria (9). One hundred healthy pediatric volunteers aged 1-18 years were enrolled in the study. The Ethics Committee approved the study by Decision No. 34, dated February 06, 2019, and the parents of the patients were informed and informed consent forms were signed.

2.2. DNA Isolation

The EZ1 DNA Blood kit (Qiagen, Valencia, CA, USA) was used to isolate DNA from blood samples according to the manufacturer's instructions. The quality of DNAs was evaluated spectrophotometrically (Thermo Scientific NanoDrop 2000), and the samples were kept at -20 °C.

2.2. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP):

Primers used F: 5'-CCACGGCTTCCTTTCTCGTA-3', R: 5'-AGTCTCACTCACCTTTGCAG-3' for the *CTLA-4* (rs231775) gene (8) and F: 5'-TAACCAGACAGCGTAGAAGG-3', R: 5'-CAATACAGAGCCCATCATCA-3' (10) for the *FOXP3* (rs3761548) gene polymorphisms. Appropriate PCR conditions for amplification of these genes regions were applied as initial denaturation at 94°C for 1 min. denaturation at 94oC for 45 sec. binding at 61°C for 45 sec. elongation at 72oC for 45 sec. and final elongation at 72°C for 5 min. The accuracy of gene regions was checked by running the PCR products on a 1.5% agarose gel. 328 bp and 503 bp amplicon sizes were evaluated for *CTLA-4* and *FOXP3*, respectively. Amplicons were incubated with restriction enzymes overnight at 37°C. BbvI (Thermo Scientific, ER1451) restriction enzyme was used for the CTLA gene while PstI restriction enzyme (Thermo Scientific, ER0611) was used for the *FOXP3*. The samples were viewed on a 2% agarose gel.

2.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package Program for the Social Sciences (SPSS) version 25.0 (SPSS Inc, Chicago, IL, USA). Whereas quantitative variables were expressed with the median, categorical variables were expressed as absolute and relative frequencies. The suitability of all genotypes for Hardy-Weinberg equilibrium was assessed. Differences in age and sex between CD patients and controls were evaluated using both the parametric T test and the nonparametric Mann-Whitney U test. Chi-square test was performed for allele and genotype polymorphism between the patient and control groups.

2.4. Ethical Approval

The Ethics Committee approved the study by Decision No. 34, dated February 06, 2019, and the parents of the patients were informed and informed consent forms were signed.

3. Results

Demographic features of celiac patients and control groups shown in Table 1. The celiac patients consisted of 58% (n:72) girls and 42% (n:53) boys, whereas the control group consisted of 46% (n:46) girls and 54% (n:54) boys in our study. The patients' mean age was 9 ±4.8 years, compared to the control group's mean age of 10 ± 5.8 years. There was no significant difference between the two study groups in terms of gender or age (Table 1). Based on the frequency of marsh stages of 125 patients, it was found that 50.4% (n:63) were type 3A, 39.2% (n:49) were type 3B, 10.4% (n:13) were type 3C. For the *CTLA-4* gene (rs231775) polymorphism, the presence of a 328 bp band was regarded as the AA

genotype, a 244 bp band as the GG genotype, and a 328 bp band as the GA genotype (Fig.1).

When the results of *CTLA-4* polymorphisms of the patient and control groups were compared, the A allele was found more frequently in the patient group (n:134) than in the control group (n:95). Additionally, we found that the patient group had a higher prevalence of the AA genotype while the control group had a higher prevalence of the AG genotype. In terms of genotype and allele frequencies, there was no discernible difference between the patient and control groups (Table 2). For the *FOXP3* (rs3761548) polymorphism, the assessment of a 503 bp band was regarded as the AA genotype, a 319 bp band, a 184 bp band, a CC genotype, and a 503 bp band, a CA genotype (Fig.2).

Table 1. The Demographic Information of the Patient and Control Groups

	Control (n=100)		Patients (n=125)		p value
	% n		% n		
Gender	Female	Male	Female	Male	>0.05
	46% (n:46)	54% (n:54)	58% (n:72)	42% (n:53)	
Mean Age	10±5.8		9±4.8		>0.05
Types of Disease					
CD	-		69.6% (n:87)		-
CD+T1DM	-		16% (n:20)		
Anemia	-		12% (n:15)		
Turner Syndrome	-		0.8% (n:1)		
Thalassemia	-		0.8% (n:1)		
Epilepsy	-		0.8% (n:1)		
Marsh Stage					
3a	-		50.4% (n:63)		-
3b	-		39.2% (n:49)		
3c	-		10.4% (n:13)		

Table 2. Comparison of genotype and allele frequencies of CTLA-4 polymorphism in control and patient groups

Polymorphism	Genotype/Allele	Control	CD-Patients	p value
rs231775	AA	42(42)	58(46.4)	0.46
	AG	47(47)	49(39.2)	
	GG	11(11)	18(14.4)	
Dominant	AA vs AG+GG	42 vs 58	58 vs 67	0.509
Recessive	AA+AG vs GG	89 vs 11	107 vs 18	0.449
MAF %	G	34.5	34.0	0.912
95%CI		23-41	23-40	

Values are n (%) Control, healthy subjects; CD-patients, patients with Celiac disease. MAF, minor allele frequency comparison.95%CI, 95% confidence interval for G-allele

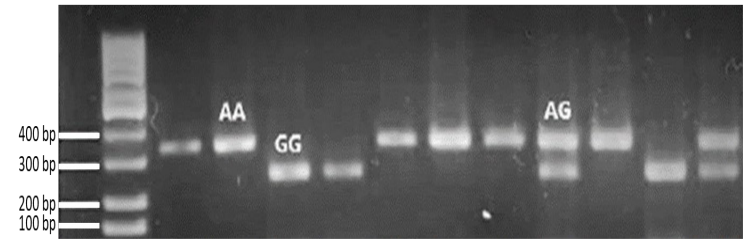

Fig. 1. CTLA-4 gene polymorphism gel image (rs231775)

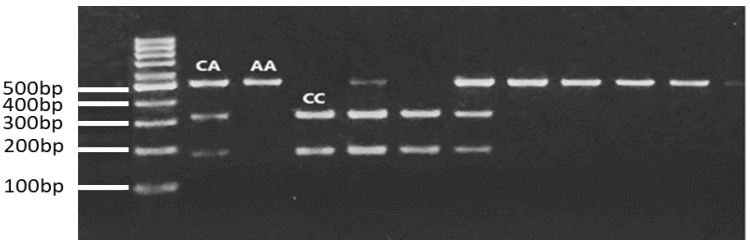

Fig. 2. FOXP3 gene polymorphism gel image (rs3761548)

When the *FOXP3* gene polymorphisms of the patient and control groups were examined, it was found that the A (n:127) allele and the CA (n:49) genotype were more common in the patient group than in the control group. The most common genotype for the control group was CC (n:43). The *FOXP3* gene's genotype and allele frequencies of the patient and control groups did not significantly differ from one another. CC and CA+AA risk alleles were significantly different between the patient and control groups (p=0.03) (Table 3).

HLA DQB1*/DQA1* allele and Marsh stage data of the patient were published in our recent study (Eldem et al, 2021). According to these results, marsh stage and *CTLA-4* gene allele and genotype frequency were not statistically significant (p>0.05). Similarly, statistical significance was not found in terms of allele and genotype frequencies in the *FOXP3* gene (p>0.05) (data not shown). Our findings revealed that there was no statistically significant link between HLA DQB1*/DQA1* and *CTLA-4* polymorphisms in CD patients and controls (Table 4).

There were not statistically significant association between these HLA DQB1*/DQA1* and *FOXP3* polymorphism the in CD patients and controls group (Table 5).

4. Discussion

Celiac disease is an immune-mediated disease defined by a genetically predisposed person's intolerance to gluten-containing cereals. For many years, there has been a strong link between CD and HLA-DQ. The greatest allele frequencies identified in DQ typing analyses in Turkish pediatric celiac patients were DQB1*02 and DQA1*05. Gluten-specific HLA class II genes HLA-DQ2 and HLA-DQ8 are necessary for CD pathogenesis (11, 1).

Table 3. Comparison of genotype and allele frequencies of FOXP3 polymorphism in control and patient groups

Polymorphism	Genotype/Allele	Control	CD-Patients	p value
rs3761548	AA	28(28)	39(31.2)	0.09
	CA	29(29)	49(39.2)	
	CC	43(43)	37(29.6)	
Dominant	CC vs CA+AA	43 vs 57	37 vs 88	**0.03**
Recessive	CC+CA vs AA	72 vs 28	86 vs 39	0.60
MAF %	A	42.5	50.8	0.08
95%CI		51-64	43-55	

Values are n (%) Control, healthy subjects; CD-patients, patients with Celiac disease. MAF, minor allele frequency comparison; 95%CI, 95% confidence interval for A-allele

Table 4. Comparison of risk genotype of HLA DQB1*/DQA1* and CTLA-4 genotypes polymorphism in control and patient groups

SNP rs231775	AA	GG	AG	p value
Controls DQB1*02/DQA1*05	3.3% (n:3)	-	4.4% (n:4)	>0.0
Patients DQB1*02/DQA1*05	43.3% (n:39)	13.3% (n:12)	35.5% (n:32)	
Controls DQB1*02/DQA1*02	5.1% (n:2)	7.6% (n:3)	-	>0.05
Patients DQB1*02/DQA1*02	35.9% (n:14)	35.9% (n:14)	15.3% (n:6)	
Controls DQB1*03/DQA1*03	16.3% (n:9)	9% (n:5)	20% (n:11)	>0.05
Patients DQB1*03/DQA1*03	25.4% (n:14)	10.9% (n:6)	18.1% (n:10)	

Table 5. Comparison of risk genotype of FOXP3 polymorphism and HLA DQB1*/DQA1* genotypes in control and patient groups

SNP rs3761548	AA	CC	CA	p value
Controls DQB1*02/DQA1*05	-	3.3% (n:3)	4.4% (n:4)	>0.05
Patients DQB1*02/DQA1*05	28.8% (n:26)	31.1% (n:28)	32.2% (n:29)	
Controls DQB1*02/DQA1*02	5.26% (n:2)	2.6% (n:1)	5.26% (n:2)	>0.05
Patients DQB1*02/DQA1*02	26.3% (n:10)	26.3% (n:10)	34.21% (n:13)	
Controls DQB1*03/DQA1*03	16.3% (n:9)	14.5% (n:8)	14.5% (n:8)	>0.05
Patients DQB1*03/DQA1*03	7.2% (n:4)	2.8% (n:12)	24.4% (n:14)	

The *CTLA-4* gene, which is one of the most essential immune system inhibitory molecules, has been investigated for numerous autoimmune disorders. A meta-analysis that included type 1 diabetes mellitus (T1DM) patients reported that GG homozygosity could increase the risk of disease 2-fold compared to healthy individuals (12). The genotype and allele frequencies of *CTLA-4* +49A/G in 1.489 patients with rheumatoid arthritis (RA) were found statistically significant compared with 1.200 healthy controls($p< 0.05$) in the Chinese population (13). Lee et al. discovered a link between the *CTLA-4* exon 1 polymorphism at the +49 position of the leader peptide and the risk of systemic lupus erythematosus (SLE) (14). Based on these reports, we aimed to determine the frequency of *CTLA-4* +49A/G polymorphism in pediatric celiac disease patients in the Turkish population. According to our results, we found that the A allele and the AA genotype were detected with higher frequency in the patients compared to the healthy control group. Mora et al. found similar results in the Italian population and reported that the A allele was more common in celiac patients (15). However, Abdullah et al. reported that the A allele is more prevalent in healthy individuals as compare to celiac patients, and the frequency of the G allele in celiac patients higher than in healthy individuals and the difference between them is statistical significant in the Iraqi population ($p< 0.05$) (16). Thus, it appears that A and G allele the frequencies may vary depending on the population.

Treg cell formation and function are dependent on the *FOXP3* gene. The amount of *FOXP3*+ T lymphocytes in celiac patients' intestinal mucosa has been reported to be considerably higher. Because of its chromosomal position and functional importance, *FOXP3* is a candidate gene for autoimmune disorders. 22 studies were analyzed in a meta-analysis that shown that the relationship between *FOXP3* polymorphism and autoimmune disorders.

On the other hand, a study of 50 Behcet's patients and 50 healthy controls showed that the rs3761548 polymorphism of *FOXP3* significantly increased the risk of Behcet's disease in the Iranian population (7). In a study that examined T1DM and CD together, it was reported that there was no link between the *FOXP3* polymorphism and these diseases (17). Li et al. found in a meta-analysis that the *FOXP3* polymorphism -3279 C/A was associated with susceptibility to autoimmune diseases in Asians but not in Caucasians (18). It has been reported that the A allele for the *FOXP3* gene alters the transcription factor binding sites E47 and c-Myc, resulting in genetic alterations that predispose the person to develop autoimmune illness (19). According to our results, the A allele was higher in children with CD compared to the control group. Furthermore, no difference was observed according to the HLA DQ types and *CTLA-4* and *FOXP3* polymorphisms.

5. Conclusion

In conclusion, this was the first study to report both *CTLA-4* gene (rs231775) and *FOXP3* gene (rs3761548) polymorphisms in Turkish children with CD. In this regard, we studied the polymorphisms that we think may show the importance of the relationship between HLA/non-HLA genes and autoimmune diseases, but we could not find any significantly result. Further research is needed to determine the significance of new potential non-HLA genes and variants.

6. Contribution to the Field

These polymorphisms have never been studied before in pediatric celiac patients. It will contribute to elucidation of pediatric celiac disease at the molecular level in the Turkish population.

Conflict of Interest

This article did not receive any financial fund. There is no conflict of interest regarding any person and/or institution.

Funding

Our study was approved and supported by Izmir Katip Celebi University Scientific Research Projects Administration by the Project number of 2020-TYL-SABE-003.

Authorship Contribution

Concept: ASY, MS; **Design:** MS; **Supervision:** MP, TKA; **Funding:** MS; **Materials:** MS, MB; **Data Collection/ Processing:** MB; **Analysis/Interpretation:** MP, TKA; **Literature Review:** AE, ASY; **Manuscript Writing:** AE, ASY; **Critical Review:** İP.

References

1. Eldem, A., Ayna, T. K., Baran, M., Soyöz, M., & Pirim, İ. Determination of High-Resolution HLA-DQB1 Suballeles and IL-17 Polymorphisms in Turkish Pediatric Patients. Journal of Pediatric Genetics. 2021; 11(3): 192–197

2. Lundin, K. E., & Wijmenga, C. Coeliac disease and autoimmune disease-genetic overlap and screening. Nature reviews Gastroenterology & hepatology. 2015;12(9), 507-515.

3. Serena, G., Lima, R., & Fasano, A. Genetic and environmental contributors for celiac disease. Current Allergy and Asthma Reports.2019; 19(9), 1-10.

4. Uhrberg, M., Parham, P., & Wernet, P. Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics.2002; 54(4), 221-229.

5. Song, G. G., Kim, J. H., Kim, Y. H., & Lee, Y. H. Association between CTLA-4 polymorphisms and susceptibility to Celiac disease: a meta-analysis. Human Immunology. 2013;74(9), 1214-1218.

6. Scazzone, C., Agnello, L., Lo Sasso, B., Salemi, G., Gambino, C. M., et.al. Foxp3 and gata3 polymorphisms, vitamin d3 and multiple sclerosis. Brain Sciences, 2021;11(4), 415.

7. Hosseini, A., Shanehbandi, D., Estiar, M. A., Gholizadeh, S., Khabbazi, A., et.al. A single nucleotide polymorphism in the FOXP3 gene associated with Behcet's disease in an Iranian population. Clin Lab.2015; 61(12), 1897-903.

8. Fathima, N., Narne, P., & Ishaq, M. Association and gene–gene interaction analyses for polymorphic variants in CTLA-4 and FOXP3 genes: role in susceptibility to autoimmune thyroid disease. Endocrine. 2019; 64(3), 591-604.

9. Husby, S., Koletzko, S., Korponay-Szabó, I., Kurppa, K., Mearin, M. L., et.al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. Journal of pediatric gastroenterology and nutrition. 2020; 70(1), 141–156.

10. Chatrabnous N, Ghaderi A, Ariafar A, et al. Serum concentration of interleukin- 35 and its association with tumor stages and FOXP3 gene polymorphism in patients with prostate cancer. Cytokine. 2019;113 :221– 227.

11. Murad, H., Jazairi, B., Khansaa, I. et al. HLA-DQ2 and -DQ8 genotype frequency in Syrian celiac disease children: HLA-DQ relative risks evaluation. BMC Gastroenterol.2018; 18, 70.

12. Chen, Z., Fei, M., Fu, D., Zhang, L., Ma, Y., Wang, Y., ... & Wang, X. Association between cytotoxic T lymphocyte antigen-4 polymorphism and type 1 diabetes: a meta-analysis. Gene. 2013;516(2), 263-270.

13. Tang, M. J., & Zhou, Z. B. Association of the CTLA-4+ 49A/G polymorphism with rheumatoid arthritis in Chinese Han population. Molecular biology reports. 2013; 40(3), 2627-2631.

14. Lee MG, Bae SC, Lee YH. Association between FOXP3 polymorphisms and susceptibility to autoimmune diseases: A meta-analysis. Autoimmunity. 2015;48(7): 445–52.

15. Mora, B., Bonamico, M., Indovina, P., Megiorni, F., Ferri, M., Carbone, M. C., ... & Mazzilli, M. C. CTLA-4+ 49 A/G dimorphism in Italian patients with celiac disease. Human immunology. 2003;64(2), 297-301.

16. Abdullah, S. H., & Al-Badran, A. I. (2022). The CTLA4 Polymorphism And Incidence Of Celiac Disease In Thi-Qar Province-South Of IRAQ. Ann. For. Res, 65(1), 8282-8294.

17. Bjørnvold M, Amundsen SS, Stene LC, et al. FOXP3 polymorphisms in type 1 diabetes and coeliac disease. J Autoimmun. 2006;27(2): 140–4. 18. Li, H. N., Li, X. R., Du, Y. Y., Yang, Z. F., & Lv, Z. T. The association between Foxp3 polymorphisms and risk of Graves' disease: a systematic review and meta-analysis of observational studies. Frontiers in endocrinology. 2020; 11, 392.

19. Shen, Z., Chen, L., Hao, F., Wang, G., Fan, P., & Liu, Y. Intron-1 rs3761548 is related to the defective transcription of Foxp3 in psoriasis through abrogating E47/c-Myb binding.2010; Jan;14(1-2):226-41