RESEARCH ARTICLE

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Investigation of TLR2 (-196 to -174del) and TLR9 (T-1486C) Gene Polymorphisms Association with Inflammatory Bowel Diseases

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Abstract

Background: Inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are diseases that result from the combined effects of a predisposing genetic background and several environmental factors, including smoking. Some genes can influence these diseases through genetic inheritance, and their regulation is explained by gene polymorphism. However, Toll-like receptor (*TLR*) genes have been identified as susceptibility genes for CD and UC. **Methods:** A case-control study was performed on a Turkish population composed of 105 healthy controls and 79 CD, 77 UC patients genotyped by Allele-specific PCR and PCR–RFLP for *TLR9* (T-1486C) and *TLR 2* (-196 to -174del) gene. Genotype and allele frequencies of *TLR9* (T-1486C) and *TLR 2* (-196 to -174del) gene polymorphisms compared to allele frequencies in CD and UC patients. **Results:** No statistically significant findings were found between the CD, UC patients, and the control group in terms of both genotype distributions and allele frequencies for TLR 9 (T-1486C; rs187084) and *TLR 2* (-196 to -174del; rs111200466) gene polymorphisms in a Turkish population ($P > 0.05$). **Conclusion:** No association was found between the *TLR2* (rs111200466) and *TLR 9* (rs187084) gene polymorphisms among IBD patients and the control groups in the Turkish population.

Keywords: Inflammatory Bowel Diseases- Polymorphism- *TLR 2* (–196 to –174del)- *TLR 9* (T-1486C).

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Introduction

Inflammatory Bowel Diseases (IBD) comprises mainly Crohn's Disease (CD) and Ulcerative Colitis (UC), and in between these two diseases is Indeterminate Colitis (IC); these diseases possess specific effects on various regions and layers of the gastrointestinal tract [1]. These diseases are known for their chronic inflammatory disorders in the gastrointestinal tract, which have been experimentally defined, and their clinical, pathological, endoscopic, and radiological features have been completely determined [2]. IBD has increased tremendously globally over the last decade, especially in the European and Asian populations, and its daily increase has been reported [3, 4]. In IBD, CD is a disease that causes treatable inflammation in any region of the intestinal epithelium along the gastrointestinal tract [1]. Although the clinical findings in CD vary depending on the localization of the inflammation. The most common symptoms are diarrhea, abdominal pain, fever, sub ileus attacks, and rectal bleeding. The disease is, however, evaluated by the patient's history alongside endoscopic, radiological, and biochemical findings of the physical examination ascertained [5]. It has been determined that infectious agents, various enteric bacteria, viruses, chlamydia, mycobacteria, and dietary factors are involved in the etiology of CD [6, 7]. The other variant of IBD, UC, is a disease that causes long-term inflammation in a part of the intestinal epithelium and mainly in the colon of the digestive tract [1]. Ulcerative colitis (UC) is a chronic inflammatory disease characterized by mucosal inflammation primarily affecting the colon. The disease is classified based on the anatomical region of the large intestine, ranging from proctitis (limited to the rectum) to pancolitis (involving the entire colon). The exact cause of UC is not fully understood, but it is believed to be influenced by a combination of environmental and genetic factors. UC is more commonly observed in developed countries. [8]. It has been suggested that environmental and genetic components affect the prevalence of IBD, such as gastroenteritis, smoking habits, and alcohol addiction. Unaccounted risk factors (e.g., dietary, obesity, antibiotic use, improved hygiene, smoking, etc.) CD incidence Some

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research shows that the intake of saturated fat and refined sugar alters the gut's microenvironment and increases the risk of developing IBD [3]. It has been determined that the most important common feature of UC and CD is a genetic predisposition, and the long duration of these diseases increases the risk of cancer in patients [9, 10]. It is interesting to know that the signs and symptoms of both diseases are very similar [11, 12]. These diseases are included in the important colon and rectal diseases group because of the complications and malignant potential they cause in extra-intestinal organs [7]. The most valid hypothesis in the pathogenesis of IBD has been expressed as an abnormal inflammatory response in the gastrointestinal tract with the effect of various factors (such as microbial antigen, autoantigen, and smoking) under genetic predisposition. In addition, in Mahid's meta-analysis of 245 studies revealed 22 articles that highlighted the link between IBD and smoking, and it has been shown that smoking affects UC and CD differently [13]. It has been shown that this inflammatory response to the protection of the immune system is controlled by suppression. Still, intestinal damage occurs because this inflammation cannot be suppressed in genetically predisposed individuals [12]. According to the data obtained because of the expression of surface antigens in a patient's peripheral blood cells with CD and UC, it has been suggested that there are important differences between these two diseases [14, 15]. In addition, according to the autoimmune hypothesis, it has been reported that autoantigens expressed in the intestinal epithelium of these patients cause different mechanisms that trigger the immune system [16]. The genetic predisposition to these diseases was revealed by the determination of mutations in the NOD2/CARD15 gene on the 16th chromosome, which affect most of the population and are generally seen in young adults, especially in the western world [17]. It has also been shown that geographical and ethnic factors are also important in the etiology of these diseases [18-20]. IBD is usually seen in young adulthood in humans, and even if most of the affected individuals are treated, it has been determined that the disease progresses and reoccurs in these individuals, thus turning into a chronic disease [2]. It has also been reported that individuals affected by CD have clinical propensity at any age [21]. The prevalence of these diseases increases over time, it characterized by an unstable inflammatory response, and it is an autoimmune disease that occurs as a product of a deviation in the autoimmune response to intestinal bacteria or other foreign biological materials [4, 22, 23].

Inflammatory bowel disease - notably Crohn's disease (CD) and ulcerative colitis (UC) tend to be facilitated by various risk factors, and smoking is chief among them. Research in this matter over the years has provided ample evidence that smokers face a notably higher chance of developing CD compared with their counterparts who do not smoke, and those who are diagnosed with the condition also have more complications. While it is less evident whether smoking induces or worsens UC. Medical analysis so far has been ambiguous. Given such potential hazards, there is an urgent need for smoking cessation measures to provide relief for individuals suffering from these conditions, whereby studies support such recommendations as stated in Targownik's work from 2009 onwards [24].

Genetic polymorphism studies in IBD have also reported that individual variations, SNPs, may contribute significantly to the formation of these diseases. Gene polymorphism studies conducted to determine genetic differences in individuals with IBD have suggested that alleles in some genes in humans may be associated with genetic susceptibility alleles, and one of these genes in polymorphisms were seen in Toll-like receptor (TLR) genes [23]. In humans, TLRs have been reported as genes forming a group of transmembrane proteins found in monocyte cells, dendritic cells, and macrophage cells that play a significant role in the innate immune system [25]. It has been determined that TLRs are generally expressed by the cell surface of innate immune cells, and they are very important regulators in maintaining the balance between the intestinal commensal bacteria and the mucosal immune system [23]. They have also been found to have superior roles as mediators of intestinal inflammatory pathways [26]. The ability of TLR gene products to react differently to their ligands is due to single-nucleotide polymorphisms (SNP) occurring in *TLR* genes, and genetic variations in these genes have been associated with susceptibility to diseases.

Research from different populations and cohorts has shown contradictory results regarding the association between inflammatory bowel disease (IBD) and polymorphisms in the *TLR2* and *TLR9* genes. Certain *TLR2* polymorphisms have been linked in certain studies to an increased risk of IBD, which may have an effect on *TLR2* function and microbial identification. Differential clinical symptoms, the location, behavior, and severity of the illness have all been associated with *TLR2* variations. *TLR9* polymorphisms, such rs5743836 and rs352140, may change *TLR9* activity and microbial DNA recognition, and they have been linked to an increased risk of developing IBD. But because of the complexity of the connection between *TLR2* and *TLR9*, further study is required to completely comprehend the processes and therapeutic implications [27].

In This case-control study, aims to investigate TLR 9 (T-1486C) and *TLR 2* (-196 to -174del) gene polymorphisms in CD and UC patients, whose clinical features and epidemiology are very similar, as an distinguishing factor in terms of these diseases using PCR and PCR-RFLP method in the Turkish population.

Materials and Methods

The peripheral blood samples used in this investigation were obtained from 156 patients who applied to Nigde State Hospital Gastroenterology Clinic between 2013 and 2015 who were diagnosed with UC (77) and CD (79) clinically, endoscopic, or histopathological, and 105 healthy individuals who had no previous bowel disease as the control group as detailed in Table 1.

Isolation of DNA

Using the DNA isolation kit (QIAGEN GmbH,

Maryland, USA), DNA was extracted from 0.2 ml blood samples according to the manufacturer's instructions protocol and eluted in 200µl D.w. The concentration and purity of the extracted DNA were determined using a Nanodrop Spectrophotometer. The samples were then stored in a refrigerator at -20°C until further analysis. Used PCR-RFLP and Allele-Specific PCR (AS_PCR) techniques, the *TLR*2 (-196 to -174 del; rs111200466) and *TLR*9 (T-1486C; rs187084) gene polymorphisms were genotyped [28, 29].

PCR and RFLP

The PCR conditions were adjusted according to the specifications outlined in the program in Table 1S (S; Supplementary material). Each reaction was conducted in a 25µl volume using 200nM of primers specified in Table 2S. The PCR reactions were prepared with the 2xTaq DNA polymerase Master Mix from (Vivantis).

Subsequently, the PCR products underwent gel analysis. *TLR*2 genotyping employed a 2% agarose gel, and the PCR fragments were visualized using RedSafe, a safe stain provided by (iNtRON Biotechnology), and visualization utilized (Fusion Fx Imaging System from Vilber Lourmat, utilizing UV light) shown in Figure1S, 2S. *TLR*9 genotyping, the PCR products digested with the AflII restriction enzyme, separated on a 3% agarose gel as shown in Figure 2, the resulting gel image captured in the documentation system.

As-PCR For *TLR*2 genotyping, the PCR products were separated on a 3% agarose gel, as shown in Figure 1; the resulting gel image was captured in the documentation system.

Statistical Analysis

The SPSS 22.0 package program was used to analyze the Inflammatory Bowel Disease (IBD) patients and control groups statistically. The age variable underwent normal distribution analysis utilizing the Kolmogorov-Smirnov test as a demographic trait and etiological data. The non-parametric Mann-Whitney U (M-W) test was used since the data did not fit the normal distribution.

Binary logistic regression and Chi-Square (X^2) analysis were used to compare the homozygous and heterozygous genotype frequencies as well as the allelic frequencies in the *TLR*2 (-196 to -174del) and *TLR*9 (T-1486C) genes between the patient groups (CD, UC) and the control group. This research ascertained the genetic association between the patient and control groups using odds ratios (OR) and 95% confidence intervals (CI). Following a significance test, the same evaluation was carried out once more while accounting for factors such as age, gender, and smoking (Figure 3). The threshold for statistical significance was set at P-value < 0.05 (P-value 0.05). Furthermore, the online computation method developed by Michael H. Court (2005-2008) was used to do the Hardy-Weinberg analysis on the patient and control groups.

Results

The study found significant differences in demographic characteristics between the control group (N=105), CD (N=77) patients, and UC (79) patients. The CD group had a slightly reduced eventuality of developing CD, UC, or IBD compared to the control group, while the UC group had a somewhat higher likelihood. These findings contribute to understanding the connection between demographic factors and the occurrence of CD, UC, and IBD results shown in Figure 3 and Table 1. a significant difference in smoking rates between the CD group and the control group, with a 1.88 odds ratio, indicating a 1.88 times higher number of smokers in the CD group.

Analysis of the TLR9 (T-1486C; rs187084) and TLR2 (-196 to -174 del; rs111200466) gene polymorphisms in CD

The study analyses *TLR*9 (T-1486C) and *TLR*2 (-196 to-174 del) gene polymorphisms in DNA samples from 79 CD and 105 control groups. Results show no significant differences in genotype distributions or allele frequencies between CD and control groups (Tables 2, 3).

Analysis of the TLR9 (T-1486C; rs187084) and TLR2 (-196 to -174 del; rs111200466) gene polymorphisms in UC patients

The study evaluated UC patients' and a control group's *TLR*9 and *TLR*2 gene polymorphism data. The results showed no significant difference in genotype and allele frequencies between the two groups. The *TLR*2 gene polymorphism genotype distributions were consistent with the Hardy-Weinberg equation, and no significant difference was observed between the patients and control

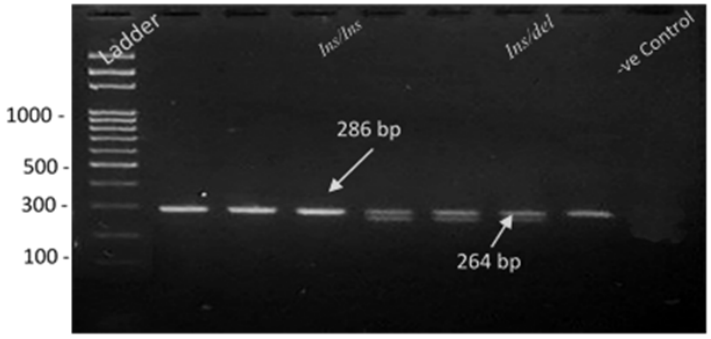

Figure 1. PCR Method *TLR 2* (-196 to -174 del) Gene 3% Gel Band Image. Ins/del: 286bp and 264bp, Ins/Ins: 286bp, del/del: 264bp and 64bp.

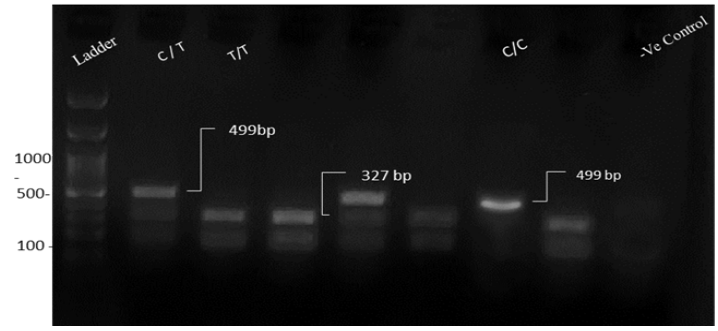

Figure 2. *TLR 9* (T-1486C) Gene Bands Cut with Afl II Enzyme in RFLP Method. The CC genotype did not reflect in this figure, however, TC- represents the three bands: 499,327 and 172. TT- represents two bands: 327 and 172.

Table 1. Demographic Factors and the Occurrence of CD, UC, IBD and Control

Variables	Patients with CD (N= 79)	p-value	Odds	Patients with UC (N= 77)	p-value	Odds	Healthy control (N= 105)	IBD (N=156)	p-value	Odds
Median (Age range)	44 (18-82)			47(17-86)			40(19-78)	43(17-86)		
Mean	44.78±12.75	0.0291	0.7	46.86±15.76	0.01	0.5	41.32± 14.22	43.96±14.4	0.005	0.7
Gender										
Males	42 (53%)	0.1306	1.57	42 (54%)	0.011	1.66	44(42%)	84 (54%)	0.06	1.61
Females	37 (47%)			35 (46%)			61(58%)	72 (46%)		
Age in years										
Age > 50	27 (33%)	0.066	1.85	31 (38%)	0.0081	2.4	23(29%)	58 (37)	0.009	2.11
Age ≤ 50	52 (28%)			46 (26%)			82 (46%)	98 (63)		
Smokers	37 (67%)			23 (30%)			25 (23%)	60 (45%)		
Non-smokers	18 (33%)	< 0.01	1.884	54 (70%)	0.4	1.1	80 (78%)	72 (55%)	<0.001	1.1
Missing data	24			-			-	-		

groups. Amplified bands indicated HOMOZYGOUS for the wild-type (ins/ins) allele, while the simultaneous presence of amplified bands indicated heterozygous (ins/del) alleles.

The study concluded that the *TLR*9 and *TLR*2 gene polymorphisms were consistent with the Hardy-Weinberg equation and did not show any deviation between the two groups (Tables 4, 5).

Discussion

There have been reports of correlations between *TLR*9 polymorphisms and atherosclerosis, SLE, IBD, and asthma [30]. Several researchers have investigated potential connections between *TLR*9 polymorphisms and IBD because the *TLR*9 gene's close to associated with Crohn's disease and ulcerative colitis susceptibility [31]. Emingil et al. (2007) examined the susceptibility of *TLR*2 (Arg753Gln and Arg677Trp) and *TLR*4 (Asp299Gly and Thr399Ile) gene polymorphisms in patients with generalized aggressive periodontitis in the Turkish population. They discovered no significant difference in distribution and allele frequencies (P>0.05) [32]. The host's innate immune system plays a critical role

Table 2. *TLR2* (-196 to -174 del) Genotype and Allele Frequencies in the UC and Control Group

Gene/ Genotypes		UC	Control	Crude Values			Adjusted Values		
		n=77(%)	n=105 (%)	P Values	Odds ratio (OR)	95% (CI)	P Values	Odds ratio (OR)	95% (CI)
TLR2	ins/ins	54 (70.13)	79 (75.24)	-		-			
-196 to -174del	ins/del	20 (25.97)	22 (20.95)	0.906	0.911	0.196-4.236	0.803	1.223	0.251-5.976
	del/del	3 (3.90)	4 (3.81)	0.423	0.752	0.374-1.510	0.594	0.823	0.402-1.685
Alleller	ins	128 (83.12)	180 (85.71)	-		-			
	del	26 (16.88)	30 (14.29)	0.497	0.821	0.463-1.454			
Resesive model									
ins/ins+ins/del		74 (96.10)	101 (96.19)	-		-			
del/del		3 (3.90)	4 (3.81)	0.976	0.977	0.212-4.497			
Dominant model									
ins/ins		54 (70.13)	79 (75.24)	-		-			
ins/del+del/del		23 (29.87)	26 (24.76)	0.443	0.773	0.400-1.494			

Table 3. *TLR9* (T-1486C) Genotype and Allele Frequencies in CD Patients and Controls

Gene/ Genotypes		CD	Control	Crude Values			Adjusted Values		
		n=79(%)	n=105(%)	P values	Odds ratio (OR)	95% (CI)	P values	Odds ratio (OR)	95% (CI)
	TT	31 (39.24)	39 (37.14)	-		-			
TLR 9 (T-1486C)	TC	37 (46.80)	49 (46.67)	0.652	1.228	0.503-3.001	0.596	1.278	0.516-3.168
	CC	11(13.90)	17 (16.19)	0.874	1.053	0.557-1.989	0.953	1.02	0.533-1.950
Allels	T	99 (62.66)	127(60.48)	-		-			
	C	59 (37.34)	83 (39.52)	0.67	1.097	0.717-1.677			
Resesive model									
TT+TC		68 (86.08)	88 (83.81)	-		-			
CC		11 (13.92)	17 (16.19)	0.672	1.194	0.525-2.716			
Dominant model									
TT		31 (39.24)	39 (37.14)	-		-			
TC+CC		48 (60.76)	66 (62.86)	0.772	1.093	0.599-1.993	-	-	-

Table 4. *TLR9* (T-1486C) Genotype and Allele Frequencies in UC Patients and Controls

Gene/ Genotypes		UC	Control	Crude Values			Adjusted Values		
		n=77(%)	n=105(%)	P values	Odds ratio (OR)	95% (CI)	P values	Odds ratio (OR)	95% (CI)
TLR 9 (T-1486 C)	TT	30 (38.96)	39 (37.14)	-		-			
	TC	33 (42.86)	49 (46.67)	0.875	0.934	0.398-2.191	0.943	1.032	0.429-2.483
Alleles	CC	14 (18.18)	17 (16.19)	0.688	1.142	0.597-2.186	0.657	1.163	0.598-2.264
	T	93 (60.39)	127(60.48)	-		-			
	C	61 (39.61)	83 (39.52)	0.987	0.996	0.651-1.524			
Resesive model									
TT+TC		63 (81.82)	88 (83.81)	-		-			
CC		14 (18.18)	17 (16.19)	0.724	0.869	0.399-1.892			
Dominant model									
TT		30 (38.96)	39 (37.14)	-		-			
TC+CC		47 (61.04)	66 (62.86)	0.803	1.08	0.590-1.979			

in the recognition and neutralization of gram-negative bacteria, and the polymorphism in the BPI gene, called Lys216Glu, has been studied in CD and UC patients in the Turkish population. This gene is on the suspect list of IBD pathogenesis. CD and UC have been identified to be related to the BPI (Lys216Glu) polymorphism [33]. This study evaluated the susceptibility status of rs187084 polymorphisms in patients with CD and UC. We on purpose chose -196 to -174del for the *TLR 2* gene and T-1486C for the *TLR* 9 gene. Our findings showed that genotypes and alleles of *TLR 2* (-196 to -174del) did not substantially differ between the patients and control groups. Similar to our work, Hishida, A. et al. 2010 looked into the association between gastric cancer risk and *TLR 2* (-196 to -174del) polymorphism in the Japanese population. They found no evidence of a connection between the two groups [34]. It was discovered that there was no statistically significant difference in the genotype and allele frequencies of the *TLR 2*, *TLR 4*, and *TLR* 9 gene polymorphisms in the 182 CD and 188 control

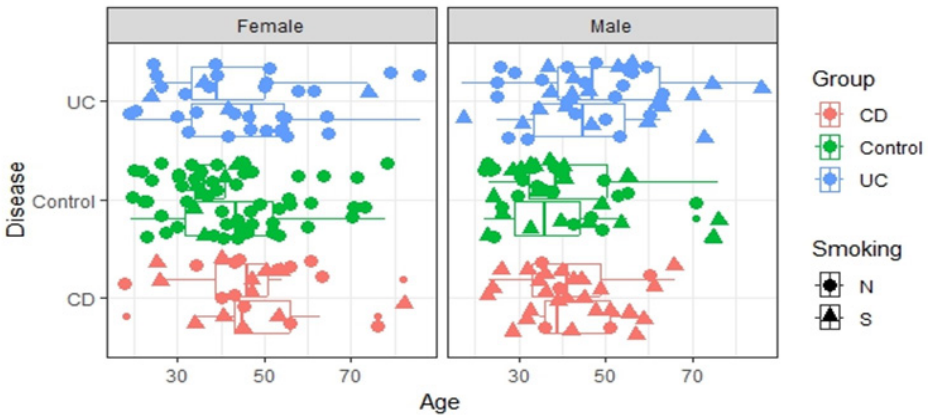

Figure 3. Demographic Factors and the Occurrence of CD, UC, and Control

Table 5. TLR2 (-196 to -174 del) genotype and allele frequency in UC patients and controls

Gene/ Genotypes		UC	Control	Crude Values			Adjusted Values		
		n=77 (%)	n=105 (%)	P Values	Odds ratio (OR)	95% (CI)	P Values	Odds ratio (OR)	95% (CI)
TLR2	ins/ins	54 (70.13)	79 (75.24)	-		-			
-196 to -174del	ins/del	20 (25.97)	22 (20.95)	0.906	0.911	0.196-4.236	0.803	1.223	0.251-5.976
	del/del	3 (3.90)	4 (3.81)	0.423	0.752	0.374-1.510	0.594	0.823	0.402-1.685
Alleller	ins	128(83.12)	180 (85.71)	-		-			
	del	26 (16.88)	30 (14.29)	0.497	0.821	0.463-1.454			
Resesive model									
ins/ins+ins/del		74 (96.10)	101 (96.19)	-		-			
del/del		3 (3.90)	4 (3.81)	0.976	0.977	0.212-4.497			
Dominant model									
ins/ins		54 (70.13)	79 (75.24)	-		-			
ins/del+del/del		23 (29.87)	26 (24.76)	0.443	0.773	0.400-1.494			

groups of the New Zealand Caucasian population [35]. 39 SNPs in 26 genes, including *TLR 2* and *TLR* 9, which govern inflammation, were examined in a cohort of 624 patients with CD, 411 patients with UC, and 795 controls in a study by Danish researchers utilizing the candidate gene technique (Bank et al. 2014). It was shown that 16 polymorphisms in 13 genes, including *TLR 2* and *TLR* 9, were related to the risk of CD and/or UC, thereby controlling inflammation.

Smoking is a known risk factor in the causation of numerous diseases, particularly cardiac and respiratory conditions. Smoking was first established as a risk factor in developing inflammatory bowel disease (IBD) study [33]. Inflammatory bowel disease includes Crohn disease (CD) and ulcerative colitis (UC). İn our result association between smokers in CD and control was a significant P value < 0.01, an increased CD risk, and a reduced UC risk. Smoking was associated with an odds ratio (OR) of 1.884 for CD compared to nonsmokers. Although the precise origin of IBD is unknown, smoking is the most widely studied and accepted behavioral risk factor. Smoking has been implicated as increasing the risk for the development of CD and as a protective factor in UC, with relative consistency in the literature [33, 36].

Further, the potential significance of *TLR*2 (-196 to -174del and Arg753Gln) variations in the pathophysiology of patients was examined in research on type 2 diabetes and its consequences in the Turkish population. There was a significant difference in the frequency of the *TLR*-2 del/del genotype (P=0.003), ins/del genotype (P=0.009), and del allele (P=0.001) in patients (100 patients) and controls (98 controls) [28]. Recent studies have shown that *TLR* genes are essential in signaling pathogen-related molecules and endogenous proteins associated with immune activation. The *TLR 2* gene, the first human *TLR* involved in host defense has two exons in 4q32 of the chromosome. The *TLR9* gene in humans is in the 3p21.2 chromosome region and has two exon numbers. Stimulation of the *TLR9* gene by its ligands triggers a signaling pathway common to all *TLR* genes, activating the transcription factor nuclear factor-kB (NF-kB) and producing pro-inflammatory cytokines.

Research has shown that the carriage of minor *TLR*2 (-196 to -174) del and *TLR3-1377 T* alleles appear to exert a significant influence on HNC risk, providing evidence of the involvement of these polymorphisms and their haplotypes as risk factors of HNC. Identifying genetic risk predictors for NPC and LC, the two major HNC types, may improve diagnosis, risk prediction, and clinical care. *TLR 9* (T1486C and T123C) seems to influence the progression of malaria under certain genetic models and in specific age groups of people from specific geographical regions. *TLR 9* (T1237C) also plays a role in susceptibility to malaria under specific genetic models in children. A case-control study in China found that *TLR 9* gene rs187084 polymorphism was positively associated with susceptibility to knee OA, especially among older adults. Balbaloglu et al. (2017) found a correlation between *TLR 9 T-1486C* gene polymorphism and advanced knee OA in the Turkish population. Still, no significant difference was found between CD and UC patients and the control group [37]. Messaritakis et al. (2018) investigated the gene polymorphisms of *TLR 2* (-196 to -174del), *TLR 4* (Asp299Gly and Thr399Ile), and *TLR 9* (T1237C and T-1486C) gene polymorphisms in the risk of development and progression of colorectal cancer in Greece. Conducting advanced genetic studies and confirming these results with a meta-analysis test will contribute more to scientific knowledge development [38].

In conclusion, the present study demonstrates that the *TLR2* –196 to –174del/del, *TLR9* (T-1486C) polymorphism is no associated with an increased risk of CD, UC and IBD. A significant difference in smoking rates between the CD group and the control group, with a 1.88 odds ratio, indicating a 1.88 times higher number of smokers in the CD group. However, we only investigated the *TLR2, TLR9* polymorphism; in two SNPs in a limited region of genes, we need sequencing of whole gene of *TLR2* and *TLR9.* In addition, because the *TLR2, TLR9* gene polymorphism may show variations in different ethnic groups, further studies will be needed in a larger and ethnically diverse population to confirm the impact of this gene on the susceptibility of IBD.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

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Ethical considerations

The IBD cohort study has been approved by the ethics committees of Erciyes University, Clinical Research Ethics Committee dated 06.12.2013 and numbered 2013/729, Investigation of Some Genetic Polymorphisms was made upon the decision dated 10.06.2016 and numbered 2016/349. These samples were also used in the "NOD2/CARD15 Gene Polymorphisms in Inflammatory Bowel Diseases" study.

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