https://doi.org/10.18549/PharmPract.2024.4.3047

Original Research

Association between Toll-Like Receptor 4 Gene and inflammatory bowel disease among Jordanian patients

Malek Zihlif , Zainab Zakaraya , Rawan Nael , Abed Al-Rahman Al-Shudiefat , Mohammad Alshhab , Ahmad Sundookah , Ahmad R. Alsayed

Received (first version): 01-Jan-2024 Accepted: 25-Feb-2024 Published online: 10-Dec-2024

Abstract

Background: Inflammatory bowel disease (IBD) is a set of chronic inflammatory gastrointestinal disorders that affects many patients worldwide, with a peak incidence in early adult life. The Toll-like receptor 4 (TLR4) binds Lipopolysaccharide (LPS) with the assistance of accessory proteins and activates signal transduction pathways of the innate immune system. TLR4 is significantly up-regulated in the active disease phase of IBD. It has been reported that the two most common single nucleotide polymorphisms (SNPs) of TLR4 (Asp299Gly and Thr399Ile) have been shown to suspend TLR4-mediated LPS signaling in vitro and are associated with endotoxin hyporesponsiveness in human beings. This study aims to determine if there is an association between certain TLR4 (Asp299Gly and Thr399Ile) polymorphisms and IBD in Jordanian patients, to determine the genotype and allele frequencies of TLR4 (Asp299Gly and Thr399Ile) polymorphisms among IBD and healthy Jordanian groups, to determine the association between the age and IBD Jordanian patients. **Methods:** 96 IBD patients aged between 17-72 years and 96 healthy subjects without genetic kinship related to the disease were enrolled and genotyped by TLR4 (Asp299Gly and Thr399Ile) polymorphisms using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assay methods. **Results:** There was no significant association between TLR4 (Asp299Gly and Thr399Ile) polymorphisms and IBD compared with healthy subjects among Jordanian subjects. **Conclusions:** IBD incidence was significantly higher in younger age compared to older age, which could be due to the higher activity of the digestive system in those patients compared to older adults.

Keywords: IBD; TLR4; genotype; polymorphism; jordan

INTRODUCTION

Worldwide, inflammatory bowel disease (IBD) considered one of the digestive system diseases that resulted in 51,000 deaths in 2013 and 55,000 deaths in 1990.[1] It is defined as a chronic disorder that affects the colon and small intestine. IBD falls within the class of autoimmune diseases in which the body's own immune system attacks parts of the digestive system,[2] caused by perturbed homeostasis between various factors in the gut of genetically susceptible hosts[3] and includes two major types; ulcerative colitis (UC) and Crohn's disease (CD).[4]

The IBD's incidence in Western population has increased with a predictable incidence of 0.35% -1.00% for CD and 0.10% -1.00% for UC throughout the last few decades.[5]

The molecular basis for the pathogenesis of IBD is unclear, suggested to involve a complex interaction of many factors like such as: environmental factors, mucosal immunity, infection, and gene variants.[4]

Innate immune cells must apply a precise and rapid process of discrimination between self and nonself based on the recognition of numerous molecular patterns like pattern recognition receptors (PRRs). Toll-like receptors (TLRs), are a class of transmembrane PRRs; play an important role in the initiation of pro/anti-inflammatory genes, that direct adaptive immune responses and maintaining mucosal and commensal homeostasis.[4]

Recent Studies[4] in different murine models of colitis have helped to understand the mechanistic importance of TLR dysfunction in IBD disease development.[4]

TLRs recognize alarm signals that can be classified into commensal/pathogen (gut microbiota and/or viral-associated) and injury-associated (endogenous/exogenous) molecular patterns.

Malek ZIHLIF*: Department of Pharmacology, School of Medicine, The University of Jordan, Amman, Jordan. m.zihlif@ju.edu.jo
Zainab ZAKARAYA: Biopharmaceutics and Clinical Pharmacy Department, Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan. z.zakaraya@ammanu.edu.jo
Rawan NAEL: Department of Laboratory medical sciences, Faculty of Allied health sciences, The Hashemite University, Zarqa, Jordan c School of Medicine. The University of Jordan, Amman, Jordan.
Abed Al-Rahman AL-SHUDIEFAT: Department of Laboratory medical sciences, Faculty of Allied health sciences, The Hashemite University, Zarqa, Jordan c School of Medicine. The University of Jordan, Amman, Jordan.
Mohammad ALSHHAB: Department of Pharmacology, School of Medicine, The University of Jordan, Amman, Jordan.
Ahmad SUNDOOKAH: Department of Clinical Nutrition and Dietetics, Applied Science Private University, Amman 11931, Jordan.
Ahmad R. ALSAYED: Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman 11937, Jordan. a_alsayed@asu.edu.jo

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

https://doi.org/10.18549/PharmPract.2024.4.3047

Molecular signatures of different classes of microorganisms or features include lipopolysaccharide: which is recognized by TLR4 localized on the cell surface, ligand binding elicits receptor activation through conformational changes. TLR4 uses both MyD88-dependent and MyD88-independent adaptor protein signaling pathways.[4]

In the normal intestine, TLR4 is present only in a small amounts on the intestinal epithelial cells (IECs) and the lamina propria .

Tlr4 will not be expressed to maintain the hypo-responsiveness to omnipresent lipopolysaccharide (LPS) in the gut lumen as well as minimizing recognition of the environment and maintaining a basal state of activation. In contrast, TLR4 is significantly increased in primary IEC and lamina propria mononuclear cells (LPMNCs) in the lower digestive tract in both human UC and CD active diseases. Increasing its responsiveness to the surroundings and leading to an irregular state of activation.[6]

The signalling of TLR4 requires three accessory molecules, LBP, CD14, and MD-2 [7]. Under healthy conditions, the expression of this receptor complex is generally low in the intestinal mucosa, but significantly up-regulated in various cell subsets in either nonactive and/or active IBD colitis in humans. MD-2/ TLR4 upregulation might also result from ligands of plenty lipopolysaccharides.[7]

Mutations in the TLR4 gene can predispose to many types of bacteria such as gram-negative like: Escherichia coli and increase susceptibility to gut infection which may prompt IBD pathogenesis.[4] Pathogenic infections have the potential to alter the composition of commensal microorganisms and disrupt the state of commensal tolerance.[4]

On the other hand, systemic administration of a TLR4-blocking antibody will change the regeneration of tissue integrity during colitis, regardless of limiting the acute inflammatory responses induced by recruited cells in an exaggerated manner.[4]

Many recent studies.[4,7] suggest that TLR signalling is important for cytoprotective functions in the gut epithelial cell (IEC) subsets. which are necessary for barrier preservation, cell stability and survival, and restitution like; inhibition of apoptosis, proliferation, and migration.[4]

TLR4 gene is localized on chromosome 9 (q32-33), a genomic region in which a CD susceptibility gene has been implicated. In active IBD, several alleles in the TLR4 gene might promote functional dysregulation of LPS sensing receptor (TLR4).[4] Mutations could functionally demonstrate proinflammatory properties in response to physiological concentrations of LPS.[4]

TLR4 is up-regulated in intestinal epithelial cells, dendritic cells, and macrophages in IBD patients. The Asp299Gly and Thr399Ile polymorphisms of the TLR4 gene were found to be associated with the low responsiveness to lipopolysaccharide and with both CD and UC.[8,9]

In view of the discrepancy in data among different studies regarding the association between key regulatory genes and IBD susceptibility; the aims of our study are: To examine the association between the two TLR4 polymorphisms, rs4986790 A>G and rs4986791 C>T, and IBD susceptibility in Jordanian patients.

MATERIALS AND METHODS

Study design

This study was conducted at the gastrointestinal unit (GIU) of Islamic Hospital as well as the University of Jordan, the study group including 96 patients who were diagnosed with IBD, including both Crohn's and ulcerative colitis have been selected randomly, on the other hand the control group of 96 healthy Jordanian subjects without genetic kinship related to the disease, who were healthy individuals or patients without functional dyspepsia, and did not have liver or gastrointestinal diseases or any other chronic disease (aged between 18 and 60) were enrolled in this study, each participant has signed consent form to participate in this study. The study and the consent form were approved by the Institutional Review Board (IRB) of THE University of Jordan, Amman, Jordan (reference Number: 64/2016). The guidelines followed for the experimentation on human subjects was according to the principles of the Declaration of Helsinki.

Data collection

Healthy Jordanian subjects and IBD patients diagnosed with IBD including Crohn's and ulcerative colitis patients were interviewed at GIU and their related information were collected and recorded in patient information form.

Sample size

The required sample size was calculated to be around 96 subjects in each group, based on information from previous studies among Caucasian populations and using the Cochran's formula:

N=(PQZ^2)/Δ^2

N =sample size, Z=standard error (z=1.96 for 95% confidence interval), P= estimated allele frequency of both Asp299Gly and Thr399Ile polymorphisms of TLR4 gene, Q=1-P, Δ=acceptable sample error 5%.

96 IBD diagnosed patients (aged between 17 and 72 years old) were enrolled in this study, and their diagnoses were relied on the results of endoscopy as being either Crohn's or ulcerative colitis patients.

The control group included 96 healthy Jordanian subjects without genetic kinship who were healthy individuals or patients without functional dyspepsia, and did not have liver or gastrointestinal diseases or any other chronic disease (aged between 18 and 60) were enrolled in this study.

Blood sample collection

Peripheral blood (3-5) ml were collected from each participant in ethylene diamine tetra-acetic acid (EDTA) tubes and then stored at 4˚C for genetic analysis.

Genomic DNA extraction

DNA was extracted from white blood cells of the collected peripheral blood using a genomic DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega, USA). To detect DNA concentration in (ng/ml), Absorbance was measured at 260 nm

https://doi.org/10.18549/PharmPract.2024.4.3047

wavelength (A260) using NanoDrop 2000 spectrophotometer (Thermo Scientific , USA).

For DNA purity evaluation, the absorbance was measured at 260 nm and divided by the absorbance at 280 nm, DNA with high quality and less contaminants will have a (A260/A280) ratio of around 1.7 to 2.0.

Genotyping of TLR4 SNPs: Asp299Gly (299 A>G, rs4986790) and Thr399Ile (399 C>T, rs4986791) alleles:

Amplification of an exact site in TLR4 gene that contains the mutation sites of interest (Asp299Gly and Thr399Ile) was carried out. Amplification was performed using a pair of oligonucleotide primers (forward and reverse) described by 10 ,which were complementary to the termini of the mutation site of interest. Primer sequence and expected product sizes for both Asp299Gly & Thr399Ile PCR amplification are shown in Table 1. PCR mixture was prepared in a total volume of 20µl and PCR was performed using PCR-Thermocycler (BIO-RAD, USA). The resultant amplified sequence length was 249 bp. It was confirmed by 1.2% agarose gel electrophoresis (Appendix-VII) and visualized using Red-Safe stain. The PCR products of Asp299Gly were digested by NcoI restriction enzyme and the PCR products of Thr399Ile were digested by HinfI restriction enzyme. The digested products of both TLR4 SNPs were mixed with 2.5µl loading dye and run on a 3.5% agarose gel electrophoresis for one hour and fifteen minutes at 90volts, and then it was photographed using UV trans-illuminator. Genotypes were recorded according to the size of the resulted fragments.

Table 1. Primer sequence and expected product sizes for both Asp299Gly & Thr399Ile PCR amplification.

Primers	Sequence (5'-3')	PCR product size (bp)
***Asp299Gly -F**	GCATACTTAGACTACTACCTCCA	249 bp
Asp299Gly -R	ACTTCTGAAAAAGCATTCCCAC	
****Thr399Ile -F**	GGTTGCTGTTCTCAAAGTG	406 bp
Thr399Ile-R	ACCTGAAGACTGGAGAGT	

* [23]
** This primer sequence designed on DNA man program v 5.2.9 and tested on primer 3 (Primer3web version 4.0.0): http://bioinfo.ut.ee/primer3/.

Statistical Analysis:

Allele and genotype frequencies of 96 IBD patients and 96 healthy controls were estimated as the following:

$$\text{Allele frequency} = \frac{\text{Number of variant alleles in the population}}{2 \times \text{population size}}$$

Normally distributed continuous variables such as age were analyzed using ANOVA test. Pearson Chi-square test and Fisher's exact test were used for tests of independence to determine the association between Asp299Gly and Thr399Ile polymorphisms in both IBD and control groups, the relation between these polymorphisms and gender and age.

A P value of 0.05 was considered significant. Odds ratio (OR) for significant association was given with 95% CI. All statistical analyses were performed using statistical package for the social sciences (SPSS Inc., Chicago, Illinois) version 22.0 software for Windows.

Ethical approval and consent to participate :

The study and the consent form were approved by the Institutional Review Board (IRB) of THE University of Jordan, Amman, Jordan (reference Number: 64/2016). Each participant has signed consent form to participate in this study.

RESULTS

Characteristics of the study population:

This study included 96 IBD patients and 96 healthy participants who visited gastrointestinal clinics at JUH and Islamic Hospital Out of the 96 healthy Jordanian subjects, 80.2% were females (n=77) and 19.8% were males (n=19) with an age range of (18-60) years and blood samples were collected from these 96 subjects. On the other hand, out of the 96 IBD patients, 52.1% were females (n=50) and 47.9% were males (n=46) with an age range of (17-72) years and blood samples were collected from these 96 patients.

Genotype frequencies of A299G polymorphism:

The wild type genotype AA was seen in 95 IBD patients (98.9%) and in 93 control subjects (96.9%), thus in 188 of IBD + Control combination (98%), the heterozygous genotype AG was seen in 1 patient of IBD patients (1.04%) and in 3 control subjects (3.13%) thus in 4 of IBD + Control combination (2.08%) and the homozygous variant genotype GG was not seen in any patient. This is shown in Table (2). Therefore, there was no significant difference between healthy and IBD patients when we compared each genotype.

Genotype frequencies of C399T polymorphism:

The wild type genotype CC was seen in 92 IBD patients (95.83%)

Table 2. Genotype frequencies of A299G polymorphism among healthy IBD combination Jordanian subjects

A299G (IBD n=96)		
IBD Genotype frequency, n (%)		
AA	**AG**	**GG**
95 (98.9%)	2(2.08%)	0(0)
95% CI		
0.9681-1.0099 ±2.09	0-0.0493 ±2.85	-
A299G (Control n=96)		
Control Genotype frequency, n (%)		
AA	**AG**	**GG**
93(96.9%)	3(3.13%)	0(0)
95% CI		
0.9343-1.0037 ±3.47	0-0.0661 ±3.48	-
P Value = 0.361		

https://doi.org/10.18549/PharmPract.2024.4.3047

and in 89 control subjects (92.71%), and thus in 181 of IBD + Control combination (94.28%), the heterozygous genotype CT was seen in 4 IBD patients (4.17%) and in 4 control subjects (4.17%) thus in 8 of IBD + Control combination (4.17%), and the homozygous variant genotype TT was seen in 3 control subjects (3.13%) only, thus in 3 IBD + Control combination (1.56%), . This is summarized in Table (3). And here also there was no significant difference between healthy and IBD patients when we compared genotypes (P >0.05).

Table 3. Genotype frequencies of C399T polymorphism among healthy IBD combination Jordanian subjects

C399T (IBD n=96)		
IBD Genotype frequency, n (%)		
CC	**CT**	**TT**
92 (95.83%)	4(4.17%)	0(0)
95% CI		
0.9187-0.9983 ±4	0.0017-0.0817 ±4	-
C399T (Control n=96)		
Control Genotype frequency, n (%)		
CC	**CT**	**TT**
89(92.71%)	4(4.17%)	3(3.13%)
95% CI		
0.8751-0.9791 ±5.2	0.0017-0.0817 ±4	-
P Value = 0.355		

Combination of A299G and C399T genotypes distribution:

The distribution of both genotypes (A299G and C399T) is shown in Table (4). The homozygous and heterozygous variant alleles for both genotypes (299GG and -399TT), (299AG and -399CT) retrospectively were not seen in any patient. Moreover, the wild alleles for both genotypes (299AA and -399CC) were seen in 91 IBD patients (94.79%) and in 86 of the controls (89.58%). And the combination has no significant difference when we compare of both IBD patients and control groups; (P>0.05).

Allele frequencies of A299G and C399T among IBD and control Jordanian patients:

Table (5) shows the allele frequencies of A299G and C399T polymorphisms in IBD and healthy Jordanian subjects. The A299G variant allele frequencies of the IBD and controls are 0.01, 0.016, respectively; while the C399T allele frequencies of the IBD and controls are 0.02, 0.05 respectively, there was no significant difference between controls and IBD patients among different Allele Frequencies (P>0.05).

Comparison between A299G and C399T allele frequencies of IBD Jordanian patients with other populations

We compared A299G and C399T allele frequencies in IBD Jordanian patients with other populations.In this study, the 299G allele frequency was 0.01 (Table 6). The variant allele frequencies among other populations ranged from 0.01 to 0.109 as shown in Table (6), the 299G allele frequency was the highest in Belgium and lowest in Jordanian.

Referring to the above mentioned table, there were no significant differences in TLR4 299G allele frequency distribution between the following populations: Hungary , CEU (Utah residents of Northern and Western European ancestry) , and YRI (oruba trios from Ibadan, Nigeri). In 399T case, the allele frequency was 0.02 (Table 6). The variant allele frequencies among other populations ranged from 0.004 to 0.071 as shown in Table 6, the 399T allele frequency was the highest in German and lowest in Greece. Referring to the above mentioned table, there were no significant differences in

Table 4. Combination of A299G and C399T genotypes distribution

AA		**IBD A299G, n (%)**		
		AG	**GG**	
IBD C399T, n (%)	**CC**	91(0.9479)	1 (0.01)	0 (0)
	CT	3 (0.031)	1 (0.01)	0 (0)
	TT	0 (0)	0 (0)	0 (0)
AA		**Control A299G, n (%)**		
		AG	**GG**	
Control C399T, n (%)	**CC**	86(0.8958)	2 (0.0208)	0 (0)
	CT	4 (0.0416)	0 (0)	0 (0)
	TT	3 (0.031)	0 (0)	0 (0)
P Value = 0.177				

Table 5. Allele frequencies of (299A, 299G, 399C, 399T) among IBD and control Jordanian patients

Allele	**Frequency (n)**	**Frequency (n)**	**P value***
	IBD	**Control**	
299A	0.99(190)	0.984(189)	
299G	0.01(2)	0.016(3)	P = 0.6042
399C	0.98(188)	0.95 (182)	
399T	0.02(4)	0.05(10)	P = 0.0928

Table 6. Comparison of A299G and C399T allele frequencies between IBD Jordanian patients and other ethnic groups

	TLR4 A<299G		**Reference**	**P-value****	**Is there a statistical difference?**
Country/ethnicity	**IBD Sample size**	**MAF*(%)**			
Jordanian	96	1	Our Present study	-	-
New Zealand Caucasian	101	6.4	[10]	0.0472	Yes
German	204	7.1	[12]	0.026	Yes

https://doi.org/10.18549/PharmPract.2024.4.3047

Scotland	234	10.3	[13]	0.0038	Yes
Hungary	527	5.1		0.074	No
Netherlands	112	10.3		0.005	Yes
Belgium	334	10.9		0.0024	Yes
CEU (Utah residents of Northern and Western European ancestry)	30	3.3	[14]	0.3749	No
YRI (oruba trios from Ibadan, Nigeri)	30	3.3		0.3749	No
Tunisian	90	7	[15]	0.0352	Yes

* Chi Square test
Note: there was no significant difference between controls and IBD patients among different Allele Fre quencies (P>0.05).

ii.	**TLR4 C< 399T**		**Reference**	**P-value****	**Is there a statistical difference?**
Country/ethnicity	**IBD Sample size**	**MAF*(%)**			
Jordanian	96	2	Our Present study	-	-
New Zealand Caucasian	106	6.7	[10]	0.0392	Yes
German	204	7.9	[12]	0.0163	Yes
Greece	120	0.4	[13]	0.5911	No
CEU (Utah residents of Northern and Western European ancestry)	30	3.3	[14]	0.3749	No
Tunisian	90	7	[15]	0.0352	Yes

***MAF: Minor Allelic Frequency**
**** t-test**

TLR4 399T allele frequency distribution between the following populations: Greece, and CEU (Utah residents of Northern and Western European ancestry).

Association of Age with IBD patients.

According to Table (7), there is a significant association between age and IBD when we compared young people (17-27) with elderly people (50-60) or (61-71), with P value <0.05.

Table 7. Association of Age with IBD patients

Age Interval	**IBD Patient Numbers**	**%**	**P-value***
17-27	27	28.13	Between 17-27 & 50-60
28-38	19	19.8	P = 0.0038
39-49	28	29.17	
50-60	11	11.46	Between 17-27 & 61-71
61-71	11	11.46	P = 0.0038

DISCUSSION

In the last few years, new insights in many present studies support the role of intestinal microbiota in developing common disorders such as obesity and inflammatory bowel disease (IBD).[10] Differences in fecal microbiota have been found in those with and without IBD, as well as defects in epithelial recognizing of bacteria are also linked with IBD. Pattern recognition receptors (PRRs) like TLR4, which identify conserved structures of microorganisms, are in charge of epithelial sensing of bacteria.[11]

The TLR system has an important role in protecting the gut epithelial barrier and maintaining tolerance against commensal microbiota, thus participating in gut homeostasis. On the other hand, it is interesting that TLR system affects the ability for front-line bacterial recognition, and thus favours to develop IBD.[11]

Under normal conditions of the gut, TLR4 is expressed only in few amounts on primary intestinal epithelial cells, however, TLR4 is significantly up-regulated in the active disease phase of IBD since it has an important role in activating signal transduction pathways of the innate immune system including the NF-κB signaling pathway and inflammatory cytokines.[11]

Our study results showed no significant association between TLR4 (Asp299Gly and Thr399Ile) polymorphisms and IBD in comparing with control healthy subjects (P > 0.05), this suggests that the TLR4 mutations could not affect the LPS responsiveness of nonhematopoietic cells such as intestinal epithelial cells in inflammatory bowel disease , and this result is similar to what was obtained from another study that was conducted by.[12,13,14,15]

On the other hand, our study contradicted with other studies reported that two common SNPs of TLR4 (Asp299Gly and Thr399Ile) have been shown to suspend TLR4-mediated LPS signaling in vitro and is associated with endotoxin hyporesponsiveness in human beings.[11] such as the study conducted by Franchimontet al.[9] who reported that the TLR4 Snp was associated with UC and CD in a Belgian study. As well as contradicting with Dutch, Greek, Australian, and German populations with CD, and an association with colonic disease

https://doi.org/10.18549/PharmPract.2024.4.3047

has been described.[16-18] In one German cohort, an association between the TLR4 Thr399Ile SNP and UC was demonstrated.[19] This is possibly due to the existence of racial and geographic differences or due to the small sample size included in the present study.

Interestingly to mention that our study revealed that IBD incidence was significantly higher in younger ages compared to older ages when we compared young people (17-25) with elderly people (50-60) or (61-71), with P value <0.05, this results agree with what is known about the epidemiology of this disease; 7–20% of IBD sufferers are children, 60–85% are adults, most of them being under 40 years of age.[19] CD typically has two incidence peaks: between 20 and 30 and between 60 and 70. The incidence of UC surges between ages 20 and 30. Recent studies suggest that the bimodal distribution of CD is typical of American cohorts. In contrast, large European and Canadian data suggest a peak incidence of CD in the 15–29 age group and a peak incidence of UC in the 20–29 age group.[20] The available epidemiological data indicate that IBD primarily affects individuals in their younger years.[20]

Relating to ethnicity, our data revealed that the variant allele frequency of Asp299Gly polymorphism (299G) in Jordanians owns the same pattern as in Caucasians, African Americans, and Hispanics. 299G variant allele frequency among many populations was similar to that found in Jordanian population (p-value>0.05) such as Hungary, CEU (Utah residents of Northern and Western European ancestry), YRI (oruba trios from Ibadan, Nigeri) but a difference in this frequency was found in New Zealand Caucasian , German, Scotland, Netherlands, Belgium, and Tunisian populations.[21-22] When comparing the 399T variant allele frequency between Jordanians and other populations, it was also similar to that found in Greece and CEU (Utah residents of Northern and Western European ancestry), However, New Zealand Caucasian , German, and Tunisian populations showed significant differences in 399T allele frequency compared to our study population.

CONCLUSIONS

Concerning the association of Asp299Gly and Thr399Ile polymorphisms of TLR4 and IBD compared with healthy subjects, no significant association was found between these polymorphisms and IBD compared with healthy subjects among Jordanian inflammatory bowel disease patients.

It is worthy that IBD incidence was significantly higher in younger age compared to older age, with P value <0.05, which could be due to the higher activity of the digestive system in those patients compared to elderly people as well as the lifestyle changes toward unhealthy dietary patterns such as high fast food consumption, this agree with many studies suggest that around a quarter of people with IBD were aged under 16 when they were diagnosed.[24]

References

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. The Lancet. 2007;369(9573):1627-40. https://doi.org/10.1016/s0140-6736(07)60750-8
2. Kittinger GW, Beamer NB, Hagemenas F, Hill JD, Baughman WL, Ochsner A. Evidence for autonomous pituitary-adrenal function in the near-term fetal rhesus (Macaca mulatta). Endocrinology. 1972;91(4):1037-44. https://doi.org/10.1210/endo-91-4-1037
3. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427-34. https://doi.org/10.1038/nature06005
4. Cario E. Toll-like receptors in inflammatory bowel diseases: a decade later. Inflammatory bowel diseases. 2010;16(9):1583-97. https://doi.org/10.1002/ibd.21282
5. Carter MJ, Lobo AJ, Travis SP. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2004;53(suppl 5):v1-v16.
6. Kaser A, Zeissig S. Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol. 2010;28:573-621. https://doi.org/10.1146/annurev-immunol-030409-101225
7. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. J Immunol. 2005;174(8):4453-60. https://doi.org/10.4049/jimmunol.174.8.4453
8. Okayama N, Fujimura K, Suehiro Y, Hamanaka Y, Fujiwara M, Matsubara T, et al. Simple genotype analysis of the Asp299Gly polymorphism of the Toll-like receptor-4 gene that is associated with lipopolysaccharide hyporesponsiveness. J Clin Lab Anal. 2002;16(1):56-8. https://doi.org/10.1002/jcla.2075
9. Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, et al. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut. 2004;53(7):987-92. https://doi.org/10.1136/gut.2003.030205
10. De Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J Biol Chem. 1994;269(22):15419-22.
11. Browning BL, Huebner C, Petermann I, Gearry RB, Barclay ML, Shelling AN, et al. Has toll-like receptor 4 been prematurely dismissed as an inflammatory bowel disease gene? Association study combined with meta-analysis shows strong evidence for association. Am J Gastroenterol. 2007;102(11):2504-12. https://doi.org/10.1111/j.1572-0241.2007.01463.x
12. Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, et al. Lack of common NOD2 variants in Japanese patients with Crohn's disease. Gastroenterology. 2002;123(1):86-91. https://doi.org/10.1053/gast.2002.34155

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

13. Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, et al. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther. 2003;17(12):1465-70. https://doi.org/10.1046/j.1365-2036.2003.01607.x
14. Wang ZW, Ji F, Teng WJ, Yuan XG, Ye XM. Risk factors and gene polymorphisms of inflammatory bowel disease in population of Zhejiang, China. World J Gastroenterol. 2011;17(1):118-22. https://doi.org/10.3748/wjg.v17.i1.118
15. Xie Z, Lv B, Zou L, Shang X, Zhou Y. Genotyping of toll-like receptor 4 in Hubei population of Chinese. Acta Medicinae Universitatis Scientiae et Technologiae Huazhong. 2004;33:630-2.
16. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet. 2007;39(5):596-604. https://doi.org/10.1038/ng2032
17. Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology. 2007;133(5):1670-89.
18. Fefferman DS, Farrell RJ. Endoscopy in inflammatory bowel disease: indications, surveillance, and use in clinical practice. Clin Gastroenterol Hepatol. 2005;3(1):11-24. https://doi.org/10.1016/s1542-3565(04)00441-0
19. Triester SL, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, et al. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. Am J Gastroenterol. 2005;100(11):2407-18. https://doi.org/10.1111/j.1572-0241.2005.00274.x
20. Hong J, Leung E, Fraser AG, Merriman TR, Vishnu P, Krissansen GW. TLR2, TLR4 and TLR9 polymorphisms and Crohn's disease in a New Zealand Caucasian cohort. J Gastroenterol Hepatol. 2007;22(11):1760-6. https://doi.org/10.1111/j.1440-1746.2006.04727.x
21. Zouiten-Mekki L, Kharrat M, Karoui S, Serghimi M, Fekih M, Matri S, et al. Tolllike receptor 4 (TLR4) polymorphisms in Tunisian patients with Crohn's disease: genotype-phenotype correlation. BMC Gastroenterol. 2009;9:62. https://doi.org/10.1186/1471-230x-9-62
22. Prelipcean CC, Mihai C, Gogalniceanu P, Mihai B. What is the impact of age on adult patients with inflammatory bowel disease? Clujul Med. 2013;86(1):3-9. http://www.ncbi.nlm.nih.gov/pmc/articles/pmc4462481/
23. Chen L, Lin MJ, Zhan LL, Lv XP. Analysis of TLR4 and TLR2 polymorphisms in inflammatory bowel disease in a Guangxi Zhuang population. World J Gastroenterol. 2012;18(46):6856-60. https://doi.org/10.3748/wjg.v18.i46.6856
24. Irving PM, Gearry RB, Sparrow MP, Gibson PR. Review article: appropriate use of corticosteroids in Crohn's disease. Aliment Pharmacol Ther. 2007;26(3):313-29. https://doi.org/10.1111/j.1365-2036.2007.03379.x