ORIGINAL RESEARCH

Correlation Between IL-8 Gene Polymorphisms and Pathogenesis of Crohn's Disease

Xiuyan Lu, MM; Shenbiao Zhao, MM; Xiaoxian Ye, MM; Pingfan Wang, BM

ABSTRACT

Context • The risk of inflammatory bowel disease (IBD) is substantially heightened if patients' first-degree relatives have it. The genetic and immune factors related to the disease have attracted great attention, including patients innate genetic polymorphisms. Interleukin-8 (IL-8) plays a vital role in digestive-system diseases, especially in gastrointestinal diseases.

Objective • The study intended to explore the expression of interleukin-8 (IL-8) in the colon tissues of patients with Crohn's disease and the correlation between its polymorphisms and the disease's occurrence.

Design • The research team performed a prospective study.

Setting • The study took place in the Department of Gastroenterology at Zhuji People's Hospital of Zhejiang Province in Zhuji, China.

Participants • Participants were 100 patients with Crohn's disease at the hospital between November 2016 and June 2018 and 100 healthy individuals. The research team assigned participants with Crohn's disease to the Crohn's disease group and the healthy participants to the control group.

Outcome Measures • The research team: (1) determined differences in the protein expression of the IL-8 between the groups; (2) examined the conformity of the data to that of the Hardy-Weinberg equilibrium; (3) analyzed the differences in the genotypes and alleles for the IL-8 single nucleotide polymorphisms (SNPs) rs102039, rs103284 and rs105432 between the groups; and (4) for the Crohn's disease group, examined the differences in the disease's location and behavior for the participants with different genotypes.

Results • The protein expression level of IL-8 in the colon tissues in Crohn's disease group was significantly higher than that in control group ($P < .05$). The genetic association analysis showed significant correlations between the polymorphisms rs103284 and rs105432 and alleles of the IL-8 gene and the occurrence of Crohn's disease ($P < .05$), but no associations existed between the gene polymorphism rs102039 and alleles and Crohn's disease ($P > .05$). Significant correlations existed between the IL-8 gene polymorphisms rs103284 and rs105432 and the disease's location and behavior ($P < .05$).

Conclusions • IL-8 had a significantly increased expression in the colon tissues of the participants with Crohn's disease, and some genotypes and alleles for the gene polymorphisms rs103284 and rs105432 were significantly higher in the Crohn's disease group than in the control group. In addition, the disease's location and behavior were significantly different for participants in the Crohn's disease group with different genotypes. (*Altern Ther Health Med.* 2023;29(5):112-120).

Xiuyan Lu, MM, Attending doctor, Department of Gastroenterology, Zhuji People's Hospital of Zhejiang Province, Zhuji, China. **Shenbiao Zhao,** MM, Attending doctor, Department of Hepatobiliary Gastrointestinal Surgery, Ningbo Yinzhou No. 2 Hospital, Ningbo, China. **Xiaoxian Ye,** MM, Attending doctor, Department of Oncology, Ningbo No. 1 Hospital, Ningbo, China. **Pingfan Wang,** BM, Chief physician, Division of Gastroenterology, Xinchang County People's Hospital Affiliated to Wenzhou Medical University, Shaoxing, China.

Corresponding author: Pingfan Wang, BM
E-mail: xcpwpf@outlook.com

Inflammatory bowel disease (IBD) is a group of chronic, nonspecific inflammatory diseases of the bowel, with an elusive etiology, and it mainly consists of two clinical phenotypes, Crohn's disease and ulcerative disease.[1] Researchers now believe that IBD can induce abnormal immune responses to intestinal flora in genetically susceptible individuals.

At present, clinicians believe that IBD's pathogenesis involves the synergistic effects of multiple complex factors, such as genetics, immunity, environment, and intestinal microecology, ultimately resulting in the bowel's uncontrollable inflammatory responses. The genetic and immune factors have attracted great attention.

Familial Risks

One epidemiological study found that 12% of patients with Crohn's disease have a family history of it, and the comorbidity rate in monozygotic twins is significantly higher than that in dizygotic twins.[2] And the risk of IBD is substantially heightened if patients' first-degree relatives have it, and the comorbidity rate of IBD in monozygotic twins is significantly higher than that in dizygotic twins.[2]

The comorbidity rates of Crohn's disease and ulcerative colitis are about 20-50%, with the rate being only 6-4% in monozygotic twins.[3] Another epidemiological survey found a familial aggregation for IBD patients, especially for patients with Crohn's disease.[4]

Some studies have discovered more than 200 IBD-associated susceptible genes, including nucleotide-binding oligomerization domain 2 (NOD2), leucine-rich repeat kinase 2 (LRRK2), interleukin-23 receptor (IL-23R), autophagy related 16 like 1 ATG16L1), signal transducer and activator of transcription 3 (STAT3), Janus kinase 2 (JAK2), and human leukocyte antigen (HLA).[5-8] Those studies also found that the genes' polymorphisms can affect the ability of hosts to recognize bacteria and have a close association with the innate immune function and the Th17 cell function of hosts. Therefore, exploration of the genetic pathogenesis of Crohn's disease is of great significance for its early prevention and precise treatment in the future.

Genetic Polymorphisms

Sandhu et al found that the site of Crohn's disease was closely associated with the innate genetic polymorphisms of participants and that disease sites may be the initiators of the abnormalities in disease behaviors.[9] Strober et al found that the leading cause of intestinal tissue fibrosis and stricture was the recurrent and uncontrollable transmural colitis of Crohn's disease, and a patient's ileum is the most vulnerable to that fibrosis.[10] Another study found that the patients with Crohn's disease in the ileum are at a higher risk of intestinal stricture than those with Crohn's disease in the colon or ileocolon.[11]

Single nucleotide polymorphism (SNP) refers to a polymorphism of DNA sequences due to variations in single deoxyribonucleotides. It's associated with many complex human diseases and phenotype differences and is one of the most common heritable variations in humans.[12] Because SNPs are highly stable genetic markers, they not only are the most reliable basis for identification of disease etiology and diagnosis but also lay a foundation for screening medications.[13]

Inflammatory Mediators

As potent inflammatory mediators, cytokines are a class of cell-secreted proteins or minor polypeptides that can serve as the media for signaling and play a pivotal role in the defense and repair in organisms. Their secretion occurs to eliminate the pathogens that invade organisms and to control the spread of inflammatory responses.

The inflammatory response is a kind of self-defense for organisms against the invasion of exogenous pathogens, but it's double-edged sword, since excessive inflammatory responses can cause hypersecretion of cytokines, leading to damage to the body and dysfunction of multiple organs.[14]

Inflammatory responses give rise to alterations in the concentrations of serum pro-inflammatory cytokines, such as IL-6, IL-8, IL-1β, and tumor necrosis factor alpha (TNF-α), and of the anti-inflammatory cytokines IL-10, IL-4, IL-1 antagonist, and TGF-β as well as other inflammatory effectors.[15]

IL-8 which was produced by neutrophils and recently recruited macrophages, also known as cell chemokine ligand 8, was the first inflammatory chemokine, discovered in 1987, and mononuclear macrophages and endothelial cells mainly secrete it.[16,17] Two studies found that IL-8 has an important role in mediating neutrophil infiltration of the gut wall in IBD, such as ulcerative colitis and Crohn's disease.[18,19]

IL-8can induce histamine release in basophilic cells, and its content is normally very low in the serum but can soar within 1-3 h after inflammation.[17] As a chemokine superfamily member, IL-8 plays a vital role in digestive-system diseases, especially in gastrointestinal diseases. Saes et al found that allele A of IL-8-251 may be an important risk factor for the gastric diseases associated with *Helicobacter pylori* infection.[20]

Current Study

However, no studies have occurred on the potential correlation between IL-8 polymorphisms and Crohn's disease. The current study intended to explore the expression of IL-8 in the colon tissues of patients with Crohn's disease and the correlation between its polymorphisms and the disease's occurrence.

METHODS

Participants

The research team performed a prospective study. The study took place in the Department of Gastroenterology at Zhuji People's Hospital of Zhejiang Province in Zhuji, China. Potential participants were patients with Crohn's disease at the hospital between November 2016 and June 2018 and healthy physical examinees.

The study included potential participants with Crohn's disease group if they: (1) had met the requirements for a diagnosis of Crohn's disease defined in the *Consensus on the management of inflammatory bowel disease in China in 2007*,[21] (2) had that diagnosis comprehensively confirmed through an evaluation of the disease's clinical manifestations, including the results of colonoscopy, enteroscopy, capsule endoscopy, gastroscopy, laboratory tests, and histopathological radiographic examinations; and (3) had complete clinical information available.

The study excluded potential participants if they: (1) Had a history of other gastrointestinal diseases or inflammatory bowel disease; (2) Had abnormal findings in colonoscopy, ultrasound, or laboratory examinations; (3) Had a history of taking antibiotics or anti-inflammatory drugs in the three months prior to the study; (4) Had a

history of allergies or autoimmune diseases. Ten of the patients refused to participate in the study, five had a previous diagnosis of ulcerative colitis, and five were taking antibiotics.

Participants signed an informed consent form. The Ethics Committee of Zhuji People's Hospital of Zhejiang Province (no. 032) approved the study's protocols

Procedures

Groups. The research team assigned participants with Crohn's disease to the Crohn's disease group and the healthy participants to the control group.

Sites and behaviors of Crohn's disease. The research team classified the sites and behaviors of Crohn's disease based on the Montreal Classification for Crohn's Disease.[21]

Blood collection. The research team: (1) drew 4 mL of venous blood from all participants after they had fasted for 8 h, (2) added 6 mg of ethylenediaminetetraacetic acid (EDTA) from Shanghai Xin Yu Biotech Co., Ltd (Shanghai, China) for anticoagulation, and (3) cryopreserved it in a refrigerator at -20°C for later use.

Western blotting. For the Crohn's disease, group, the research team: (1) took the freshly frozen colon tissues from an ileocecal biopsy from a refrigerator at -80°C; (2) initially cut them into pieces using scissors and then fully ground them using a grinder; (3) ultrasonicated the tissues and centrifuged the lysate; and (4) aspirated the supernatant and aliquoted it into Eppendorf (EP) tubes from Taixing Qiujing Instrument Plant (Taizhou , Jiangsu, China).

The research team: (1) measured the concentration of proteins using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA) and an ultraviolet spectrometer from Techcomp Scientific Limited (Beijing, China); (2) diluted the proteins from all samples into the same constant concentration, aliquoted them, and preserved them in a refrigerator at -80°C; (3) subjected the extracted total proteins to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) from Thermo Fisher Scientific (Waltham, MA, USA); (4) transferred them onto cellulose acetate/ polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA); (5) incubated them with the primary antibody against IL-8 from Abcam (Cambridge, MA, USA) at 4°C overnight and then with the goat anti-rabbit secondary antibody from Abcam (Cambridge, MA, USA) in the dark for one h; and (6) scanned the protein bands using an Odyssey scanner from Linco Research (St Charles, MO, USA); and quantified the data.

Immunohistochemical staining. The research team: (1) de-paraffinized the colon tissue sections obtained from the ileocecal biopsy in an incubator at 60°C for 30 min; (2) performed antigen retrieval using a citrate buffer from 3B Pharmachem (Wuhan) International Co.,Ltd. (Wuhan, Hubei, China) with the high-pressure method; (3) immersed the colon tissues in 3% hydrogen peroxide, incubated them for 20 min, and sealed them with 8% goat serum for 30 min; and (4) added the resulting tissues dropwise and soaked them with primary antibody against IL-8 diluted 1:200 in phosphate buffered saline (PBS) and incubated them in a refrigerator at 4°C overnight.

The research team: (1) rewarmed each group of sections the next day, added them to the secondary antibody B solution in drops, and incubated them at room temperature for 30 min; (2) added the rinsed sections dropwise to the developer diaminobenzidine (DAB) working buffer (Solarbio, Beijing, China); (3) developed color for a strictly controlled period under a light microscope from NIKON instruments (Shanghai) Co., Ltd. (Shanghai, China); (4) counter-stained the sections in each group with hematoxylin from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China), dehydrated them in alcohol at gradient concentrations and mounted them; and (5) photographed the resulting sections under the light microscope at 200× in a randomly selected 10 fields of view.

Deoxyribonucleic acid (DNA) extraction and polymorphism chain reaction (PCR) amplification. The research team: (1) collected 4 mL of the EDTA-anti-coagulated blood to extract genomic DNAs according to the steps in the instructions of the DNA extraction kit (Google Biology, Wuhan, Hubei, China); (2) measured the mass of 2 μL of a DNA sample using 1.5% agarose gel electrophoresis from Hangzhou Aikerui Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China) and measured the concentration of the extracted DNA using the ultraviolet spectrophotometer; (3) designed and amplified the primers for the IL-8 gene polymorphisms rs102039, rs103284, and rs105432; (4) conducted PCR in the reaction system (20 μL) consisting of 2.0 μL of DNA template, 10.0 μL of 2× Mix from Thermo Fisher Scientific (Waltham, MA, USA), 0.4 μL each of the forward and reverse primers, and 7.2 μL of double-distilled water (ddH2O) under the following conditions: 35 cycles of 95°C for 120 s, 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s and an extension at 72°C for 10 min; (5) detected the amplification of gene fragments using the agarose gel electrophoresis. Table 1 lists the primer sequences and product sizes from the PCR.

Ligase chain reaction. The BGI Group (Shenzhen, Guangdong, China) designed and synthesized the forward and reverse probes used in the reaction. The research team: (1) following the 5'-terminal phosphorylation, prepared all the forward probes into the probe mixture at a concentration of 12.5 pmol/μL; (2) completed the ligase chain reaction in a

Table 1. Primer Sequences and Product Size For PCR

Polymorphism	Primer sequence	Product, bp
Rs102039	Forward: ACGTTATGTCGTAGTGCTC	221
	Reverse: AGTCGTAGTGTCGTAGTGT	
Rs103284	Forward: CGTAGTCGTAGTCGTGTAC	187
	Reverse: ACGTAGCTGTTAGACCCAC	
Rs105432	Forward: CACGTAGTCGATCGTGATGT	169
	Reverse: CGTAGTCGTAGTGTGTCGAA	

Abbreviations: bp, base pair; PCR, polymorphism chain reaction.

Table 2. Ligase Chain Reaction Probe Sequences and Product Sizes of Different IL-8 Gene Polymorphisms

Polymorphism	Probe	Primer sequence 5'-3'	Product (bp)
Rs102039	Rs102039	P-ATCGTAGTCGCACCATTTTTTTTTTTTTT-FAM	211
	Rs102039-C	TTTACGTACGTACACGTTCGAATTTTTTTTTAT	
	Rs102039-G	TTTTTTCACGTGTACATGTGCATTTTTTTTTAAA	
Rs103284	Rs103284	P-ATAGTCCACGTAGTGTTTTTTTTTTTTTT-FAM	185
	Rs103284-A	TTTTTTTTTTTTACACTGAATCGTAGTCGTAGTC	
	Rs103284-T	TTTTTTTTTTTACATCGTGCCGTAGTCGATACG	
Rs105432	Rs105432	P-AAGACTGTGTGTGTGCACTTTTTTTTTT-FAM	192
	Rs105432-A	TTTTTTTACGTAGCTGTGTGTTGATGCTGATGC	
	Rs105432-G	TTTTTTTTACAGTGTACGTGTGGTCGATGCTGAT	

Abbreviations: bp, base pair.

reaction system (3.05 μL) containing 0.05 μL of ligase, 1 μL of buffer, 1 μL of PCR products, and 1 μL of probe mixture, under the following conditions: 95°C for 120 s, 94°C for 15 s, and 57°C for 25 s; (3) after 30 cycles of PCR, used the ultraviolet spectrophotometer to determine the concentration; and (4) entrusted the BGI Group with the sequencing and fragment analysis of the target gene. The research team analyzed all data using the GeneMapper software (Thermo Fisher Scientific , Waltham, MA, USA). Table 2 presents the ligase chain reaction probe sequences and the product sizes of the different IL-8 gene polymorphisms.

Outcome measures. The research team: (1) determined differences in the protein expression of the IL-8 between the groups; (2) examined the conformity of the data to that of the Hardy-Weinberg equilibrium; (3) analyzed the differences in the genotypes and alleles for the IL-8 single nucleotide polymorphisms (SNPs) rs102039, rs103284 and rs105432 between the groups; and (4) for the Crohn's disease group, examined the differences in the disease's location and behavior for the participants with different genotypes.

Outcome Measures

Protein expression of IL-8. The research team performed the Western blotting and immunohistochemical staining to determine the protein expression of the IL-8 and compared the expression between the groups.

Hardy-Weinberg equilibrium. The research team examined the conformity of the data to that of the Hardy-Weinberg equilibrium. The team detected the linkage disequilibrium of IL-8 gene polymorphisms using the Hardy-Weinberg equilibrium equation. To determine whether the IL-8 gene polymorphisms in the study population were in Hardy-Weinberg equilibrium, the research team likely used the Hardy-Weinberg equation: $p^2 + 2pq + q^2 = 1$, where p and q represent the frequencies of two alleles at a particular gene locus and p^2, 2pq, and q^2 represent the frequencies of the three possible genotypes (homozygous dominant, heterozygous, and homozygous recessive). By comparing the observed genotype frequencies in the study population to the expected frequencies under the Hardy-Weinberg equilibrium, the research team could determine whether the gene polymorphisms were in equilibrium or whether evolutionary forces were at play. This equilibrium is a principle stating that the genotype frequencies in a population remain constant between generations in the absence of disturbing factors.

Polymorphisms and genotypes. To analyze the differences between the groups, the research team performed the deoxyribonucleic acid (DNA) extraction and polymorphism chain reaction (PCR) amplification and Ligase chain reaction to determine the polymorphisms and genotypes expression of the IL-8 and compared the expression between the groups.

Polymorphisms and alleles. To analyze the differences between the groups, the research team performed the deoxyribonucleic acid (DNA) extraction and polymorphism chain reaction (PCR) amplification and Ligase chain reaction to determine the polymorphisms and alleles expression of the IL-8 and compared the expression between the groups.

Polymorphisms and disease sites. To analyze the differences between the groups, the research team performed the deoxyribonucleic acid (DNA) extraction and polymorphism chain reaction (PCR) amplification and Ligase chain reaction to determine the polymorphisms and disease sites expression of the IL-8 and compared the expression between the groups.

Polymorphisms and disease behavior. To analyze the differences between the groups, the research team performed the deoxyribonucleic acid (DNA) extraction and polymorphism chain reaction (PCR) amplification and Ligase chain reaction to determine the polymorphisms expression of the IL-8 and compared the expression and disease behavior between the groups.

Statistical Analysis

The research team analyzed all data using the Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA). The team: (1) expressed the enumeration data and measurement data as the frequencies and percentages (%) and the means ± standard deviations (SDs), respectively, and (2) performed the χ^2 test and multiple comparisons for the enumeration data and conducted the *t* test and analysis of variance (ANOVA) for the measurement data. $P < .05$ indicated a statistically significant difference.

Table 3. Participants' Demographic and Clinical Characteristics at Baseline (N = 200)

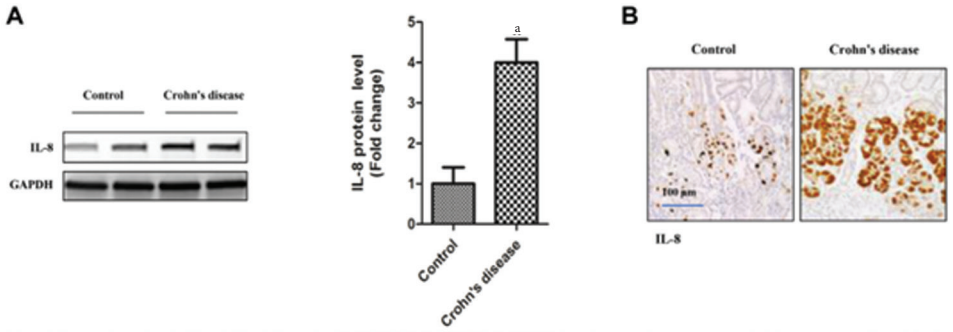
Characteristics	Crohn's Disease Group n = 100 Mean ± SD n (%)	Control Group n = 100 Mean ± SD n (%)	*P* value
Age, y	43.11 ± 1.83	42.89 ± 2.06	.863
Gender			.234
Male	76 (76.0)	70 (70.0)	
Female	24 (24.0)	30 (30.0)	
Smoking Status			.552
Yes	47 (47.0)	46 (46.0)	
No	53 (53.0)	54 (54.0)	
Drinking Status			.248
Yes	31 (31.0)	29 (29.0)	
No	69 (69.0)	71 (71.0)	
Disease Site			
Terminal ileum	41 (41.0)	0 (0.0)	
Colon	20 (20.0)	0 (0.0)	
Ileocolon	39 (39.0)	0 (0.0)	
Disease Behavior			
Penetrating	55 (55.0)	0 (0.0)	
Stricturing	23 (23.0)	0 (0.0)	
Nonpenetrating, nonstricturing	22 (22.0)	0 (0.0)	
Treatment[a]			
5-Aminosalicylic Acid	78 (78.0)		
Hormone	53 (53.0)		
Antibiotic	70 (70.0)		
Immunosuppressant	31 (31.0)		
Infliximab	18 (18.0)		
Surgery	13 (13.0)		

[a]Some participants received multiple treatments

Table 4. Linkage Disequilibrium Between IL-8 Gene Polymorphisms (N=200)

	r^2		
Polymorphism	Rs102039	Rs103284	Rs105432
Rs102039	-	0.002	0.158
Rs103284	0.002	-	0.229
Rs105432	0.229	0.158	-

Figure 1. Protein Expression of IL-8 in the Colon Tissues of the Crohn's Disease and Control Groups. Figure 1A shows the results of Western blotting of IL-8, and Figure 1B shows the protein expression of IL-8 detected using immunohistochemical staining.



[a]$P < .05$, indicating that the protein expression level of IL-8 in the colon tissues of the Crohn's disease group was significantly higher than that of the control group

RESULTS

Participants

Table 3 shows that no significant differences existed in age, gender, smoking status, or drinking status between the groups at baseline ($P > .05$). Among the 100 participants in the Crohn's disease group, 41 had Crohn's disease in the terminal ileum (41.0%), 20 in the colon (20.0%), and 39 in the ileocolon (39.0%). Regarding the disease behaviors, 55 participants in Crohn's disease group had penetrating Crohn's disease (550%), 23 had stricturing Crohn's disease (23.0%), and 22 had nonpenetrating, nonstricturing Crohn's disease (22.0%).

Protein Expression of IL-8

Figure 1A shows the Western blotting results, and Figure 1B shows the immunohistochemical staining results (Figure 1B). The protein expression level of IL-8 in the colon tissues

Table 5. Comparison of the IL-8 Gene Polymorphism Genotypes of the Crohn's Disease and Control Groups (N=200)

	Rs102039			Rs103284			Rs105432		
Group	CC n (%)	CG n (%)	GG n (%)	AA n (%)	AT n (%)	TT n (%)	AA n (%)	AG n (%)	GG n (%)
Crohn's disease, n=100	35 (35.0)	33 (33.0)	32 (32.0)	11 (11.0)	25 (25.0)	64 (64.0)	58 (58.0)	32 (32.0)	10 (10.0)
Control, n=100	33 (33.0)	34 (34.0)	33 (33.0)	28 (28.0)	46 (46.0)	26 (26.0)	20 (20.0)	42 (42.0)	38 (38.0)
χ^2	0.345			0.892			0.293		
P value	.763			<.001[a]			.001[a]		

Comparison of Differences Between Crohn's Disease and Control Groups by Genotype Group																		
Rs102039						Rs103284						Rs105432						
CC Group		CG Group		GG Group		AA Group		AT Group		TT Group		AA Group		AG Group		CG Group		
χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	
0.089	.765	0.022	.881	0.023	.880	9.205	.002	9.630	.002	29.171	<.001[b]	30.349	<.001[b]	2.145	.143	21.491	<.001	

[a]*P* < .05, indicating that the IL-8 gene polymorphisms rs103284 and rs105432 were significantly more frequent in the Crohn's disease group than in the control groups
[b]*P* < .05, indicating that the Crohn's disease group had a significantly higher number of participants with the polymorphism rs10328 genotype TT and the rs105432 genotype AA than did the control group

Table 6. Comparison of the IL-8 Polymorphism Alleles of the Crohn's Disease and Control Groups (N=200)

	Rs102039		Rs103284		Rs105432	
Groups	C n (%)	G n (%)	A n (%)	G n (%)	A n (%)	T n (%)
Crohn's disease, n=100	52 (52.0)	48 (48.0)	23 (23.0)	77 (77.0)	74 (74.0)	26 (26.0)
Control, n=100	50 (50.0)	50 (50.0)	51 (51.0)	49 (49.0)	38 (38.0)	62 (62.0)
χ^2	2.039		0.982		0.872	
P value	.451		<.001[a]		<.001[a]	

Comparison of Differences Between Crohn's Disease and Control Groups by Allele Group												
Rs102039				Rs103284				Rs105432				
C Group		G Group		A Group		G Group		A Group		T Group		
χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value	
0.080	.777	0.08	.777	16.817	.000	16.817	<.001[b]	26.299	.000[b]	26.299	.000	

[a]*P* < .05, indicating that the IL-8 polymorphisms rs103284 and rs105432 were significantly more frequent in the Crohn's disease group than in the control group
[b]*P* < .05, indicating that the Crohn's disease group had a significantly higher number of participants with the polymorphism rs10328 allele G and the rs105432 allele A than did the control group

of the Crohn's disease group was significantly higher than that of the control group ($P < .05$).

Hardy-Weinberg Equilibrium

Table 4 shows that the Hardy-Weinberg equilibrium equation found that the gene polymorphisms conformed to the Hardy-Weinberg equilibrium ($r^2 < 0.33$).

Polymorphisms and Genotypes

Table 5 shows the frequencies of all of the genotypes of the IL-8 gene polymorphisms in the groups. No significant differences existed in the IL-8 rs102039 polymorphism genotypes between the groups ($P > .05$). The IL-8 gene polymorphisms rs103284 and rs105432 were significantly more frequent in the Crohn's disease group than in the control group, with $P < .001$ and $P = .001$, respectively.

Specifically, the Crohn's disease group had a significantly higher number of participants with the rs103284 genotype TT ($P < .001$) and the rs105432 genotype AA ($P < .001$) than did the control group.

Polymorphisms and Alleles

Table 6 shows the frequencies of the alleles of the IL-8 gene polymorphism in the groups. No significant differences existed in the IL-8 rs102039 polymorphism alleles between the groups ($P > .05$). The IL-8 rs103284 and rs105432 polymorphisms were significantly more frequent in the Crohn's disease group than in the control group (both $P < .001$).

Specifically, the Crohn's disease group had a significantly higher number of participants with the rs103284 allele G ($P < .001$) and the rs105432 genotype allele A ($P < .001$) than did the control group.

Polymorphisms and Disease Sites

Table 7 shows that the number of participants in the Crohn's disease group with disease in the terminal ileum was

Table 7. Comparison of IL-8 Rs103284 and Rs105432 Gene Polymorphisms and Disease Site for Participants in the Crohn's Disease Group With Disease in the Terminal Ileum (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Terminal Ileum n = 41 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Rs103284	AA, n = 11	3 (27.3)	2.372	.123	3.158	.0756	17.11	<.001[a]
	AG, n = 25	2 (8.0)						
	GG, n = 64	36 (56.3)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	n (%)	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
	AA, n = 58	25 (43.1)	3.386	1.84	0.209	.648	2.670	.1022
	AT, n = 32	10 (31.25)						
	TT, n = 10	6 (60.0)						

[a]*P* < .05, indicating that the number of participants in the Crohn's disease group with disease in the terminal ileum was significantly higher for those with the Rs103284 genotype GG than for those with the AA and AG genotypes and for those with the Rs105432 genotype AA than for those with the AT or TT genotypes

Table 8. Comparison of IL-8 Rs103284 and Rs105432 Gene Polymorphisms and Disease Site for Participants in the Crohn's Disease Group With Disease in the Ileocolon (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Ileocolon n = 39 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Rs103284	AA, n = 11	5 (45.4)	4.591	.032	1.948	.163	18.41	<.001[a]
	AG, n = 25	18 (72.0)						
	GG, n = 64	16 (25.0)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	n (%)	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
	AA, n = 58	23 (39.7)	0.143	.706	1.417	.234	1.822	.177
	AT, n = 32	14 (43.8)						
	TT, n = 10	2 (20.0)						

[a]*P* < .05, indicating that the number of participants in the Crohn's disease group with disease in the ileocolon was significantly higher for those with the rs103284 genotypes AA and AG than for those with the GG genotype

Table 9. Comparison of IL-8 Rs103284 and Rs105432 Gene Polymorphisms and Disease Site for Participants in the Crohn's Disease Group With Disease in the Colon (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Colon n = 20 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Rs103284	AA, n=11	3 (27.3)	0.234	.629	0.426	.514	2.44	.118
	AG, n=25	5 (20.0)						
	GG, n=64	12 (18.7)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	n (%)	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
	AA, n=58	10 (17.2)	0.776	.378	1.337	.248	0.721	.396
	AT, n=32	8 (25.0)						
	TT, n=10	2 (20.0)						

significantly higher for those with the Rs103284 genotype GG AG than for those with the AA (*P* < .001) or AG (*P* < .001) genotypes. It also was significantly higher for participants with the Rs105432 genotype AA than for those with the AT (*P* < .001) or TT (*P* < .001) genotypes.

Table 8 shows that the number of participants in the Crohn's disease group with disease in the ileocolon was significantly higher for those with the rs103284 genotype AA (*P* < .001) and AG (*P* < .001) than for those with the GG genotype. It also was significantly higher for participants with the rs105432 genotypes AA (*P* < .001) and AT (*P* < .001) than for those with TT genotype.

Table 9 shows that no significant differences existed between the genotype groups for participants in the Crohn's disease group with disease in the colon.

Polymorphisms and Disease Behavior

Table 10 shows that the number of participants in the Crohn's disease group with penetrating Crohn's disease was

Table 10. Comparison of IL-8 Rs103284 and Rs105432 Gene Polymorphisms and Disease Behavior for Participants in the Crohn's Disease Group With Penetrating Disease (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Penetrating n = 55 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Rs103284	AA, n = 11	5 (45.5)	7.186	.007[a]	1.86	.173	5.333	.021
	AG, n = 25	2 (8.0)						
	GG, n = 64	48 (75.0)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	N (%)	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
	AA, n = 58	38 (65.5)	2.96	.085	17.34	<.001[a]	7.088	.008
	AT, n = 32	15 (46.9)						
	TT, n = 10	2 (20.0)						

[a]*P* < .05, indicating that the number of participants in the Crohn's disease group with penetrating disease was significantly higher for those with the rs103284 genotype GG than for those with the AA or AG genotypes and for those with the rs105432 genotype AA than in for those with the AT or TT genotypes

Table 11. Association Between IL-8 Gene Polymorphisms and Disease Behavior for Participants in the Crohn's Disease Group With Penetrating and Stricturing Disease (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Penetrating & Stricturing n = 23 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Rs103284	AA, n = 11	5 (45.5)	1.275	.259	3.819	.051	5.46	.02[a]
	AG, n = 25	6 (36.0)						
	GG, n = 64	12 (18.8)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	n(%)	χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
	AA, n = 58	6 (10.3)	15.39	<.001[a]	0.766	.382	2.284	.131
	AT, n = 32	15 (46.9)						
	TT, n = 10	2 (20.0)						

[a]*P* < .05, indicating that the number of participants in the Crohn's disease group with penetrating and stricturing disease was significantly higher for those with the rs103284 genotype AA than for those with the AG or GG genotypes

Table 12. Association Between IL-8 Gene Polymorphisms and Disease Behavior for Participants in the Crohn's Disease Group With Nonpenetrating, Nonstricturing Disease (n = 100)

IL-8 Gene Polymorphism	Participants in Genotype	Nonpenetrating Nonstricturing n = 22 n (%)	Comparisons Between Genotypes					
			AA and AG Genotypes		AA and GG Genotypes		AG and GG Genotypes	
			χ2	*P* value	χ2	*P* value	χ2	*P* value
Rs103284	AA, n = 11	1 (9.0)	10.6	.001[a]	0.128	.727	38.02	<.001[a]
	AG, n = 25	17 (80.0)						
	GG, n = 64	4 (6.2)						
			AA and AT Genotypes		AA and TT Genotypes		AT and TT Genotypes	
Rs105432	Participants in Genotype	n (%)	χ2	*P* value	χ2	*P* value	χ2	*P* value
	AA, n = 58	14 (24.2)	4.514	.034	5.284	.022[a]	20.78	<.001[a]
	AT, n = 32	2 (6.2)						
	TT, n = 10	6 (60.0)						

[a]*P* < .05, indicating that the number of participants in the Crohn's disease group with nonpenetrating, nonstricturing disease was significantly higher for those with the rs103284 genotype AG than for those with the AA or GG genotypes and for those with the rs105432 genotype TT than for those with the AA or AT genotypes

significantly higher for participants with the rs103284 genotype GG than for those with the AA (*P* < .001) or AG (*P* < .001) genotypes. It also was significantly higher for participants with the rs105432 genotype AA than for those with the AT (*P* < .001) or TT (*P* < .001) genotypes.

Table 11 shows that the number of participants in the Crohn's disease group with penetrating and stricturing Crohn's disease was significantly higher for participants with the rs103284 genotype AA than for those with the AG (*P* < .001) or GG (*P* < .001) genotypes. It also was significantly higher for participants with the rs105432 genotype AT than for those with the AA (*P* < .001) or TT (*P* < .001) genotypes.

Table 12 shows that the number of participants in the Crohn's disease group with nonpenetrating, nonstricturing Crohn's disease was significantly higher for participants with the genotype AG than for those with the AA (*P* < .001) or GG

($P < .001$) genotypes. It also was significantly higher for participants with the rs105432 genotype TT than for those with the AA ($P < .001$) or AT ($P < .001$) genotypes.

DISCUSSION

The current study found that the protein expression level of IL-8 in the Crohn's disease group was significantly higher than that in the control group, suggesting that an increase in IL-8 may be a mechanism by which inflammation is activated in Crohn's disease. The current study also found that the frequency of certain genotypes and alleles for the IL-8 polymorphisms rs103284 and rs105432 were significantly higher for the Crohn's disease group than for the control group.

Specifically, the number of participants in the Crohn's disease group carrying the IL-8 rs103284 genotype TT and the G allele. Moreover, the disease's location and behavior were significantly different for participants in the Crohn's disease group with different genotypes.

The study had a few limitations. The study included only 100 patients with Crohn's disease and 100 healthy individuals. This small sample size may limit the generalizability of the findings. The study was conducted at a single hospital in China, which may limit the generalizability of the findings to other populations. The study was cross-sectional in nature and did not follow participants over time. And ,this limited the ability to determine causality and the potential impact of IL-8 gene polymorphisms on the development and progression of Crohn's disease. Also, the study only examined the association between IL-8 gene polymorphisms and Crohn's disease. Other genetic factors that may play a role in the development of Crohn's disease were not considered.

CONCLUSIONS

L-8 had a significantly increased expression in the colon tissues of the participants with Crohn's disease, and some genotypes and alleles for the gene polymorphisms rs103284 and rs105432 were significantly higher in the Crohn's disease group than in the control group. In addition, the disease's location and behavior was significantly different for participants in the Crohn's disease group with different genotypes.

AUTHORS' DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest related to the study.

REFERENCES

1. Hanauer SB, Hommes DW. Inflammatory bowel disease. *Expert Rev Clin Immunol*. 2010;6(4):499-500. doi:10.1586/eci.10.52
2. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology*. 1998;115(1):182-205. doi:10.1016/S0016-5085(98)70381-6
3. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol*. 2003;3(7):521-533. doi:10.1038/nri1132
4. Mizoguchi A, Mizoguchi E, Chiba C, et al. Cytokine imbalance and autoantibody production in T cell receptor-alpha mutant mice with inflammatory bowel disease. *J Exp Med*. 1996;183(3):847-856. doi:10.1084/jem.183.3.847
5. Cummings JR, Jewell DP. Clinical implications of inflammatory bowel disease genetics on phenotype. *Inflamm Bowel Dis*. 2005;11(1):56-61. doi:10.1097/00054725-200501000-00008
6. Naito T, Yokoyama N, Kakuta Y, et al. Clinical and genetic risk factors for decreased bone mineral density in Japanese patients with inflammatory bowel disease. *J Gastroenterol Hepatol*. 2018;33(11):1873-1881. doi:10.1111/jgh.14149
7. Van Limbergen J, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet*. 2009; 10(89-116. doi:10.1146/annurev-genom-082908-150013
8. Dinu I, Mahasirimongkol S, Liu Q, et al. SNP-SNP interactions discovered by logic regression explain Crohn's disease genetics. *PLoS One*. 2012;7(10):e43035. doi:10.1371/journal.pone.0043035
9. Sandhu BK, Fell JM, Beattie RM, Mitton SG, Wilson DC, Jenkins H. Guidelines for the management of inflammatory bowel disease in children in the United Kingdom. *J Pediatr Gastroenterol Nutr*. 2010; 50 Suppl 1(S1-S13. doi:10.1097/MPG.0b013e3181c92c53
10. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest*. 2007;117(3):514-521. doi:10.1172/JCI30587
11. Cho JH, Nicolae DL, Gold LH, et al. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA*. 1998;95(13):7502-7507. doi:10.1073/pnas.95.13.7502
12. Simeonov A, Nikiforov TT. Single nucleotide polymorphism genotyping using short, fluorescently labeled locked nucleic acid (LNA) probes and fluorescence polarization detection. *Nucleic Acids Res*. 2002;30(17):e91. doi:10.1093/nar/gnf090
13. Morino H, Kawarai T, Izumi Y, et al. A single nucleotide polymorphism of dopamine transporter gene is associated with Parkinson's disease. *Ann Neurol*. 2000;47(4):528-531. doi:10.1002/1531-8249(200004)47:4<528::AID-ANA20>3.0.CO;2-R
14. Odeh M. Role of cytokines in rheumatoid arthritis. *Drug News Perspect*. 1998;11(6):331-341. doi:10.1358/dnp.1998.11.6.661380
15. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol*. 2004; 22(929-979. doi:10.1146/annurev.immunol.22.012703.104622
16. Middleton J, Neil S, Wintle J, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell*. 1997;91(3):385-395. doi:10.1016/S0092-8674(00)80422-5
17. Ozkaramanli Gur D, Guzel S, Akyuz A, Alpsoy S, Guler N. The role of novel cytokines in inflammation: defining peripheral artery disease among patients with coronary artery disease. *Vasc Med*. 2018;23(5):428-436. doi:10.1177/1358863X18763096
18. Mitsuyama K, Toyonaga A, Sasaki E, et al. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clin Exp Immunol*. 1994;96(3):432-436. PMID:8004812 doi:10.1111/j.1365-2249.1994.tb06047.x
19. Grimm MC, Elsbury SK, Pavli P, Doe WF. Interleukin 8: cells of origin in inflammatory bowel disease. *Gut*. 1996;38(1):90-98. PMID:8566866 doi:10.1136/gut.38.1.90
20. Saes M, de Labio RW, Rasmussen LT, Payao S. Interleukin 8 (-251 T>A) polymorphism in children and teenagers infected with Helicobacter pylori. *J Venom Anim Toxins Incl Trop Dis*. 2017; 23(23. doi:10.1186/s40409-017-0113-z
21. Ouyang Q, Hu PJ, Qian JM, Zheng JJ, Hu RW. Chinese Cooperative Group For The Study On Ibd; Chinese Society Of Gastroenterology. Consensus on the management of inflammatory bowel disease in China in 2007. *J Dig Dis*. 2008;9(1):52-62. doi:10.1111/j.1443-9573.2007.00320.x