ORIGINAL ARTICLE

Toll-like receptor 9 gene mutations and polymorphisms in Japanese ulcerative colitis patients

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Abstract

AIM: To investigated gene mutations and polymorphisms of *TLR9* in Japanese ulcerative colitis (UC) patients.

METHODS: Three single nucleotide polymorphisms (SNPs) in *TLR9* were identified in healthy controls, and were assessed in 48 UC patients and 47 healthy controls. Control subjects were matched for age, sex and date of blood sampling from among a subgroup of participants.

RESULTS: *TLR9* -1486CC, 1174GG and 2848AA increase the risk of UC [odds ratio (OR) 2.64, 95% confidence interval (95% CI): 1.73-6.53, *P* = 0.042], and *TLR9* -1486TT, 1174AA and 2848GG decrease the risk of UC (OR 0.30, 95% CI: 0.10-0.94, *P* = 0.039), although there were no correlations between SNPs and disease phenotype or *TLR9* mRNA expression.

CONCLUSION: *TLR9* polymorphisms are associated with increased susceptibility to UC.

INTRODUCTION

Inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders of the digestive tract. Although the pathogenesis of IBDs is complex and remains unclear, it has been suggested that immunologic, environmental and genetic factors contribute to their etiology[1]. Both clinical studies of IBD and studies of animal colitis models implicate luminal bacteria as necessary for initiating and perpetuating intestinal inflammation[2,3].

Among several candidate IBD-related chromosomal regions and genes, the caspase recruitment domain 15 (*CARD15*) gene coding for the nucleotide oligomerization domain 2 (*NOD2*) gene was identified as having the strongest linkage for CD susceptibility[4,5]. *NOD2/CARD15* is an intracytoplasmic receptor that binds bacterial peptidoglycan-derived muramyl dipeptide. While three common variants of this gene (R702W, G908R and L1007fsinsC) have been reported in CD patients in Western countries, there were no variants found in Japanese patients[6].

Similarly to NOD2, Toll-like receptors (TLRs) are essential components of innate immunity that recognize microbial compounds from bacteria, fungi and viruses[7-9]. While TLR activation leads to transcription of inflammatory and immunoregulatory genes, recent studies have demonstrated that TLR signaling in intestinal sites can inhibit inflammatory responses and maintain colonic homeostasis[10-12]. *TLR9*, which recognizes unmethylated CpG DNA in bacteria and viruses[13,14], and its signaling pathway protect mice from experimental colitis through production of type I interferons (IFN)[15]. Therefore, we need to know whether *TLR9* is a protective molecule not only in a mouse model but also in human IBD.

As the relationships between UC and *TLR9* gene variation have not been reported to date, we focused on *TLR9* gene mutations or polymorphisms in UC. Our results demonstrate that *TLR9* genetic polymorphisms are associated with an increased risk of UC in the Japanese population.

MATERIALS AND METHODS

Human subjects

After obtaining written informed consent based on the Declaration of Helsinki of the World Medical Association, 48 UC patients and 47 healthy controls were enrolled in this study. All subjects were Japanese, and visited our hospital between December 2005 and January 2007. UC diagnoses were confirmed by review of medical charts by clinicians. Standard clinical, endoscopic and histological criteria were used[16-18]. The following clinical characteristics were analyzed: sex, age at diagnosis, disease location and disease severity. Control subjects were matched for age, sex and date of blood sampling from a subgroup of participants who were healthy volunteers, free from IBD and malignant tumors. Clinical characteristics of these subjects are shown in Table 1. This study was approved by the Ethics Committee of Fukushima Medical University.

Quantitative real-time reverse transcription-polymerase chain reaction

Peripheral blood mononuclear cells (PBMC) from UC patients and healthy controls were isolated from heparinized blood by Ficoll Paque density-gradient centrifugation (Lymphoprep, Axis-Shield PoC AS, Oslo, Norway). Total cellular RNA was extracted from isolated PBMC using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA).

cDNA was generated by using the HotStarTaq Master Mix Kit (QIAGEN GmbH, Hilden, Germany). Quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using a TaqMan® fast universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and on-demand gene-specific primers, assessed using StepOne™ real time PCR system (Applied Biosystems). The primers were as follows: *TLR9* (Hs00370913_s1) and *GAPDH* (Hs02786624_g1). Both primers were purchased from Applied Biosystems. Rela-

	UC (*n* = 48)	Controls (*n* = 47)
Age (yr)	43.49 ± 15.48	41.21 ± 15.36
Sex (M/F)	21 (43.8)/27 (56.2)	21 (44.7)/26 (55.3)
Age at diagnosis (yr)	35.07 ± 14.39	
Disease duration (yr)	8.42 ± 8.49	
Familial disease	0 (0)	0 (0)
Disease location		
Proctitis	3 (6.25)	
Left colitis	27 (56.3)	
Right colitis	1 (2.1)	
Pancolitis	17 (35.4)	
Disease severity		
Light	17 (35.4)	
Mild	18 (37.5)	
Severe	13 (27.1)	
History of colectomy	5 (10.4)	
History of immunosuppressant	6 (12.5)	
History of colon cancer	3 (6.3)	

Table 1 Basic subject data (mean ± SD) *n* (%)

UC: Ulcerative colitis.

tive quantification was achieved by normalizing to the values of the *GAPDH* gene.

SNP discovery

Genomic DNA from UC patients and healthy controls was extracted from heparinized whole blood samples using the PUREGENE® DNA purification kit (Gentra Systems, Minneapolis, MN, USA).

All exons, introns and approximately 1500 bases of the 5'-flanking region and 1500 bases of the 3'-flanking region of the *TLR9* gene were amplified from the genomic DNA of one healthy control. The genomic sequences were based on the GenBank reference sequence NM_017442 and AC097637.2 for *TLR9*. The subject was selected at random. Thirteen *TLR9* gene segments were amplified by PCR with the primer pairs shown in Table 2, *Taq* DNA polymerase (QIAGEN GmbH, Hilden, Germany) and genomic DNA from a healthy control as a template. Amplified products were purified using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences UK Limited, Buckinghamshire, UK), and were subjected to direct sequencing with the GenomeLab™ DTCS-Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) on a CEQ2000 DNA Sequencer (Beckman Coulter). SNP offsets were calculated relative to the A base of the *TLR9* ATG start codon, such that SNPs in the promoter region upstream of the first intron have negative position numbers.

Genotyping

Three common SNPs (-1486T/C, 1174A/G, 2848G/A) were analyzed for genotyping. The -1486T/C SNP was genotyped by restriction fragment length polymorphism (RFLP). Briefly, genomic DNA fragments containing -1486T/C SNP were amplified by PCR. PCR products were digested with the restriction enzyme Afl II (TAKARA SHUZO Co., Ltd., Shiga, Japan), run on a 3% aga-

Table 2 Primers used in polymerase chain reaction for genotyping *Toll-like receptor 9* polymorphisms and three single nucleotide polymorphisms

Fragment (product)	Forward	Reverse
TLR9 polymorphisms		
-2109 to -1293 (817 bp)	5'-CCAAGGGACTCTGGGAAAG-3'	5'-CATGTCACCCTCTCAACAGGG-3'
-1626 to -1095 (532 bp)	5'-CAGCCTTCACTCAGAAATACCC-3'	5'-GGCCAACAAGGCCCTATG-3'
-1296 to -553 (662 bp)	5'-CATGGGAGCAGAGACATAATG-3'	5'-GCCAGGGTGTAGCTTGA-3'
-795 to -134 (662 bp)	5'-GAGTCTCCTCACCTAGATCAG-3'	5'-TATACCAGCCTAGTAGC-3'
79 to 962 (884 bp)	5'-CTGCAAGCAACAGTGACGG-3'	5'-AGCTTTCACTTAACCAATCCC-3'
-393 to 1698 (2092 bp)	5'-TACCCGCTACTGGTGCTATC-3'	5'-TGGCAGAGTCTAGCATCAGG-3'
996 to 1687 (692 bp)	5'-CTGGTTCTGAAGCCTAATTC-3'	5'-AGCATGAGGATGTTGGTATGG-3'
976 to 1899 (924 bp)	5'-CTGGATTCTAGGTCTCAGTCC-3'	5'-CTGGATTCTAGGTCTCAGTCC-3'
1540 to 2368 (829 bp)	5'-CCTGCCACATGACCATCGAG-3'	5'-CCTGCCACATGACCATCGAG-3'
1812 to 2520 (709 bp)	5'-AACCTCACCCACCTGTCAC-3'	5'-AACCTCACCCACCTGTCAC-3'
2407 to 3350 (944 bp)	5'-TGCAGATGAACTTCATCAACC-3'	5'-GCTGTTGCAGCTGACATC-3'
3267 to 4057 (791 bp)	5'-CAGGAAACCAGCTGAAGG-3'	5'-CAGGAAACCAGCTGAAGG-3'
3867 to 4701 (835 bp)	5'-GACTGGGTGTACAACGAGCTT-3'	5'-TTCTGCATGGGAAAGGTAGG-3'
SNP		
-1486	5'-CAGCCTTCACTCAGAAATACCC-3'	5'-GGCCAACAAGGCCCTATG-3'
1174	5'-CTGGTTCTGAAGCCTAATTC-3'	5'-AGCATGAGGATGTTGGTATGG-3'
2848	5'-TGCAGATGAACTTCATCAACC-3'	5'-GCTGTTGCAGCTGACATC-3'

TLR9: Toll-like receptor 9; SNP: Single nucleotide polymorphism.

Table 3 Allele frequencies in ulcerative colitis cases and disease-free controls for *Toll-like receptor 9* single nucleotide polymorphisms at -1486, 1174, 2848

SNP[1]	Allele	UC (*n* = 48)	Controls (*n* = 47)	dbSNP[2]	JSNP[3]
-1486	T/C	0.344/0.656[a]	0.532/0.468	0.647/0.353	ND
1174	A/G	0.344/0.656[a]	0.532/0.468	0.512/0.488	0.517/0.483
2848	G/A	0.354/0.646[a]	0.543/0.457	0.481/0.519	0.517/0.483
					[a]*P* = 0.013

[1]Positions were calculated taking the A of the Toll-like receptor 9 (TLR9) ATG start codon as position 1 based on Genbank Accession No. NM_017442; [2]dbSNP public database: http://www.ncbi.nlm.nih.gov/SNP. [3]JSNP is a database of Japanese SNPs: http://snp.ims.u-tokyo. ac.jp/index_ja.html; [a]*P* value by Fisher's Exact test, cases *vs* controls. SNP: Single nucleotide polymorphisms; UC: Ulcerative colitis; ND: No data.

rose gel and subsequently stained with ethidium bromide to visualize the bands. The other two SNPs (1174A/G and 2848G/A) were confirmed by direct sequencing. The primers used for PCR or sequencing are shown in Table 2.

Statistical analysis

Statistical analyses were performed using SPSS version 11.0.1 and SNPAlyze version 6.0 (Dynacom Co., Ltd. Yokohama, Japan). Cases and controls were compared using Fisher's exact test (Tables 3-6) for categorical items. Simple logistic regression models were used to analyze Genotype-Diplotype associations. The results are expressed as odds ratio (OR) with corresponding 95% confidence intervals (95% CI). The level of significance was set at 5%. We examined Lewontin's D' (|D'|) and the linkage disequilibrium coefficient r^2 between each pair of SNPs using the expectation-maximization (EM) algorithm in SNPAlyze software.

RESULTS

TLR9 mRNA expression

In order to confirm whether there were differences in

TLR9 expression, we performed quantitative RT-PCR of *TLR9* using PBMC from UC patients and healthy controls. There were no significant differences in *TLR9* mRNA expression (Figure 1).

SNPs and their allele frequencies

We sequenced both exons and approximately 1500 bases of the 5'- and 3'-regions of *TLR9* in one healthy control. Offsets for each SNP were calculated relative to the transcription start site of *TLR9* mRNA (GenBank Accession No. NM_017442). We identified three SNPs in the regions sequenced. Their locations in chromosome 3 and in the *TLR9* gene are illustrated in Figure 2. These SNPs were confirmed in dbSNP (http://www.ncbi.nlm. nih.gov/SNP/) and JSNP (http://snp.ims.u-tokyo.ac.jp/ index_ja.html). The remainder of the analysis will focus on these three relatively common SNPs.

We found a marginally significant association between the SNPs at -1486, 1174, 2848 and UC. Elevated risk for UC was associated with a C allele at -1486 (Fisher's exact test, *P* = 0.013), a G allele at 1174 (Fisher's exact test, *P* = 0.013), and an A allele at 2848 (Fisher's exact test, *P* = 0.013) (Table 3).

Table 4 Genotype frequencies in ulcerative colitis cases and disease-free controls for *Toll-like receptor 9* single nucleotide polymorphisms at -1486, 1174, 2848 *n* (%)

Genotype at *TLR9*		UC (*n* = 48)	Controls (*n* = 47)
-1486	CC	20 (41.7)[a]	10 (21.3)
	CT	23 (47.9)	24 (51.1)
	TT	5 (10.4)[a]	13 (27.7)
1174	GG	20 (41.7)[a]	10 (21.3)
	GA	23 (47.9)	24 (51.1)
	AA	5 (10.4)[a]	13 (27.7)
2848	AA	19 (39.6)[a]	9 (19.1)
	AG	24 (51.1)	25 (53.2)
	GG	5 (10.4)[a]	13 (27.7)
			[a]P < 0.05

[a]P value by Fisher's exact test, ulcerative colitis (UC) cases *vs* controls. TLR9: Toll-like receptor 9.

Table 5 Haplotype frequencies in ulcerative colitis cases and disease-free controls for *Toll-like receptor 9* single nucleotide polymorphisms at -1486, 1174, 2848 *n* (%)

Haplotype			UC (*n* = 96)	Controls (*n* = 94)
-1486	1174	2848		
C	G	A	62 (64.6)[a]	43 (45.7)
T	A	G	33 (34.4)[a]	50 (53.2)
C	G	G	1 (1.04)	1 (1.06)
				[a]P < 0.05

[a]P value by Fisher's exact test, ulcerative colitis (UC) cases *vs* controls.

Table 6 Diplotype frequencies in ulcerative colitis cases and disease-free controls for *Toll-like receptor 9* single nucleotide polymorphisms at -1486, 1174, 2848 *n* (%)

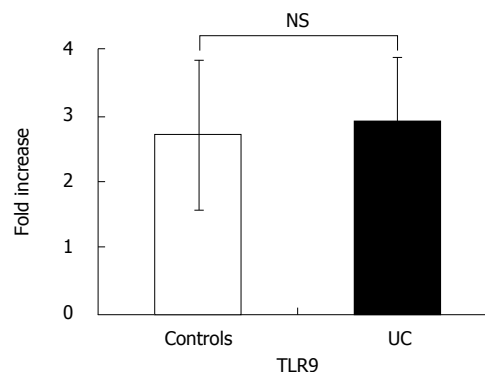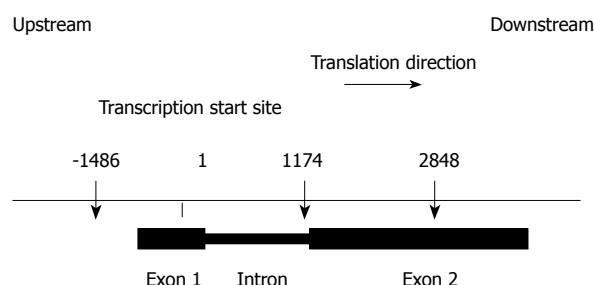| Diplotype			UC (*n* = 48)	Controls (*n* = 47)
-1486	1174	2848		
CC	GG	AA	19 (39.6)[a]	9 (19.1)
CC	GG	AG	1 (2.1)	1 (2.1)
CT	GA	AG	23 (47.9)	24 (51.1)
TT	AA	GG	5 (10.4)[a]	13 (27.7)
				[a]P < 0.05

[a]P value by Fisher's exact test, ulcerative colitis (UC) cases *vs* controls.

Genotype frequencies

We then analyzed genotype frequencies between UC patients and healthy controls. As shown in Table 4, a pattern of increased prevalence of CC genotypes at -1486 (Fisher's exact test, P = 0.047), GG genotypes at 1174 (Fisher's exact test, P = 0.047), AA genotypes at 2848 (Fisher's exact test, P = 0.042), and a decreased prevalence of TT at -1486 (Fisher's exact test, P = 0.039), AA genotypes at 1174 (Fisher's exact test, P = 0.039), GG genotypes at 2848 (Fisher's exact test, P = 0.039) was observed in UC patients, as compared with controls.

Pairwise linkage disequilibrium and estimation of haplotypes

We observed absolutely identical allele frequencies for the

Figure 1 *Toll-like receptor 9* mRNA expression in peripheral blood mononuclear cells from ulcerative colitis patients and controls. Total RNA was prepared from peripheral blood mononuclear cells and Toll-like receptor 9 (TLR9) mRNA expression was analyzed by quantitative reverse transcriptase-polymerase chain reaction. There were no significant differences between ulcerative colitis (UC) patients and controls. NS: No significance.

Figure 2 Gene structure and location of identified *Toll-like receptor 9* single nucleotide polymorphisms. We sequenced both exons and approximately 1500 bases of the 5'- and 3'-regions of *Toll-like receptor 9* in one healthy control. We identified three single nucleotide polymorphisms (-1486, 1174, 2848) in the regions sequenced.

Table 7 Linkage disequilibrium among single nucleotide polymorphisms of *Toll-like receptor 9* in ulcerative colitis cases and disease-free controls

SNPs		UC (*n* = 48)		Controls (*n* = 47)	
		|D'|	r^2	|D'|	r^2
-1486 T/C	1174 A/G	1	1	1	1
-1486 T/C	2848 G/A	1	0.9522	1	0.9581
1174 A/G	2848 G/A	1	0.9522	1	0.9581

Lewontin's D' (|D'|) and the linkage disequilibrium coefficient r^2 between each pair of single nucleotide polymorphisms (SNPs) were examined by the expectation-maximization algorithm of SNPAlyze software. UC: Ulcerative colitis.

three common SNPs. Table 7 shows that three SNPs are in strong linkage disequilibrium (LD) in both UC patients and healthy controls. We also inferred three SNP haplotypes by using an expectation-maximization algorithm. The number of statistically inferred haplotypes is eight (i.e. 2^3). A total of three of the eight possible haplotypes are observed in Table 5. The usage of haplotype C-G-A was more frequent in UC patients when compared with healthy controls, and the difference was statistically significant. (Fisher's exact test, P = 0.013, Table 5).
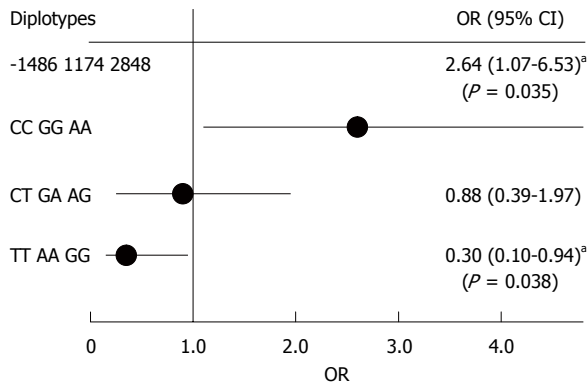
Diplotypes		OR (95% CI)
-1486 1174 2848		2.64 (1.07-6.53)[a]
		(*P* = 0.035)
CC GG AA		
CT GA AG		0.88 (0.39-1.97)
TT AA GG		0.30 (0.10-0.94)[a]
		(*P* = 0.038)

Figure 3 *Toll-like receptor 9* **gene diplotypes and disease sensitivity (expressed as odds ratios).** There was an increased risk of ulcerative colitis with the CCGGAA diplotype, and a decreased risk with the TTAAGG diplotype, as compared with controls. OR: Odds ratio; CI: Confidence interval. [a]*P* < 0.05.

Diplotype frequencies

We next analyzed diplotype frequencies as pairs of haplotypes. Table 6 shows the distribution of three diplotype loci by disease status; an increased prevalence of CCGGAA diplotypes (Fisher's exact test, *P* = 0.042) and a decreased prevalence of TTAAGG diplotypes (Fisher's exact test, *P* = 0.039) was noted among UC cases, as compared with controls. Thus, we found an increased UC risk among CCGGAA diplotypes (OR 2.64, 95% CI: 1.73-6.53, *P* = 0.042) and a decreased UC risk among TTAAGG diplotypes (OR 0.30, 95% CI: 0.10-0.94, *P* = 0.039) (Figure 3). These data suggest that CCGGAA diplotypes are more susceptible to UC.

Genetic association with clinical phenotypes

In order to further examine the contribution of genetic variations in *TLR9* to UC phenotypes, we analyzed various clinical characteristics among UC patients: sex, age at diagnosis, disease location and disease severity. However, we did not observe any statistically significant correlations between disease status and diplotype, genotype or specific *TLR9* allele.

DISCUSSION

In this study, we found that genetic variations of *TLR9* are associated with an increased risk of UC in the Japanese population. *TLR9*, which is activated by unmethylated CpG DNA, triggers innate immune responses[19,20]. Although no variants have been found in Japanese patients, the *NOD2/CARD15* gene was found to indicate CD susceptibility in Western populations[5,6], suggesting that abnormal innate immune responses toward luminal bacteria are involved in pathogenesis of IBD. With regard to TLRs, previous reports have indicated that *TLR4* Asp299Gly polymorphism is associated with CD and UC in Caucasian populations[21]. Török *et al*[22] investigated possible associations between genetic variations in *TLR9* and IBD in the German population, but did not detect any associations between *TLR9* gene variations and UC susceptibility. Thus, as UC is a heterogeneous polygenic disease, association studies

are expected to reveal various sets of susceptibility genes depending on the ethnic background of the study populations. Further genetic studies in different ethnic groups will resolve the role of the TLRs in UC susceptibility.

Associations between TLR9 and several autoimmune diseases or infectious diseases have been studied[23-26]. These reports have suggested that the -1237T/C SNP in *TLR9* is associated with an increased risk of allergic asthma[27], while 2848G/A was associated with myocardial infarction, deep vein thrombosis and chronic obstructive pulmonary disease[27], and 1635A/G was associated with HIV phenotype[28]. In addition, there were no associations between *TLR9* gene polymorphisms and susceptibility to systemic lupus erythematosus (SLE) and related phenotypes in Caucasian American subjects[23], but such an association was observed in a Japanese population[24]. These observations show that several candidate genes showing initial positive associations have generated negative findings in replication studies due to issues related to insufficient power or sample heterogeneity.

In this study, although relatively low numbers of subjects were enrolled, we observed statistically significant differences. According to the statistical methods used in this research, statistical differences in a category that has more than one subject indicate a reliable result. Moreover, we can expect to obtain the same results if more samples are added to the experiment. Therefore, we did not increase the power of this study, although more studies with large cohorts are necessary to characterize the role of *TLR9* SNPs in the etiology of UC.

We demonstrated that a C allele at -1486 (located in promoter region), a G allele at 1174 (located in intron 1) and an A allele at 2848 are associated with an increased risk of UC. SNPs in promoter regions potentially affect gene expression levels by altering the binding of gene transcription factors and SNPs in introns, thereby affecting mRNA splicing and/or enhancement of gene transcription. We were unable to identify down-regulation in *TLR9* expression in UC patients. Evaluating the relationship between the *TLR9* polymorphism and predefined clinical characteristics or biological markers also failed to demonstrate any impact on a particular UC phenotype. Taken together, these results indicate that *TLR9* polymorphism is associated with the development of disease itself, which is probably based on functional impairment of *TLR9*.

A recent study suggested that TLR9-triggered type-1 IFN protects mice from experimental colitis[15]. It also underscores the important protective role of type-1 IFN in intestinal homeostasis, suggesting that type-1 IFN produced by *TLR9* signaling affects the pathogenesis of IBD. Although further experiments are needed in order to identify the functional roles of *TLR9* polymorphism, either downstream molecules of the *TLR9* signaling pathway or cytokine production participating in the development of IBD is expected to be affected by these gene polymorphisms.

In conclusion, we identified an association between *TLR9* polymorphisms and UC in Japanese patients. Our findings indicate the importance of *TLR9* in the genetic

control of reactions to intestinal microbes in UC. Studying SNPs among the molecules involved in bacterial recognition will be essential to understanding the individual responses to bacterial components and to define the genetic background associated with risk of IBD.

ACKNOWLEDGMENTS

COMMENTS

Background

The pathogenesis of inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD), is multi-factorial, involving susceptibility genes as well as immunological and environmental factors. Recent studies on IBD have provided some evidence that commensal bacteria play a key role in the pathogenesis of the disease, and that the Toll-like receptor (TLR) signaling pathway activated by commensal bacteria plays an important role in maintaining colonic homeostasis. The signaling pathway of *TLR9*, which recognizes unmethylated CpG DNA in bacteria and viruses, protects mice from experimental colitis. As the relationships between UC and *TLR9* gene variation have not been reported to date, in this report we focused on *TLR9* gene mutations or polymorphisms in UC.

Research frontiers

Among several candidate IBD-related chromosomal regions and genes, the caspase recruitment domain 15 (*CARD15*) gene coding for the nucleotide oligomerization domain 2 (*NOD2*) gene was identified as having the strongest linkage for CD susceptibility. Similarly to NOD2, TLRs are essential components of innate immunity that recognize microbial compounds from bacteria, fungi and viruses. While TLR activation leads to transcription of inflammatory and immunoregulatory genes, recent studies have demonstrated that TLR signaling in intestinal sites can inhibit inflammatory responses and maintain colonic homeostasis. A gene variation in *NOD2/CARD15* has been reported in CD patients in Western countries, but this variation has not been identified in Japanese CD patients.

Innovations and breakthroughs

As the relationships between UC and *TLR9* gene variation have not been reported to date, in this report we focused on *TLR9* gene mutations or polymorphisms in UC. Three single nucleotide polymorphisms (SNPs) in *TLR9* were identified in healthy controls, and were assessed in 48 UC patients. The authors found that *TLR9* -1486CC, 1174GG and 2848AA increase the risk of UC, and *TLR9* -1486TT, 1174AA and 2848GG decrease the risk of UC.

Applications

The authors' findings indicate the importance of *TLR9* in the genetic control of reactions to intestinal microbes in UC. Studying SNPs among the molecules involved in bacterial recognition will be essential to understanding the individual responses to bacterial components and to define the genetic background associated with risk of IBD.

Terminology

TLR9 is a mammalian Toll-like receptor homologue that appears to function as an innate immune pattern recognition protein for motifs that are far more common in bacterial than in mammalian DNA.

Peer review

It is interesting that the authors identified an association between *TLR9* polymorphisms and UC in Japanese patients. My main concern is the sample size (not too large) and the fact that the P values obtained are not extremely significant (almost marginally significant on some occasions). This may limit the validity of the present work.

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