

Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis

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Abstract

Background—Recently, the association of a polymorphism in the gene coding for the anti-inflammatory cytokine interleukin-1 receptor antagonist with ulcerative colitis has been reported. This was interpreted as a possible genetic predisposition for severity of the inflammatory response.

Aims—To examine this polymorphism in a southern German population.

Subjects—The study included 234 healthy controls, 57 patients with ulcerative colitis, including 31 patients with pancolitis, 44 first degree healthy relatives of patients with ulcerative colitis, and 65 patients with Crohn's disease.

Methods—Genotypes were determined by a polymerase chain reaction amplification of the intron 2 fragment harbouring a variable number of tandem repeat nucleotide sequences. Amplification products were separated on a 2% agarose gel.

Results—The allele frequency for allele 2 was 27% in healthy controls, 28% in Crohn's disease, and 21% in patients with ulcerative colitis. The same allele frequency (21%) was found in a subgroup of patients with ulcerative colitis affecting the whole colon. Thus for allele 2 as well as for all other alleles, genotypes, or carriage rates no significant differences were found compared with controls. All allele frequencies in the control population were similar to those in earlier studies.

Conclusions—No association of a polymorphism in the interleukin-1 receptor antagonist gene with ulcerative colitis could be identified in this southern German population. The findings of an earlier study reporting an increased frequency of allele 2, particularly in patients with pancolitis, could not be confirmed.

(Gut 1997; 40: 623-627)

Keywords: cytokine, interleukin-1 receptor antagonist, polymorphism, inflammatory bowel disease, ulcerative colitis, genetics.

Clear evidence exists for a genetic predisposition to inflammatory bowel disease. The prevalence of ulcerative colitis and Crohn's disease is different between distinct ethnic groups.¹ Relatives of patients with ulcerative colitis and Crohn's disease have an increased

risk for developing inflammatory bowel disease.²⁻⁵ Furthermore, twin studies have provided evidence for genetic factors, particularly in Crohn's disease.⁶ Genetic markers for inflammatory bowel disease have been identified in the major histocompatibility complex.⁷ An association between the HLA-DR2 allele and ulcerative colitis has been described.⁸ In Crohn's disease the haplotype HLA-DR1 DQ_{wp5} was found with increased frequency.⁸

Interleukin-1 is a cytokine with potent pro-inflammatory properties and plays a central part in various inflammatory diseases.⁹⁻¹¹ It is the only cytokine for which a physiological antagonist – interleukin-1 receptor antagonist – has been identified.¹²⁻¹⁷ The results of a study by Casini *et al* point to a role for the balance between the proinflammatory cytokine interleukin-1 and its receptor antagonist in inflammatory bowel disease.¹⁸

A polymorphism in the gene coding for interleukin-1 receptor antagonist has been described¹⁹ (Table I). In the second intron of this gene five alleles are defined by different numbers (two to six) of repeats of a 86 bp segment (variable number of tandem repeats). Recently, an association between this polymorphism and ulcerative colitis has been described.²⁰ An increase in the frequency of allele 2 was reported in patients with ulcerative colitis, especially in those with involvement of the entire colon. This was the first observed association of ulcerative colitis with a gene outside the major histocompatibility complex. There are interindividually stable differences in the production of certain cytokines.^{21, 22} The genotype of the polymorphism in the interleukin-1 receptor antagonist gene may influence its production, as possible binding sites for transcription factors have been characterised in this region.¹⁹ Recently, an influence of the allele status on the production of interleukin-1

TABLE I Polymorphism of the interleukin-1 receptor antagonist gene

Name of allele	Number of repeats (R)	PCR product: number of base pairs
A1	RRRR (4)	410
A2	RR (2)	240
A3	RRRRR (5)	500
A4	RRR (3)	325
A5	RRRRRR (6)	595

Five alleles can be distinguished in intron 2 of the interleukin-1 receptor antagonist gene polymorphism. The table shows the nomenclature, the number of repeats found in these alleles, and the size of the products by PCR amplification.

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Accepted for publication
23 December 1996

receptor antagonist was noted. Allele 2 of the polymorphism was associated with an increased production of interleukin-1 receptor antagonist protein in healthy humans when mononuclear cells were stimulated with GM-CSF.²³ It was speculated that a genetic influence – caused by the polymorphism in the interleukin-1 receptor antagonist gene – on the production of interleukin-1 receptor antagonist could contribute to the mechanism of inflammatory disease. In the present study we attempted to confirm the recently described²⁰ association of the polymorphism in the interleukin-1 receptor antagonist gene in a large southern German patient population with ulcerative colitis.

Methods

PATIENTS

The study comprised 234 unrelated healthy volunteers, 57 patients with ulcerative colitis, and 65 patients with Crohn's disease. Furthermore, 44 first degree relatives of patients with ulcerative colitis were studied. The diagnosis and extent of disease had been determined by radiological, endoscopic, and histological criteria (Table II). Informed consent was obtained from all patients.

TABLE II Patient characteristics

	Ulcerative colitis	Crohn's disease
Total number (n)	57	65
Sex F/M (n)	31/26	47/18
Age (years):		
(mean (SD))	39.9 (11.9)	35.1 (10.5)
(median/range)	40/17–66	33/19–69
Age at onset of symptoms:		
(mean (SD))	31.0 (11.8)	23.7 (6.9)
(median)	29	22
Duration of symptoms:		
(mean (SD))	9.2 (6.3)	11.5 (7.9)
(median)	8	9
Site of disease:		
Small bowel involved	0	53
Terminal ileum	0	48
Other small bowel	0	5
Colon involved	57	55
Right colon	0	20
Left colon	8	19
Distal colon	16	4
Rectum	2	3
Total colon	31	9

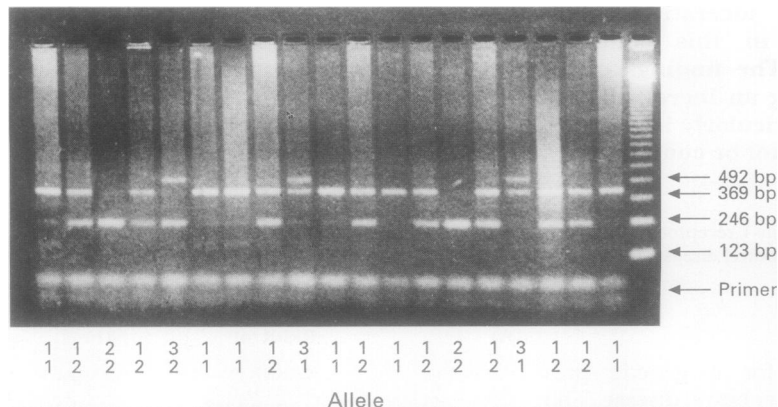


Figure 1: Agarose gel (2%) stained with ethidium bromide. PCR products (8 μ l) of 19 unrelated, healthy controls were separated by electrophoresis. The marker ladder ranges from 123 to 4182 base pairs (bp) in steps of 123 bp. In the samples fragments of 240 bp, 410 bp, and 500 bp can be identified, which represent alleles 2, 1, and 3 respectively. The deduced genotypes are indicated below each lane.

ISOLATION OF DNA

Genomic DNA was extracted from 10 ml peripheral blood collected into tubes containing ethylenediaminetetra-acetic acid (EDTA), using a salting out procedure described elsewhere.²⁴ The DNA samples of the patient groups in this study have not been previously investigated.

POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) was used to identify the genotypic status of the polymorphism in intron 2 of the interleukin-1 receptor antagonist gene.²⁵ Negative controls were included within each of the reactions. Approximately 250 ng DNA was subjected to PCR amplification of the 86 bp tandem repeat polymorphism in a GeneAmp PCR System 9600 (Perkin Elmer, Weiterstadt, Germany). Primers as described by Tarlow *et al*¹⁹ (5'CTC-AGCAACTCCTAT3' and 5'TCCTG-GTCTGCAGGTAA3' (Pharmacia Biotech Europe GmbH, Freiburg, Germany)) were used at a final concentration of 1 μ M each. PCR Buffer II 10 \times (Perkin Elmer, Weiterstadt, Germany) contained 100 mM Tris-HCl at pH 8.3 (25°C) and 500 mM KCl. The final MgCl₂ concentration in the PCR reaction was 3 mM. Dimethyl sulphoxide (DMSO; 2%; Sigma, Munich, Germany) was added. The reaction volume was 20 μ l. The incubation parameters were: first denaturation at 94°C for three minutes followed by 94°C (one minute), 65°C (one minute), and 72°C (one minute) for two cycles, 94°C (one minute), 63°C (one minute), and 72°C (one minute) for two cycles and 94°C (one minute), 60°C (one minute), and 72°C (one minute) for 30 cycles. The final extension was carried out at 72°C for five minutes. The PCR product was separated by electrophoresis on a 2% agarose gel stained with ethidium bromide. A molecular ladder in steps of 123 bp ranging from 123 to 4182 bp (Gibco BRL, Berlin, Germany) was used to determine the size of the PCR products (Fig 1).

STATISTICS

The allele frequencies and carriage rates were calculated from the numbers of genotypes. For statistical analyses the χ^2 test was performed on allele frequencies, and genotypes and carriage rates.

Results

In healthy controls (n=234) allele frequencies were 69% for allele 1, 27% for allele 2, and 4% for the sum of alleles 3, 4, and 5 (Table III). In patients with ulcerative colitis the frequency of allele 2 was decreased compared with the controls (21% *v* 27%) as was the carriage rate of allele 2 (39% *v* 49%). The frequency of allele 1 was increased (77% *v* 69%) compared with controls (Fig 2). Subdividing the patients with ulcerative colitis by extent of disease, the frequency of allele 2 was 22% in disease affecting the distal colon, 19% in disease affecting the left hemicolon, and 21% in

TABLE III Genotype numbers in patients and controls (total numbers) and calculated allele frequencies (%)

	Genotypes (n)							Alleles (%)		
	1,1	1,2	2,2	1,3	2,3	1,5	1,4	1	2	3, 4, or 5
Healthy controls (n=234)	107	97	13	10	4	2	1	69	27	4
Crohn's disease (n=65)	35	24	6	0	0	0	0	72	28	0
Ulcerative colitis (n=57)	33	20	2	2	0	0	0	77	21	2
Relatives of patients with ulcerative colitis (n=44)	21	16	6	1	0	0	0	67	32	1

patients with an involvement of the entire colon (Fig 3). The allele frequencies, genotype frequencies, and carriage rates showed no significant differences in these subgroups of patients with ulcerative colitis compared with controls. Also in the first degree healthy relatives of patients with ulcerative colitis, allele and genotype frequencies were similar to controls (Table III).

In patients with Crohn's disease the allele frequencies were 72% for allele 1 and 28% for allele 2 (Table III). A carriage rate for allele 2 of 46% compared with 49% in controls was calculated. Using the χ^2 test no significant differences in genotype frequencies, allele frequencies, or carriage rates could be found in patients with Crohn's disease compared with

healthy controls. This was in accordance with previous findings.²⁰

Thus in this study no significant association between a polymorphism in the interleukin-1 receptor antagonist gene and either ulcerative colitis or Crohn's disease could be identified. In particular we found no increase in the frequency of allele 2 in the subgroup of 31 patients with pancolitis, compared with the 20 patients in the study by Mansfield *et al.*²⁰ The genotype frequencies of all groups (healthy controls, patients with Crohn's disease, patients with ulcerative colitis, and first degree healthy relatives of patients with ulcerative colitis) were consistent with Hardy-Weinberg equilibrium.

Discussion

The present study found a decreased allele frequency and carriage rate of allele 2 of the interleukin-1 receptor antagonist polymorphism in 57 patients with ulcerative colitis compared with 234 healthy controls. This corresponded with an increased allele frequency and carriage rate of allele 1 in this patient group. The differences determined, however, did not reach significance. In the control group, genotype and allele frequencies were similar to those described in other healthy controls.^{19, 20} In 65 patients with Crohn's disease allele frequencies were almost equal to those in the healthy control group.

In the study of Mansfield *et al* the uncommon allele 2 of this polymorphism in the interleukin-1 receptor antagonist gene was more frequent in patients with ulcerative colitis than in healthy controls.²⁰ This increase could be attributed entirely to the subgroup of 20 patients with pancolitis, in which the frequency of allele 2 was 52.5%.

Our present results confirm the findings of Mansfield *et al*,²⁰ firstly, for healthy controls and, secondly, for patients with Crohn's disease, in whom no correlation with any allele of this interleukin-1 receptor antagonist polymorphism was found. By contrast, our results were different for patients with ulcerative colitis. In particular, in the subgroup with ulcerative colitis affecting the whole colon, we found an allele 2 frequency of 21%, which was not different from that in all patients with ulcerative colitis (21%) and was decreased (non-significantly) compared with healthy controls (27%). Methodical reasons are unlikely to be responsible for the differences, because the results found in the healthy controls were similar in both studies. These findings raise the question whether the association may only be found in distinct ethnic groups and may be absent in others. It has been shown recently that the association of allele 2 of the interleukin-1 receptor antagonist polymorphism with ulcerative colitis was only detectable in a Jewish subgroup of a patient population in Los Angeles, but not in the whole study cohort.²⁰ Furthermore, in preliminary reports, the allele 2 frequency was not different from control groups in a Spanish and a Dutch population of patients with ulcerative

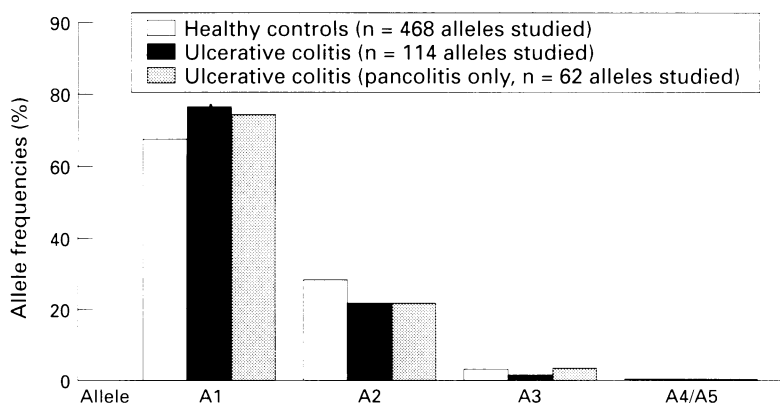


Figure 2: Allele frequencies of the interleukin-1 receptor antagonist polymorphism in patients with ulcerative colitis and in the subgroup with pancolitis in comparison with healthy controls.

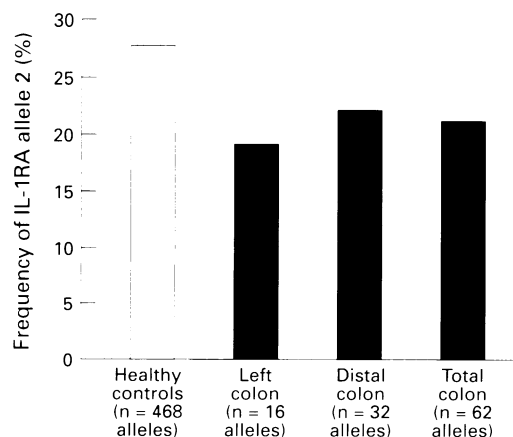


Figure 3: Allele frequencies of the interleukin-1 receptor antagonist (IL-1RA) gene polymorphism in the subgroups of patients with ulcerative colitis determined by site of disease.

colitis.²⁷ In view of the data, it seems unlikely that an association of the intron 2 interleukin-1 receptor antagonist polymorphism with ulcerative colitis exists in the general population of patients with this disease.

Both in peripheral blood and in mucosal biopsy samples of patients with inflammatory bowel disease increased amounts of interleukin-1 and interleukin-1 receptor antagonist have been measured.²⁸⁻³¹ In a study by Casini *et al* the ratio of interleukin-1 receptor antagonist versus interleukin-1 was decreased in the mucosa of both ulcerative colitis and Crohn's disease but not in self limiting colitis.¹⁸ It was hypothesised that defective interleukin-1 receptor antagonist production could explain disease reactivation and chronicity.³² In animal models neutralisation of endogenous interleukin-1 receptor antagonist with specific antibodies led to exacerbation and prolongation of inflammation.³³ Furthermore, recombinant interleukin-1 receptor antagonist has been successfully applied in animal models of inflammatory bowel disease.^{34 35}

The influence of the polymorphism investigated here on production of interleukin-1 receptor antagonist protein has recently been studied.²³ After stimulation with GM-CSF, mononuclear cells from healthy persons carrying allele 2 in one or two copies produced more interleukin-1 receptor antagonist and less proinflammatory interleukin-1 α compared with those carrying one or no copies of allele 2.²³ From these data it would not be expected that carriage of allele 2 would confer increased susceptibility to an inflammatory response. By contrast, a recent presentation described decreased levels of interleukin-1 receptor antagonist in inflamed bowel mucosa of carriers of allele 2 compared with non-carriers of this allele.³⁶ Together, the published data on the influence of this polymorphism on production of interleukin-1 receptor antagonist protein are not yet conclusive.

In summary, we found no association between ulcerative colitis and the intron 2 polymorphism in the gene coding for the interleukin-1 receptor antagonist in a southern German group of patients. It seems unlikely that allele 2 of this polymorphism represents a marker for ulcerative colitis in the general population.

We thank Dr Gunther Hartmann, Dr Jochen Moeller, Désirée Zarskyj, Uta Emmerich, Anne Krug, and Britta Siegmund, for most helpful discussions. The experimental data of this study are part of the dissertation of C Bidlingmaier (Medizinische Fakultät der Ludwig-Maximilians-Universität München, in preparation). The work was supported by grant En 169-2/3 and SFB 217 from the Deutsche Forschungsgemeinschaft.

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