Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease
Stokkers, P.C.F.; van Aken, B.E.; Basoski, N.M.; Reitsma, P.H.; Tytgat, G.N.J.; van Deventer, S.J.H.

Published in:
Gut

DOI:
10.1136/gut.43.1.33

Link to publication

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease

P C F Stokkers, B E van Aken, N Basoski, P H Reitsma, G N J Tytgat and S J H van Deventer

Gut 1998;43:33-39

Updated information and services can be found at:
http://gut.bmj.com/cgi/content/full/43/1/33

These include:

References
This article cites 40 articles, 12 of which can be accessed free at:
http://gut.bmj.com/cgi/content/full/43/1/33#BIBL

6 online articles that cite this article can be accessed at:
http://gut.bmj.com/cgi/content/full/43/1/33#otherarticles

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

To order reprints of this article go to:
http://www.bmjjournals.com/cgi/reprintform

To subscribe to Gut go to:
http://www.bmjjournals.com/subscriptions/
Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease

P C F Stokkers, B E van Aken, N Basoski, P H Reitsma, G N J Tytgat, S J H van Deventer

Abstract

Background—An imbalance between the proinflammatory cytokine interleukin 1β (IL-1β) and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra) has been postulated as a pathogenic factor in inflammatory bowel disease (IBD).

Aims—To study allelic frequencies of novel polymorphisms in the genes for IL-1β and IL-1ra in patients with IBD and to assess the relation between ex vivo cytokine production and allelic variants of the IL-1β and IL-1ra genes.

Subjects—Two hundred and seventy healthy controls, 74 patients with ulcerative colitis (UC), 72 with Crohn’s disease (CD), 40 with primary sclerosing cholangitis for the allelic frequencies, and 60 healthy individuals for the ex vivo stimulation test.

Methods—Genotyping was performed by polymerase chain reaction and subsequent cleavage with specific endonucleases (Mwo1, MspAI1, Alu1, Taq1, BsoF1) for five novel restriction fragment length polymorphisms (RFLPs) in the genes for IL-1ra and IL-1β.

Results—No significant differences were found in the allelic frequencies or allele carriage rates of the markers in the IL-1β and IL-1ra genes between CD, UC, and healthy controls. No association between the genetic markers and cytokine production levels was observed. Patients with UC carried the combination of both the infrequent allele of the Taq1 RFLP and the Mwo1 RFLP significantly more frequently (35.2% in UC versus 71.1% in controls).

Conclusions—UC is associated with carriage of both infrequent alleles of the Taq1 and Mwo1 RFLPs. However, it could not be confirmed whether the association reflects a pathogenic mechanism underlying UC.

(Gut 1998;43:33–39)

Keywords: cytokine gene polymorphisms; interleukin 1 receptor antagonist; interleukin 1β; Crohn’s disease; ulcerative colitis

The genetic background of inflammatory bowel disease (IBD) has been intensively studied in recent years. In particular genes on chromosome 6, including the HLA class 2 genes and the genes for tumour necrosis factor (TNF) α and β have been studied as candidate genes. These efforts have yielded several associations between alleles of these genes and either IBD, ulcerative colitis (UC), or Crohn’s disease (CD).1,2 Recent linkage analyses have confirmed a role for the HLA region in UC but not CD.1,4 However, an extended haplotype of the TNF genes on chromosome 6 has been associated with CD.5 Recently genome wide screenings have yielded susceptibility loci for IBD on chromosomes 3, 7, and 12 and for CD on chromosome 16.6–8

The interleukin 1 (IL-1) gene cluster on chromosome 2 has received particular attention as a candidate locus for a role in the pathogenesis of IBD. IL-1β is a potent proinflammatory cytokine6 and IL-1 receptor antagonist (IL-1ra) is a naturally occurring antagonist; both are expressed in the mucosa of patients with IBD.9–12 An imbalance between mucosal levels of IL-1β and IL-1ra in patients with IBD has been claimed to play a role in the pathogenesis of IBD.13 This imbalance may very well be under genetic control of the genes encoding these cytokines. Indeed, an association between UC and a variable number of tandem repeats (VNTR) in intron 2 of the IL-1ra gene (IL-1RN) has been reported16 although others did not completely confirm this finding.17–21 Additionally, the production rates of the proinflammatory cytokine IL-1β and anti-inflammatory cytokine IL-1ra seem to be genetically determined and are associated with genetic markers.22–24

The genes of IL-1α (IL1A), IL-1β (IL1B), and IL-1ra (IL1RN) are located close to each other on chromosome 2q12–21 and their genomic sequence is known.25–31 Recently several novel restriction fragment length polymorphisms (RFLPs) in IL1B and IL1RN have been reported that are more informative than the previously recognised polymorphisms.32

Our aim was to determine the allelic frequencies for these novel polymorphisms in a panel of patients with IBD from the department of gastroenterology and in healthy controls. We also investigated the relation between the genetic markers and cytokine production in healthy volunteers by means of ex vivo whole blood stimulation assays.

Materials and methods

Patients

Blood samples were obtained from 273 healthy individuals recruited among the employees of our hospital, and from 75 patients with UC, 74 with CD, and 40 with primary sclerosing cholangitis (PSC). Twenty seven patients with PSC had concomitant UC, and 10 had concomitant CD. The patients with UC (41 men, 34 women) had a median age of 38.4 years, with a median age of disease onset of 28.9 years, and a median disease duration of 9.1 years. The patients with CD (19 men, 55
women) had a median age of 38.6 years, a median age of disease onset of 27.7 years, and a median disease duration of 10.9 years. The patients with PSC (23 men, 17 women) had a median age of 45.2 years (median age of onset and disease duration are not known for this group). For all patients with IBD the family history for IBD was negative. All patients were unrelated inhabitants of the Amsterdam area. The diagnosis of IBD had been made prior to the study by conventional radiological, endoscopic, and histological criteria. Table 1 presents details of disease extent.

DNA ISOLATION
White blood cells were isolated from 10 ml EDTA blood samples followed by sodium dodecyl sulphate (SDS)/protease K digestion. DNA extraction was performed by means of phenol/high salt extraction and ethanol precipitation. The DNA was resuspended in TE (Tris/EDTA) buffer (pH 8) and the samples were stored at 4°C.

POLYMORPHISM SCREENING
For all polymorphisms the polymerase reaction was used followed by digestion with specific enzymes as described previously. All reactions were performed blinded with respect to patient status.

IL-1β gene
Two novel polymorphisms were studied in addition to the previously reported TaqI polymorphism in the IL-1β gene (IL1B)—that is, a BosF1 RFLP in intron 4 and an Alu1 RFLP in the promoter region of the gene.

IL-1ra gene
We recently reported four novel polymorphisms in the IL-1ra gene (IL1RN): an MspI RFLP in exon 2, an Mwo1 RFLP in intron 2, an SspI RFLP in intron 3, and an MspAI RFLP in exon 4. The first three polymorphisms and the VNTR in intron 2 are in tight linkage disequilibrium (fig 1). We therefore selected the MspAI and Mwo1 polymorphisms for further study.

WHOLE BLOOD STIMULATION
To limit circadian variation in cytokine production, blood from the 60 healthy participants was obtained between 09 00 and 10 00 am. Blood was collected into pyrogen free tubes (Sarstedt, Numbrecht, Germany), containing pyrogen free heparin (Thromboline, Organon, Oss, The Netherlands; final concentration 30 IU/ml). Heparinised blood aliquots (1 ml) were diluted 1/1 in Hank’s buffered saline solution (Biowhittaker, Heidelberg, Germany) in pyrogen free tubes (Falcon, Becton Dickinson, Mountain View, California, USA) and incubated with Escherichia coli 0111:B4 lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Michigan, USA) at a final concentration of 10 ng/ml. After 24 hours incubation at 37°C in a CO2 incubator, plasma was prepared by centrifugation at 800 g for 10 minutes at 4°C. Samples were diluted in enzyme linked immunosorbent assay (ELISA) dilution buffer (CLB, Amsterdam, The Netherlands), and analysed in parallel for IL-1β and IL-1ra. A response time curve showed that after 8 to 12 hours’ stimulation with LPS the IL-1β and IL-1ra response reaches a plateau which remains unchanged for 24 hours, in accordance with findings previously reported.

IL-1β was determined by ELISA using mouse antihuman IL-1β (58.121.08, Biosource, Brussels, Belgium) as capture antibody. The standard curve was prepared from recombinant human IL-1β (58.121.10, Biosource); biotinylated mouse antihuman IL-1β (58.121.03, Biosource) was used as the detection antibody and was conjugated with HRP-streptavidin (CLB).

IL-1ra was assayed by ELISA using mouse antihuman IL-1ra (Antibody Solutions, SARL Illkirch, France). The standard curve was prepared from recombinant human IL-1RA (280-RA-101, R&D Systems Europe, Abington, UK); biotinylated goat antihuman IL-1ra (BAF280, R&D) was used as the detection antibody.

For leucocyte counts, blood was drawn into EDTA(K3) tubes, and numbers were assessed routinely with the use of a flow cytometer (Technicon H1 system, Technicon Instruments, Tarrytown, USA).

STATISTICS
The results are presented as genotypes and allele frequencies for each polymorphism. χ2 Tests for statistical significance were performed on genotype frequencies. The significance was assessed with the CLUMP computer program, which uses a Monte Carlo approach. The CLUMP program performs repeated simulations to generate tables having the same totals as the tested results, and counts the number of times that the found χ2 value is achieved by the randomly simulated data. This method has the advantage that a Bonferroni correction is not necessary. Carriers of both infrequent alleles of the two RFLPs were defined as those patients that were heterozygous for at least both infrequent alleles. Risk estimates were made on 2×2 contingency tables and 95% confidence intervals (CI) were calculated for the different disease classifications in carriers of specific alleles.

Statistical analysis of the cytokine production in relation to the different genotypes was done by linear regression and one way analysis of variance, assuming a normal distribution, after performing a Shapiro and Wilk test and removing outlying values. A t test was used for the comparison of cytokine production between two genotypes. Probability values from
this test were corrected for multiple comparisons by multiplying the p value by the number of comparisons that were made (Bonferroni correction). Calculation of confidence intervals for the cytokine production assays was done by grouping the tested persons in allele 1 homozygotes and allele 2 carriers and subsequent calculation of the mean difference and standard error of the mean difference. Unless otherwise indicated data are presented as mean (SEM).

**Results**

**Allele frequencies of the RFLPs in relation to UC, CD, and PSC**

Tables 2 and 3 respectively present the allele frequencies of the Taq1 polymorphism in IL-1B and the Mwo1 polymorphism in IL-1RN (data on other RFLPs not shown). We were particularly interested in carriage rates of both the infrequent allele of the Mwo1 RFLP and the infrequent allele of the Taq1 RFLP, as these RFLPs are reported to be linked with cytokine production. Carriage of both infrequent alleles seemed to be associated with UC, yielding a relative risk of 2.6 (95% CI 1.33 to 5.18) (fig 2). All combinations between the other markers in IL1B and IL-1RN were tested, but no associations with the disease groups were found (data not shown).

**GENETIC MARKERS IN RELATION TO CYTOKINE PRODUCTION**

No relation was found between IL-1B production and the genotypes of the BsoF1 RFLP (1/1: 1.6 (0.2), 1/2: 1.2 (0.1), 2/2: 2.6 (0.6) ng/10⁶ cells, p=0.33; see fig 3B) or for the genotypes of the Alu1 RFLP (1/1: 1.6 (0.2), 1/2: 1.4 (0.2), 2/2: 1.6 (0.4) ng/10⁶ cells, p=0.69; see fig 3C) following ex vivo stimulation of whole blood with LPS. There was also no relation between IL-1ra levels and the genotypes of the MspA1 RFLP (1/1: 6.3 (0.5), 1/2: 6.3 (0.6), 2/2: 9.1 (1.7) ng/10⁶ cells,

### Table 2 Taq1 restriction fragment length polymorphism in the interleukin-1β gene: genotype frequencies, allele frequencies and carriage rates in IBD

<table>
<thead>
<tr>
<th>Taq1</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
<th>Carriage rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1</td>
<td>1/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Healthy (n=256)</td>
<td>57.8</td>
<td>35.9</td>
<td>6.3</td>
</tr>
<tr>
<td>CD (n=64)</td>
<td>72.3</td>
<td>23.1</td>
<td>4.6</td>
</tr>
<tr>
<td>CD+PSC (n=74)</td>
<td>70.3</td>
<td>25.7</td>
<td>4.1</td>
</tr>
<tr>
<td>UC (n=54)</td>
<td>42.6</td>
<td>50.0</td>
<td>7.4</td>
</tr>
<tr>
<td>UC+PSC (n=74)</td>
<td>48.6</td>
<td>45.9</td>
<td>5.4</td>
</tr>
<tr>
<td>PSC (n=40)</td>
<td>65.0</td>
<td>32.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Disease group v controls.
CD, Crohn’s disease; UC, ulcerative colitis; PSC, primary sclerosing cholangitis.*
Furthermore, the reported relation between the Taq1 RFLP genotypes and IL-1β production (1/1: 1.5 (0.2), 1/2: 1.6 (0.2), 2/2: 0.9 (0.4) ng/10⁶ cells, p=0.50; see fig 3A) or Mwo1 RFLP genotypes and IL-1ra production (1/1: 6.3 (0.5), 1/2: 7.0 (0.6), 2/2: 7.3 (0.6) ng/10⁶ cells, p=0.34; see fig 4B) could not be confirmed.

Homozygotes for allele 2 of the BsoF1 RFLP tended to have higher IL-1β levels (fig 3B), and homozygotes for the MspA1 RFLP, and Mwo2 tended to have higher IL-1ra levels (fig 4A and B), but this did not reach significance. Between carriers of allele 1 or allele 2 of the Alu1 RFLP the mean difference in IL-1β production was 0.19 (0.27) ng/10⁶ cells (CI −0.35 to 0.73 ng/10⁶ cells). Numbers for the BsoF1 RFLP and Taq1 RFLP were 0.07 (0.27) (CI −0.61 to 0.47) ng/10⁶ cells and 0.06 (0.27) (CI −0.48 to 0.60) ng/10⁶ cells respectively. Between carriers of allele 1 or allele 2 the mean difference in IL-1ra production was 0.71 (0.79) (CI −0.87 to 2.3) ng/10⁶ cells higher for the MspA1 RFLP and 0.72 (0.76) (CI −0.80 to 2.2) ng/10⁶ cells higher for the Mwo1 RFLP. Additionally we were interested whether the carriage of both infrequent alleles of the Taq1 and Mwo1 RFLPs, that are associated with UC, was reflected in the ratio between IL-1β and IL-1ra production. Although carriers of both infrequent alleles of these RFLPs had a lower ratio of IL-1ra and IL-1β production levels than non-carriers of these alleles, this difference did not reach significance (p=0.08) (fig 5).

### Table 3  Mwo1 restriction fragment length polymorphism in the interleukin-1 receptor antagonist gene: genotype frequencies, allele frequencies and carriage rates in IBD

<table>
<thead>
<tr>
<th>Mwo1</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
<th>Carriage rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1</td>
<td>1/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Healthy (n=270)</td>
<td>51.5</td>
<td>38.9</td>
<td>9.6</td>
</tr>
<tr>
<td>CD (n=72)</td>
<td>41.3</td>
<td>47.6</td>
<td>11.1</td>
</tr>
<tr>
<td>CD+PSC (n=72)</td>
<td>45.8</td>
<td>44.4</td>
<td>9.7</td>
</tr>
<tr>
<td>UC (n=55)</td>
<td>49.1</td>
<td>47.3</td>
<td>3.6</td>
</tr>
<tr>
<td>UC+PSC (n=75)</td>
<td>44.0</td>
<td>50.7</td>
<td>5.3</td>
</tr>
<tr>
<td>PSC (n=40)</td>
<td>50.0</td>
<td>45.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Disease group v controls.
CD, Crohn’s disease; UC, ulcerative colitis; PSC, primary sclerosing cholangitis.
is a competitive inhibitor of IL-1 that binds to the IL-1 receptor but does not elicit post-receptor signalling. An imbalance between IL-1 and IL-1ra may cause a proinflammatory state and has been suggested to underlie the mucosal inflammation found in IBD.

We have used novel genetic markers to investigate whether IL-1B or IL-1RN determine genetic susceptibility to IBD, either alone or in conjunction. The Mwo1 RFLP, one of these markers, is in tight linkage disequilibrium with the other polymorphic markers in this region of IL1RN. Guash et al described an Msp1 RFLP at nt 8006 in exon 2, an Mwo1 RFLP at nt 8061 in intron 2, and an Ssp1 RFLP at nt 9586 in intron 3 and found a complete linkage disequilibrium between these three markers. The VNTR in intron 2 is situated between the Mwo1 RFLP and the Ssp1 RFLP of IL1RN. More recently, this finding was confirmed by another study. Therefore, allele 2 of the Mwo1 RFLP is on the same haplotype as allele 2 of the VNTR.

This study focuses on two issues: the relation of the markers in IL1B and IL1RN and IBD; and the relation between the markers and cytokine production.

IL1B AND IL1RN RFLP IN IBD
Patients with UC more frequently carried the combination of both the allele 2 of the Taq1 RFLP in IL-1B and the allele 2 of the Mwo1 RFLP in IL-1RN. We could not confirm the reported association between UC and the polymorphism in intron 2 of the IL-1ra gene alone. Nor did we find any additional association between the RFLPs and IBD, UC, or CD. Our results are in sharp contrast to those of Bioque et al and Hersbach et al, who found a notable decrease in the number of individuals carrying the infrequent alleles of both the Taq1 RFLP in IL1B and the VNTR in IL1RN. Apparently this difference is caused by a high number of heterozygotes for the Taq1 RFLP in our population of patients with UC. Ethnic differences or patient selection on disease severity might contribute to this discrepancy.

Several studies have focused on the association between UC and allele 2 of the IL-1ra gene (which equates to our Mwo1 RFLP). Initially, two studies confirmed this association in a group of Hispanic patients with UC in Los Angeles and in a population from Pittsburgh in the United States. Another study only showed an increased frequency of allele 2 in patients with more severe UC but this result was not significant. The association with more severe disease in UC was also described by Roussomoustakaki et al in 107 patients with UC undergoing colectomy. In this study patients with more extensive colitis significantly more often carried allele 2 than patients with distal disease at the time of surgery. However, it should be noted that UC disease localisation seems to be a dynamic entity changing over time. Therefore associations with disease localisation should be interpreted with care. Furthermore, our data are in accordance with more recent studies from Europe that failed to show a significant increase in the allele frequency of allele 2 in UC.

IL1B AND IL1RN RFLP AND CYTOKINE PRODUCTION
In a cohort of 60 healthy individuals we did not observe dependence of ex vivo cytokine production and IL1B or IL1RN genotypes. We were particularly interested in individuals carrying both infrequent alleles of the Taq1 RFLP and the Mwo1 RFLP, because we found an association between UC and carriage of both these alleles. Although these individuals had a lower ratio for IL-1ra:IL-1β production levels this difference did not reach statistical significance. It should be noted that only eight
individuals of the cohort of 60 were allele 2/2 carriers and hence our study might lack power. Oth


