Genetic variants in the region harbouring IL2/IL21 associated with ulcerative colitis


DOI
10.1136/gut.2008.166918

Publication date
2009

Document Version
Final published version

Published in
Gut

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: https://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.
Genetic variants in the region harbouring \textit{IL2/IL21} associated with ulcerative colitis

E A M Festen,\textsuperscript{1} P Goyette,\textsuperscript{2} R Scott,\textsuperscript{3} V Annese,\textsuperscript{4} A Zhernakova,\textsuperscript{5} J Lian,\textsuperscript{6} S R Brant,\textsuperscript{6} J H Cho,\textsuperscript{7} M S Silverberg,\textsuperscript{8} K D Taylor,\textsuperscript{9} D J de Jong,\textsuperscript{10} P C Stokkers,\textsuperscript{11} D McGovern,\textsuperscript{11} O Palmieri,\textsuperscript{12} J-P Achkar,\textsuperscript{12} R J Xavier,\textsuperscript{13} M J Daly,\textsuperscript{14} R H Duerr,\textsuperscript{3} C Wijnenga,\textsuperscript{2} R K Weersma,\textsuperscript{1} and both forms of IBD,\textsuperscript{1,6}J Lian,\textsuperscript{6}K D Taylor,\textsuperscript{9}Finally,\textsuperscript{1,6}All four SNPs were strongly associated with UC\textsuperscript{6}A strong association for the\textsuperscript{IL2}\textsuperscript{6–11}are\textsuperscript{locus}\textsuperscript{14}\textsuperscript{17}locus most associated with coeliac\textsuperscript{locus}\textsuperscript{and IBD.}\textsuperscript{IL18RAP}\textsuperscript{while2009;R H Duerr,\textsuperscript{8}P C Stokkers,\textsuperscript{16}D J de Jong,\textsuperscript{1}systemic lupus erythematosus,\textsuperscript{13}Far fewer have been found for UC. Recently\textsuperscript{Additional tables are Ulcerative colitis\textsuperscript{R J Xavier,}.\textsuperscript{7}region variants identified in\textsuperscript{locus on 4q27 is\textsuperscript{C Lefe}\textsuperscript{is another shared\textsuperscript{1}locus and the IBDs.}\textsuperscript{IL12B}\textsuperscript{O Palmieri,}\textsuperscript{This overexpression is most marked in\textsuperscript{mice}\textsuperscript{Interestingly, there appear to\textsuperscript{end of article}\textsuperscript{For numbered affiliations see}}
analysis to identify association with specific subsets of IBD. Our data unequivocally show that the IL2/IL21 locus is strongly associated with UC. We confirmed this finding in multiple IBD populations.

METHODS

Subjects
For the first phase, the cases consisted of a cohort of 1590 patients with IBD (777 CD and 813 UC) collected from the outpatient clinics of the Departments of Gastroenterology and Hepatology at the Amsterdam Medical Center (n = 752), the Radboud University Medical Center, Nijmegen (n = 275) and the University Medical Center Groningen, The Netherlands (n = 585). The control cohort consisted of 929 healthy Dutch individuals who were blood donors.6

To replicate the findings from the first phase, two independent cohorts were examined. The first replication cohort consists of an IBD case–control cohort (2387 cases of which 654 were CD and 1735 UC, and 1266 controls) collected through the North American NIDDK IBD Genetics Consortium (IBDGC) as described previously.20,21 Cases and geographically matched controls were ascertained through the University of Montreal, Cedars-Sinai Medical Center, Johns Hopkins University, University of Chicago, University of Pittsburgh and the University of Toronto Genetics Research Centers (GRCs). This NIDDK-IBDGC IBD cohort contained five related pairs of cases between UC and CD samples. All cases were included in the subphenotype analysis, but in the IBD analysis one member of each pair (five cases) was removed. The second replication cohort consists of an Italian IBD case–control cohort (805 cases, of which 157 were CD and 648 UC, and 421 controls) collected at the S. Giovanni Rotondo “CSS” (SGRC) Hospital in Italy. This cohort has previously been used and characterised in several association reports from our group.22,23 A fourth cohort consisting of 398 cases and 418 controls of Jewish descent from the USA was also included; this cohort was also collected by the NIDDK-IBDGC and has previously been characterised.20,21

All patients and controls were of European Caucasian descent. The diagnosis of IBD required (1) one or more symptoms of diarrhoea, rectal bleeding, abdominal pain, fever or complicated perianal disease, (2) occurrence of symptoms on two or more occasions separated by at least 8 weeks or ongoing symptoms of at least 6 weeks duration, and (3) objective evidence of inflammation from radiological, endoscopic and histopathological evaluation. All affected subjects fulfil clinical criteria for IBD. For patients with CD, phenotypic details were registered according to the Vienna classification. However, perianal disease was scored as an independent variable and not included in the group with penetrating disease behaviour. For patients with UC, phenotypes were described according to age of onset, maximum extent of disease (proctitis, left-sided or extensive), necessity for colectomy and the occurrence of malignancy and extraintestinal manifestations. A summary of the phenotype information available for each cohort can be found in Supplementary table 1 (CD) and Supplementary table 2 (UC).

Genotyping
We analysed the four most strongly associated single nucleotide polymorphisms (SNPs) in IL2/IL21 found by Van Heel et al: rs6822844, rs13151961, rs13119723 and rs6840978.6 Genotyping of the Dutch cohort was performed using TaqMan technology, while SNP genotyping assays for PCR were supplied by Applied Biosystems (Foster City, California, USA), as described.6 The patient and control DNA samples were processed in 384-well plates and each plate also contained 16 genotyping controls (4 duplicates of 4 Centre d’Etude du Polymorphisme Humain (CEPH) DNA).

Genotyping of 1577 samples from the North American IBD cohort was performed using primer extension chemistry and mass spectrometric analysis (iPlex assay, Sequenom, San Diego, California, USA) on the Sequenom MassArray. This was performed at the Laboratory for Genetics and Genomic Medicine of Inflammation (www.inflammgen.org) of the Université de Montréal and at The University of Pittsburgh. Data from an additional set of 2917 North American IBD samples were also obtained from genotyping on Illumina HumanHap300 or HumanHap550 Genotyping BeadChips (Illumina, San Diego, California, USA) as was previously reported in the IBDGC’s CD and UC genome-wide association studies.24

Genotyping for the Italian cohort was also performed at the Laboratory for Genetics and Genomic Medicine of Inflammation, using primer extension chemistry and mass spectrometric analysis on the Sequenom MassArray. The patient and control DNA samples were again processed in 384-well plates and each plate also contained 16 genotyping controls (4 duplicates of 4 CEPH DNA). All SNPs were validated, and we obtained >99.9% concordance between our genotype data and the CEU data available from HapMap.

Statistical analysis
Hardy–Weinberg equilibrium (HWE) was tested by comparing the expected and observed genotypes in a 2 × 3 χ2 table. Controls did not show deviation from HWE (p value (HWE) > 0.001). Differences in allele and genotype distribution in the cases and controls of the individual cohorts were tested for significance by the χ2 test. Analyses for association between genotype and subphenotypes were also performed with the χ2 test. A significant threshold for p values was determined at <0.05. Odds ratios (ORs) were calculated and the CIs were approximated using Woolf’s method with Haldane’s correction. Power calculations were performed using the online Genetic Power Calculator by Shaun Purcell (http://pngu.mgh.harvard.edu/~purcell/gpc/).24

Combined analysis of the different cohorts was performed by Cochran–Mantel–Haenszel meta-analysis.

RESULTS
Initially the rs13151961, rs13119723, rs6840978 and rs6822844 SNPs were tested in 1590 Dutch patients (777 patients with CD and 813 patients with UC) and 929 healthy controls. The minor alleles of all four SNPs tested were associated with IBD with a p value range between 0.0005 and 0.00039 and an OR between 0.76 and 0.78. This association was even stronger in the UC subgroup of the cohort (p value range 0.0005–0.00001 and OR range 0.71–0.67). In the CD subgroup, the rs15119723 SNP was borderline significant with a p value of 0.0527, while only a trend towards association was observed for the other SNPs. This indicated that the association of the IL2/IL21 locus with IBD was coming predominantly from the UC subgroup. The results are shown in table 1.

In all cases, informed consent was obtained using protocols approved by the local institutional review board in all


<table>
<thead>
<tr>
<th>SNP</th>
<th>A1</th>
<th>A2</th>
<th>MAF controls</th>
<th>MAF cases</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>MAF controls</th>
<th>MAF cases</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>MAF controls</th>
<th>MAF cases</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>Combined p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs13151961</td>
<td>G</td>
<td>A</td>
<td>0.19</td>
<td>0.15</td>
<td>0.0039</td>
<td>0.85 (0.71 to 1.02)</td>
<td>0.17</td>
<td>0.14</td>
<td>0.0002</td>
<td>0.78 (0.68 to 0.89)</td>
<td>0.16</td>
<td>0.12</td>
<td>0.0007</td>
<td>0.66 (0.52 to 0.84)</td>
<td>1.41 × 10⁻⁷</td>
</tr>
<tr>
<td>rs13151961</td>
<td>G</td>
<td>A</td>
<td>0.16</td>
<td>0.13</td>
<td>0.0093</td>
<td>0.76 (0.65 to 0.89)</td>
<td>0.16</td>
<td>0.13</td>
<td>0.0005</td>
<td>0.78 (0.68 to 0.89)</td>
<td>0.17</td>
<td>0.12</td>
<td>0.0028</td>
<td>0.70 (0.55 to 0.89)</td>
<td>1.32 × 10⁻⁶</td>
</tr>
<tr>
<td>rs6840978</td>
<td>T</td>
<td>C</td>
<td>0.22</td>
<td>0.18</td>
<td>0.0067</td>
<td>0.78 (0.67 to 0.90)</td>
<td>0.21</td>
<td>0.17</td>
<td>0.0006</td>
<td>0.81 (0.71 to 0.91)</td>
<td>0.20</td>
<td>0.16</td>
<td>0.0160</td>
<td>0.77 (0.62 to 0.95)</td>
<td>6.17 × 10⁻⁴</td>
</tr>
<tr>
<td>rs6822844</td>
<td>T</td>
<td>G</td>
<td>0.19</td>
<td>0.15</td>
<td>0.00070</td>
<td>0.77 (0.66 to 0.89)</td>
<td>0.17</td>
<td>0.14</td>
<td>0.0005</td>
<td>0.79 (0.69 to 0.90)</td>
<td>0.16</td>
<td>0.11</td>
<td>0.0005</td>
<td>0.65 (0.51–0.83)</td>
<td>7.45 × 10⁻⁵</td>
</tr>
</tbody>
</table>

Table 1 Summary of the association results in our screening (Dutch) and replication (North American and Italian) cohorts, as well as the combined results following the Cochran–Mantel–Haenszel meta-analysis.

Original Dutch cohort (1590 IBD (777 CD, 813 UC), 929 controls), replication cohorts: North American (2387 IBD (553 CD, 1733 UC), 1266 controls); Italian (805 IBD (157 CD, 648 UC), 421 controls). All p values are two-tailed. CD, Crohn’s disease; IBD, inflammatory bowel disease; MAF, minor allele frequency; SNP, single nucleotide polymorphism; UC, ulcerative colitis.
participating institutions. All DNA samples and data in this study were denonimalised.

To replicate these findings, we studied two independent cohorts. In the North American cohort (2387 IBD cases (654 CD and 1733 UC) and 1266 controls), we observed association with the same alleles of all SNPs in IBD (p value range 0.0011–0.0003 and OR range 0.77–0.81). As in the original cohort, this effect was strongest in the UC subgroup of the cohort (p value range 0.0046–0.0004 and OR range 0.77–0.81). In the CD subgroup of the North American cohort, a moderate association with the same alleles was also observed (p value range 0.0123–0.0011). Testing of all four SNPs in the Italian cohort (805 IBD cases (157 CD, 648 UC) and 421 controls) showed the same strong association of the minor alleles in UC as seen in the original cohort, with a p value range between 0.0123 and 0.0002 and an OR range between 0.75 and 0.62. The CD subgroup of the Italian cohort showed only a trend towards association with the same alleles, which was not significant, with a p value range between 0.3495 and 0.0873. The results are shown in table 1.

A Cochran–Mantel–Haenszel meta-analysis of the results from all three cohorts showed a very convincing association of all IL2/IL21 SNPs in IBD (p value range 7.45×10⁻⁸–1.41×10⁻¹⁰). In UC, this effect also reached genome-wide significance, with a p value of 5.07×10⁻⁹ for rs6840978 and a p value of 1.35×10⁻¹⁰ for rs13151961. The meta-analysis showed a moderate association with CD for all four SNPs with the same alleles (p value range 0.0016–9.86×10⁻¹⁰).

The fourth cohort consisting of patients with a Jewish background was analysed separately; these results are depicted in table 2. We did not find a significant association between any of the SNPs and CD in this cohort. We were reluctant to add this cohort to the meta-analysis for all patients with CD because of the large discrepancy in minor allele frequency (MAF) between Jewish controls and controls from the other cohorts: the MAF for SNP rs13119723 in Jewish controls was 0.06, while the MAF in the other cohorts was between 0.16 and 0.17. We performed a meta-analysis of all CD cohorts including the Jewish cohort (data not shown), which yielded a p value of 1.4×10⁻⁵ for SNP rs13151961, a p value of 1.0×10⁻⁴ for SNP rs13151973, a p value of 4.1×10⁻⁶ for SNP rs6822844 and a p value of 1.4×10⁻⁷ for SNP rs6840978.

Because the association of the IL2/IL21 locus with CD is much more moderate than that with UC it might be that the association is mainly with colonic disease. If this were the case, then we would predict that the association signal from CD comes exclusively from disease localised in the colon. To test this hypothesis, we performed a within-cases analysis for the association in colonic and non-colonic CD. However, this did not yield any significant results. Further genotype-phenotype analysis for disease localisation or extent, disease behaviour, necessity for operation, the occurrence of malignancy and extraintestinal manifestations did not yield any phenotype-specific associations (data not shown). Although phenotype data were available for a large proportion of cases (80% for both CD and UC) this might still be due to a lack of power in each specific subgroup to detect true genotype-phenotype associations.

Another possible explanation for the comparatively modest association with CD is the relatively low total number of patients with CD: 1588 patients with CD compared with 3194 patients with UC. This, however, does not appear likely as the power calculations showed that with the 1588 patients with CD we have in our study there is 95% power to detect an effect with an OR of 0.85, which is similar to that observed in UC.

**DISCUSSION**

In the current study we have identified and replicated a novel association between genetic variants in the IL2/IL21 locus and IBD (OR 0.66; p value 1.4×10⁻¹⁰), with the strongest evidence of association in UC (OR 0.62; p value 1.35×10⁻¹⁰). This association is consistent with the recent findings of a common protective allele in coeliac disease, rheumatoid arthritis, psoriasis and type 1 diabetes, and thus confirms this locus as a general risk locus for inflammatory disease.²⁻⁶⁻¹⁰

This locus on chromosome 4q27 comprises a region of 480 kb of extensive linkage disequilibrium (LD) that harbours the testis nuclear RNA-binding protein (TENR) gene, a gene encoding a protein of unknown function (KIAA1109), and genes encoding the IL2 and IL21 cytokines. TENR is expressed primarily in testis, and KIAA1109 transcripts are ubiquitous, hence their roles in inflammatory diseases are not particularly compelling, which leaves IL2 and IL21 as the most likely candidates for disease association in the region.³ As previously reported in other immune diseases, the four SNPs tested and found to be associated with IBD in this study are correlated to each other (with r² correlation coefficients ranging from 0.5 to 0.97) and are all located in non-coding regions within this 480 kb LD block. Two SNPs, rs13151961 and rs13119723, are situated in intronic regions of the KIAA1109 gene. SNP rs6822844 is located in the intergenic region between IL2 and IL21, and SNP rs6840978 is located downstream of IL21. These SNPs are not known to have an effect on expression of the genes in the IL2–IL21 region.²¹

IL2 is secreted in an autocrine fashion by antigen-stimulated T cells, and stimulates T cell activation and proliferation. In these T cells, IL2 stimulates the production of the proinflammatory cytokines interferon γ and IL4. Furthermore, IL2 has an important role in regulating the adaptive immune response by stimulating T regulatory (CD4⁺ CD25⁺) cells and by its ability to stimulate activation-induced cell death in antigen-activated T cells.²² IL21 is also a T cell-derived cytokine; it stimulates class switching to immunoglobulin G (IgG) in B cells and regulates natural killer cell proliferation and differentiation. IL21 augments proliferation in cells of the monocyte–macrophage lineages and induces an immunosuppressive phenotype by stimulating the formation of immature monocytes that inhibit antigen-specific T cell proliferation. During inflammatory processes, the receptor for IL21, IL21R, can be found on non-immune cells, such as colon epithelial cells or fibroblasts. When stimulated by IL21, these cells secrete proteins that mobilise T cells to areas of immune challenge.²³

The overexpression of IL21 in patients with IBD compared with healthy controls and patients with diverticulitis shows the importance of this interleukin in the inflammatory process of

| Table 2 Association of the IL2/IL21 SNPs in a Jewish cohort |
|------------------|------------------|------------------|
| SNP             | A1   | A2   | MAF controls | MAF cases | p Value | OR (95% CI) |
| rs13151961      | G    | A    | 0.07          | 0.06       | 0.5691  | 0.89 (0.60 to 1.30) |
| rs13119723      | G    | A    | 0.06          | 0.05       | 0.6388  | 0.89 (0.58 to 1.37) |
| rs6840978       | T    | C    | 0.07          | 0.06       | 0.3790  | 0.83 (0.56 to 1.23) |
| rs6822844       | T    | G    | 0.14          | 0.14       | 0.7468  | 0.96 (0.72 to 1.27) |

Jewish CD cohort (398 cases, 418 controls). All p values are two-tailed.

CD, Crohn’s disease; IL2, interleukin 2; IL21, interleukin 21; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
Interestingly, Monteleone et al observed the increase in IL2 expression level predominantly in the CD subgroup of patients with IBD, whereas we here observed a stronger association of the IL2/IL21 locus with UC rather than CD. Although speculative, this prioritises the IL2 gene as the more likely to be involved. IL2 is an attractive functional candidate gene for UC pathogenesis, as the IL2/−/− mouse develops a disease similar to UC, supporting an association between IL2 and UC. The fact that calcineurin inhibitors, which mainly suppress the expression of IL2, are effective in treatment-resistant UC, but not in CD, might also point to a key role for this interleukin in UC. Further support for the importance of IL2 in UC comes from the fact that a pilot trial with antibodies against the IL2 receptor in treatment-resistant UC was successful. The fact that both a lack of IL2 and an excess of IL2 predispose to colitis is however puzzling. Further functional studies on these genetic variants are needed to define the specific role for the IL2/IL21 locus in the pathogenesis of IBDs.

An equivalent protective association signal of the IL2/IL21 locus with coeliac disease, rheumatoid arthritis, type 1 diabetes and psoriasis has previously been reported. This shows that this locus plays an important role in inflammatory diseases. Previously MAGIC, PARD3, MYO1B and IL18RAP were reported to be associated with both coeliac disease and UC. The IL2/IL21 locus is now the fifth locus to be associated with both diseases, further supporting a model where a common set of biological pathways lead to coeliac disease and UC. Interestingly, multiple SNPs in this same region, that are independent of the SNPs studied herein, have recently been reported to confer risk of type 1 diabetes and potentially of coeliac disease. Although these SNPs conferring risk were not tested in the current study a published study in CD (rs17388568, p = 1.7 × 10−4; rs716501, p = 3.8 × 10−4) potentially supports the presence of alleles conferring increased risk for disease. Further examination of these risk-conferring alleles are warranted in CD and UC.

Extensive sequencing in coeliac cases and matched controls, as well as functional studies, will be needed to find the true causal variant in the IL2/IL21 locus and determine the molecular mechanisms by which this locus influences an individual’s risk of multiple immune-mediated diseases.

Author affiliations: 1 Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 2 Laboratory in Genetics and Genomic Medicine of Inflammation, Montreal Heart Institute, Université de Montréal, Montreal, Canada; 3 Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; 4 UU.DO. Gastroentero logia ed Endoscopia Digestiva, Ospedale Casa Sollievo della Sofferenza, RDCs, San Giovanni Rotondo, Italy; 5 Complex Genetics Section, Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 6 Harvey M and Lyn P Meyerhoff Inflammatory Bowel Disease Center, Department of Medicine, Johns Hopkins University, Baltimore, Maryland, USA; 7 Departments of Medicine and Genetics, Division of Gastroenterology, Inflammatory Bowel Disease (IBD) Center, Yale University, New Haven, Connecticut, USA; 8 Mount Sinai Hospital IBD Centre, University of Toronto, Toronto, Ontario, Canada; 9 Medical Genetics Institute and Inflammatory Bowel Disease (IBD) Center, Cedars-Sinai Medical Center, Los Angeles, California, USA; 10 Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 11 Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands; 12 Center for Inflammatory Bowel Disease, Department of Gastroenterology & Hepatology, Cleveland Clinic, Cleveland, Ohio, USA; 13 Center for Computational and Integrative Biology and Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Massachusetts, USA; 14 Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Acknowledgements: We thank all the patients and controls who participated in this study, and Jackie Senior for checking the final text.

Funding: This study was made possible by a VICI grant from the Netherlands Organization for Scientific Research (918.66.620) to CW, and an AGIKO-grant from the Netherlands Organization for Scientific Research to EF.

Competing interests: None.

Patient consent: Informed consent was obtained using protocols approved by the local institutional review board in all participating institutions.

REFERENCES


**Editor’s quiz: GI snapshot**

**ANSWER**

*From the question on page 744*

The CT scan (fig 1) revealed a thickened terminal ileum. The endoscopic biopsy revealed inflammation only. She received right hemicolectomy and partial ileum resection for persistent ileal obstruction. During the operation, segmental stiffness of the terminal ileum was noted (fig 2). Pathological examination (fig 3) showed metastatic lobular carcinoma, which is compatible with the previous histological finding of breast cancer. The cancer cells involved submucosa and muscularis propria but sparing the mucosa, which explained the inconclusive finding by endoscopic biopsy.

A thickened terminal ileum should remind clinicians of Crohn’s disease, tuberculosis, ischaemia, adenocarcinoma, lymphoma and rarely metastatic cancer. Metastatic breast cancer is the leading cause of small intestinal obstruction resulting from metastatic cancers, with an incidence of up to 16%. The interval between the primary tumour and gastrointestinal tract metastasis may span >10 years. Most of these cases were disseminated and lobular in type. Metastatic cancer should be considered in such patients with a history of lobular carcinoma of the breast.

Gut 2009;58:804. doi:10.1136/gut.2008.164913a