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

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## ABSTRACT

**Objectives:** Genetic susceptibility is known to play a large part in the predisposition to the inflammatory bowel diseases (IBDs) known as Crohn's disease (CD) and ulcerative colitis (UC). The *IL2/IL21* locus on 4q27 is known to be a common risk locus for inflammatory disease (shown in coeliac disease, type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus and psoriasis), while the roles that interleukin 2 (IL2) and IL21 play in the immune response also make them attractive candidates for IBD. The objective of this study was to test for association between the *IL2/IL21* locus and the IBDs.

**Methods:** The four single nucleotide polymorphisms (SNPs) in the *IL2/IL21* locus most associated with coeliac disease were genotyped in 1590 subjects with IBD and 929 controls from The Netherlands, and then replicated in a North American cohort (2387 cases and 1266 controls) and an Italian cohort (805 cases and 421 controls), yielding a total of 4782 cases (3194 UC, 1588 CD) and 2616 controls. Allelic association testing and a pooled analysis using a Cochran–Mantel–Haenszel test were performed.

**Results:** All four SNPs were strongly associated with UC in all three cohorts and reached genome-wide significance in the pooled analysis (rs13151961 $p = 1.35\times10^{-10}$, rs13119723 $p = 8.60\times10^{-8}$, rs6840978 $p = 3.07\times10^{-8}$, rs6822844 $p = 2.77\times10^{-9}$). A moderate association with CD was also found in the pooled analysis (p value range $0.0016–9.86\times10^{-5}$).

**Conclusions:** A strong association for the *IL2/IL21* locus with UC was found, which also confirms it as a general susceptibility locus for inflammatory disease.

Inflammatory bowel diseases (IBDs) are the most common chronic inflammatory diseases in the Western world after rheumatoid arthritis, with an incidence of about 40 per 100 000 in North America and Western Europe.[1] Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types of IBD, both characterised by recurring inflammation of the digestive tract. In CD the inflammation can occur throughout the gastrointestinal tract, most commonly affecting the terminal part of the small intestine and causing weight loss and abdominal pain. In UC the disease is confined to the colon, and patients usually present with bloody diarrhoea and abdominal cramping.[2]

Genetic susceptibility plays an important role in the pathogenesis of IBD. CD and UC are complex diseases with numerous genetic and environmental factors leading to disease. Epidemiological studies suggest stronger heritability in CD compared with UC.[1]

Many genetic factors contributing to CD pathogenesis have been identified during the last decade. There are currently >30 genes or loci associated with CD, the majority having been identified since the introduction of genome-wide association studies.[3] Far fewer have been found for UC. Recently the first genome-wide association study in UC was published identifying several new loci, and another genome-wide association study will be published shortly.[4 5]

Genetic studies have also shown that susceptibility genes are commonly shared between inflammatory diseases. For example, the *IL2/IL21* (interleukin 2/interleukin 21) locus on chromosome 4q27 has been shown to be associated with coeliac disease, type 1 diabetes, Grave's disease, systemic lupus erythematosus, rheumatoid arthritis and psoriasis.[6–11] Interestingly, there appear to be at least two independent association signals in this region, one conferring an increased risk of disease and the other conferring a protective effect.[8 11]

There are several reasons why the *IL2/IL21* locus could also represent an interesting locus for IBD. First, a number of shared autoimmune and inflammatory genes show an association with IBD: *IL12B*, for example, is associated with psoriasis,[10] systemic lupus erythematosus,[12] asthma[13] and both forms of IBD,[14] while *IL18RAP* was found to be associated with both coeliac disease[15] and IBD.[16] *IL2/IL21* is another shared inflammatory locus, and both *IL2* and *IL21* are attractive functional candidate genes for association with IBD. Overexpression of IL21 in inflamed regions of the bowel of patients with IBD has been reported.[17] This overexpression is most marked in CD, but a significant overexpression compared with that in diverticular disease and healthy controls is also present in UC.[17] Finally, *Il2*$^{-/-}$ mice develop IBD most reminiscent of UC.[18]

Given all these observations, and that IBD and coeliac disease are chronic inflammatory diseases of the gut, we were specifically interested in testing whether the *IL2/IL21* region variants identified in the coeliac genome-wide association studies also have a role to play in IBD. This was achieved via a case–control association study with a three-stage design in a large cohort of patients with IBD. In addition, we performed genotype–phenotype

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 = 732), the Radboud University Medical Centre, Nijmegen (n = 273) and the University Medical Center Groningen, The Netherlands (n = 585).[19] 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 1733 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.[5 21]

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 $2\times3$ $\chi^2$ 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 $\chi^2$ test. Analyses for association between genotype and subphenotypes were also performed with the $\chi^2$ 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 0.00093 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 0.00038–0.00001 and OR range 0.71–0.67). In the CD subgroup, the rs13119723 SNP was borderline significant with a p value of 0.0327, 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 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

| | | | Dutch IBD: 1590 cases, 929 controls | | | | North American IBD: 2387 cases, 1266 controls | | | | Italian IBD: 805 cases, 421 controls | | | | Meta analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | A1 | A2 | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | Combined p value |
| rs13151961 | G | A | 0.19 | 0.15 | **0.00039** | 0.85 (0.71 to 1.02) | 0.17 | 0.14 | **0.0002** | 0.78 (0.68 to 0.89) | 0.16 | 0.12 | **0.0007** | 0.66 (0.52 to 0.84) | $\mathbf{1.41\times10^{-9}}$ |
| rs13119723 | G | A | 0.16 | 0.13 | **0.00093** | 0.76 (0.65 to 0.89) | 0.16 | 0.13 | **0.0005** | 0.78 (0.68 to 0.90) | 0.17 | 0.12 | **0.0028** | 0.70 (0.55 to 0.89) | $\mathbf{1.32\times10^{-8}}$ |
| rs6840978 | T | C | 0.22 | 0.18 | **0.00067** | 0.78 (0.67 to 0.90) | 0.21 | 0.17 | **0.0006** | 0.81 (0.71 to 0.91) | 0.20 | 0.16 | **0.0160** | 0.77 (0.62 to 0.95) | $\mathbf{6.17\times10^{-8}}$ |
| rs6822844 | T | G | 0.19 | 0.15 | **0.00070** | 0.77 (0.66 to 0.89) | 0.17 | 0.14 | **0.0005** | 0.79 (0.69 to 0.90) | 0.16 | 0.11 | **0.0005** | 0.65 (0.51–0.83) | $\mathbf{7.45\times10^{-6}}$ |
| | | | Dutch CD: 777 cases, 929 controls | | | | North American CD: 654 cases, 1266 controls | | | | Italian CD: 157 cases, 421 controls | | | | Meta analysis |
| SNP | A1 | A2 | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | Combined p value |
| rs13151961 | G | A | 0.19 | 0.17 | 0.0761 | 0.85 (0.71 to 1.02) | 0.17 | 0.15 | **0.0123** | 0.79 (0.65 to 0.95) | 0.16 | 0.14 | 0.3454 | 0.84 (0.58 to 1.21) | **0.0016** |
| rs13119723 | G | A | 0.16 | 0.14 | **0.0327** | 0.81 (0.67 to 0.98) | 0.16 | 0.12 | **0.0011** | 0.72 (0.59 to 0.88) | 0.17 | 0.14 | 0.3495 | 0.84 (0.58 to 1.21) | $\mathbf{9.86\times10^{-5}}$ |
| rs6840978 | T | C | 0.22 | 0.20 | 0.1454 | 0.88 (0.75 to 1.04) | 0.21 | 0.16 | **0.0063** | 0.76 (0.64 to 0.90) | 0.20 | 0.18 | 0.3209 | 0.84 (0.60 to 1.18) | **0.0007** |
| rs6822844 | T | G | 0.19 | 0.16 | 0.1221 | 0.87 (0.73 to 1.04) | 0.17 | 0.14 | **0.0020** | 0.77 (0.64 to 0.93) | 0.16 | 0.12 | 0.0873 | 0.71 (0.48 to 1.05) | **0.0009** |
| | | | Dutch UC: 813 cases, 929 controls | | | | North American UC: 1733 cases, 1266 controls | | | | Italian UC: 648 cases, 421 controls | | | | Meta analysis |
| SNP | A1 | A2 | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | MAF controls | MAF cases | p Value | OR (95% CI) | Combined p value |
| rs13151961 | G | A | 0.19 | 0.14 | **0.00003** | 0.67 (0.56 to 0.81) | 0.17 | 0.14 | **0.0004** | 0.77 (0.67 to 0.89) | 0.16 | 0.11 | **0.0002** | 0.62 (0.48–0.80) | $\mathbf{1.35\times10^{-10}}$ |
| rs13119723 | G | A | 0.16 | 0.12 | **0.00038** | 0.71 (0.58 to 0.86) | 0.16 | 0.13 | **0.0046** | 0.81 (0.70 to 0.94) | 0.17 | 0.12 | **0.0013** | 0.67 (0.52 to 0.85) | $\mathbf{8.60\times10^{-8}}$ |
| rs6840978 | T | C | 0.22 | 0.16 | **0.00001** | 0.68 (0.57 to 0.81) | 0.21 | 0.18 | **0.0040** | 0.83 (0.73 to 0.94) | 0.20 | 0.16 | **0.0123** | 0.75 (0.60 to 0.94) | $\mathbf{3.07\times10^{-8}}$ |
| rs6822844 | T | G | 0.19 | 0.13 | **0.00004** | 0.68 (0.57 to 0.82) | 0.17 | 0.14 | **0.0018** | 0.80 (0.69 to 0.92) | 0.16 | 0.11 | **0.0005** | 0.64 (0.49 to 0.82) | $\mathbf{2.77\times10^{-9}}$ |

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 alllele frequency; SNP, single nucleotide polymorphism; UC, ulcerative colitis.

participating institutions. All DNA samples and data in this study were denominalised.

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 $7.45\times10^{-6}$–$1.41\times10^{-9}$). In UC this effect also reached genome-wide significance, with a p value of $3.07\times10^{-8}$ for rs6840978 and a p value of $1.35\times10^{-10}$ for rs13151961. The meta-analysis showed a moderate association with CD for all four SNPs with the same alleles (p value range 0.0016–$9.86\times10^{-5}$).

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 $1.4\times10^{-3}$ for SNP rs13151961, a p value of $1.0\times10^{-4}$ for SNP rs13119723, a p value of $4.1\times10^{-4}$ for SNP rs6822844 and a p value of $1.4\times10^{-3}$ 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 occurence 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.

**Table 2** Association of the *IL2/IL21* SNPs in a Jewish cohort

| | | | Jewish CD: 398 cases, 418 controls | | | |
|---|---|---|---|---|---|---|
| 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 alllele frequency; SNP, single nucleotide polymorphism.

## 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 $1.4\times10^{-9}$), with the strongest evidence of association in UC (OR 0.62; p value $1.35\times10^{-10}$). 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.[6 8–10]

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.[6] 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 $r^2$ 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 rs6840987 is located downstream of *IL21*. These SNPs are not known to have an effect on expression of the genes in the *IL2–IL21* region.[25]

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.[26] 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.[27]

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

both CD and UC.[17] Interestingly, Monteleone *et al* observed the increase in IL21 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 gene 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.[18] 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.[28] 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.[29] 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 *MAGI2*, *PARD3*, *MYOIXB* and *IL18RAP* were reported to be associated with both coeliac disease and UC.[15 16 30–32] 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 ot type 1 diabetes and potentially of coeliac disease.[11] Although these SNPs conferring risk were not tested in the current study a published study in CD (rs17388568, $p = 1.7\times10^{-4}$; rs716501, $p = 3.8\times10^{-4}$) potentially supports the presence of alleles conferring increased risk for disease.[3] 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.

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## REFERENCES

1. **Baumgart DC,** Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;**369**:1627–40.
2. **Baumgart DC,** Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 2007;**369**:1641–57.
3. **Barrett JC,** Hansoul S, Nicolae DL, *et al*. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;**40**:955–62.
4. **Franke A,** Balschun T, Karlsen TH, *et al*. Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat Genet* 2008;**40**:1319–23.
5. **Silverberg MS,** Cho JH, Rioux JD, *et al*. Ulcerative colitis-linked loci on chromosome 1p36 and 12q15 found by genome-wide association study. *Nat Genet* 2009;**41**:216–20.
6. **van Heel DA,** Franke L, Hunt KA, *et al*. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nat Genet* 2007;**39**:827–9.
7. **Todd JA,** Walker NM, Cooper JD, *et al*. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 2007;**39**:857–64.
8. **Zhernakova A,** Alizadeh BZ, Bevova M, *et al*. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. *Am J Hum Genet* 2007;**81**:1284–8.
9. **Sawalha AH,** Kaufman KM, Kelly JA, *et al*. Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus. *Ann Rheum Dis* 2008;**67**:458–61.
10. **Liu Y,** Helms C, Liao W, *et al*. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* 2008;**4**(3):e1000041.
11. **Smyth DJ,** Plagnol V, Walker NM, *et al*. Shared and distinct genetic variants in type 1 diabetes and celiac disease. *N Engl J Med* 2008;**359**:2767–77.
12. **Sanchez E,** Morales S, Paco L, *et al*. Interleukin 12 (IL12B), interleukin 12 receptor (IL12RB1) and interleukin 23 (IL23A) gene polymorphism in systemic lupus erythematosus. *Rheumatology (Oxford)* 2005;**44**:1136–9.
13. **Randolph AG,** Lange C, Silverman EK, *et al*. The IL12B gene is associated with asthma. *Am J Hum Genet* 2004;**75**:709–15.
14. **Fisher SA,** Tremelling M, Anderson CA, *et al*. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat Genet* 2008;**40**:710–2.
15. **Hunt KA,** Zhernakova A, Turner G, *et al*. Newly identified genetic risk variants for celiac disease related to the immune response. *Nat Genet* 2008;**40**:395–402.
16. **Zhernakova A,** Festen EM, Franke L, *et al*. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. *Am J Hum Genet* 2008;**82**:1202–10.
17. **Monteleone G,** Monteleone I, Fina D, *et al*. Interleukin-21 enhances T-helper cell type I signaling and interferon-gamma production in Crohn's disease. *Gastroenterology* 2005;**128**:687–94.
18. **Sadlack B,** Merz H, Schorle H, *et al*. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993;**75**:253–61.
19. **Weersma RK,** Stokkers PC, van Bodegraven AA, *et al*. Molecular prediction of disease risk and severity in a large Dutch Crohn's disease cohort. *Gut* 2009;**58**:388–95.
20. **Duerr RH,** Taylor KD, Brant SR, *et al*. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;**314**:1461–3.
21. **Rioux JD,** Xavier RJ, Taylor KD, *et al*. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007;**39**:596–604.
22. **Tello-Ruiz MK,** Curley C, DelMonte T, *et al*. Haplotype-based association analysis of 56 functional candidate genes in the IBD6 locus on chromosome 19. *Eur J Hum Genet* 2006;**14**:780–90.
23. **De Jager PL,** Franchimont D, Waliszewska A, *et al*. The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun* 2007;**8**:387–97.
24. **Purcell S,** Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;**19**:149–50.
25. **Dixon AL,** Liang L, Moffatt MF, *et al*. A genome-wide association study of global gene expression. *Nat Genet* 2007;**39**:1202–7.
26. **Thornton AM,** Donovan EE, Piccirillo CA, *et al*. Cutting edge: IL-2 is critically required for the in vitro activation of CD4+CD25+ T cell suppressor function. *J Immunol* 2004;**172**:6519–23.
27. **Leonard WJ,** Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. *Nat Rev Immunol* 2005;**5**:688–98.
28. **Lichtiger S,** Present DH, Kornbluth A, *et al*. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994;**330**:1841–5.

29. **Van Assche G,** Dalle I, Noman M, *et al*. A pilot study on the use of the humanized anti-interleukin-2 receptor antibody daclizumab in active ulcerative colitis. *Am J Gastroenterol* 2003;**98**:369–76.
30. **Wapenaar MC,** Monsuur AJ, van Bodegraven AA, *et al*. Associations with tight junction genes PARD3 and MAGI2 in Dutch patients point to a common barrier defect for coeliac disease and ulcerative colitis. *Gut* 2008;**57**:463–7.
31. **Monsuur AJ,** de Bakker PI, Alizadeh BZ, *et al*. Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. *Nat Genet* 2005;**37**:1341–4.
32. **van Bodegraven AA,** Curley CR, Hunt KA, *et al*. Genetic variation in myosin IXB is associated with ulcerative colitis. *Gastroenterology* 2006;**131**:1768–74.

# Editor's quiz: GI snapshot

## ANSWER

*From the question on page 741*

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,[1] with an incidence of up to 16%.[2] The interval between the primary tumour and gastrointestinal tract metastasis may span >10 years.[1] Most of these cases were disseminated and lobular in type.[3] 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

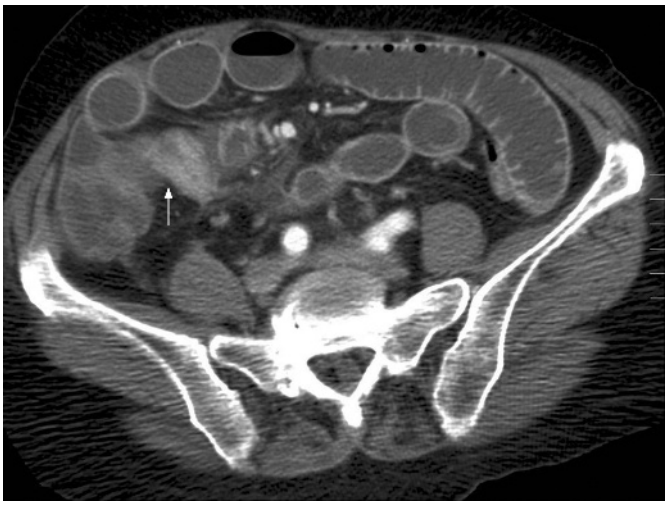

**Figure 1** Abdominal CT revealed a diffuse dilated small bowel with a transitional zone at the terminal ileum; the terminal ileum wall was very enhanced and thickened (arrow).

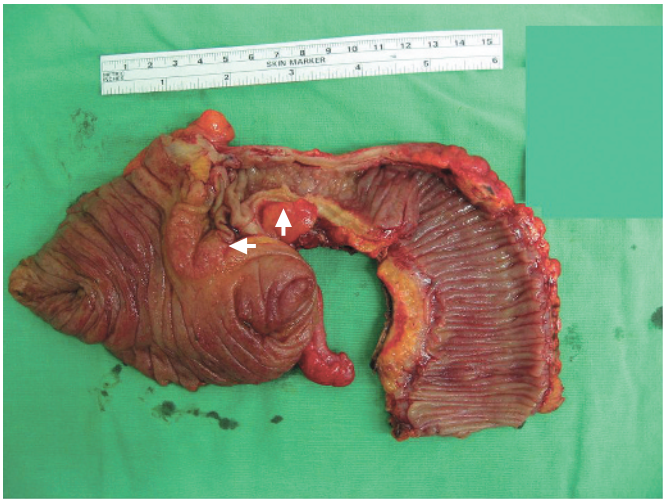

**Figure 2** Operation specimens reveal segmental stiffness with a nodular surface of the terminal ileum (arrows).

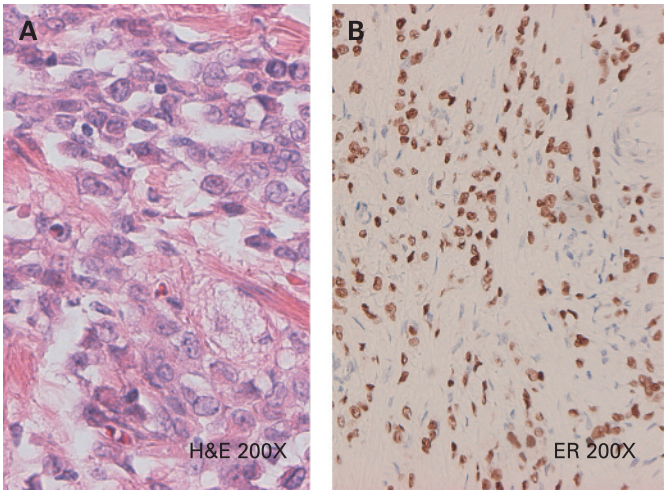


**Figure 3** Pathology revealed (A) uniform-sized discohesive cells with round cell morphology and concentric vesicular nuclei, which had characteristics of lobular breast carcinoma. (B) Diffuse expression of oestrogen receptor protein.

## REFERENCES

1. **Idekevich E.** Small bowel obstruction caused by secondary tumors. *Surg Oncol* 2006;**15**:29–32.
2. **Cifuentes N.** Metastases from carcinoma of mammary gland: an autopsy study. *J Surg Oncol* 1979;**11**:193–205.
3. **Hsieh PS.** Ileocecal breast carcinoma metastasis. *Int J Colorectal Dis* 2004;**19**:607–8.