Genetic susceptibility for inflammatory bowel disease across ethnicities and diseases

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HNF4α and CDH1 are associated with ulcerative colitis in a Dutch cohort

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ABSTRACT

Background and Aims: Inflammatory Bowel Disease (IBD) consisting of ulcerative colitis (UC) and Crohn’s disease (CD) are complex disorders with multiple genes contributing to disease pathogenesis. A recent genome wide association scan identified three novel susceptibility loci for UC comprising HNF4α, CDH1 and LAMB1. We performed an analysis of these three loci in an independent cohort.

Materials and methods: 821 UC patients and 1,260 healthy controls of central European Caucasian descent were genotyped for SNPs: rs6017342 (HNF4α), rs1728785 (CDH1) and rs6949033 (LAMB1). Differences in allele and genotype distribution in cases and controls were tested for significance by the χ²-test.

Results: Allelic association analysis showed that SNP rs6017342 in the HNF4α locus was strongly associated with UC (p-value = 1.04 × 10^{-11}, OR = 1.56, CI = 1.37–1.77) and SNP rs1728785 (CDH1) was associated with a P-value of 0.01 (OR = 1.23, CI = 1.05–1.44). SNP rs6949033 in LAMB1 was not associated in our cohort (p-value = 0.12, OR = 1.11, CI = 0.97–1.26). We found an association for SNP rs6949033 (LAMB1) for disease limited to the rectum limited disease (P-value = 0.02). However this association was lost after correcting for multiple testing. No further specific subphenotype associations were identified.

Conclusion: This is the first independent study to replicate the HNF4α and CDH1 loci as susceptibility loci for UC. The main candidate genes in these risk loci play important roles in the maintenance of the integrity of the epithelial barrier, highlighting the importance of the mucosal barrier function for UC pathogenesis.
INTRODUCTION

Inflammatory Bowel Disease (IBD) are common, chronic gastrointestinal inflammatory disorders with a prevalence of 100–200/100.000 in the developed countries. They comprise two major forms; Crohn’s disease (CD) and ulcerative colitis (UC).\(^1,2\) The aetiology of CD and UC is complex and consists of an aberrant immune response to the commensal bacterial flora in a genetically susceptible host. It is thought that this aberrant response is due to a combination of factors including environmental and genetic factors, causing defects in both innate and adaptive immunity and epithelial barrier function.\(^2\)

The genetic basis of IBD had long been appreciated through family studies with an approximately 30,0% concordance of IBD among monozygotic twins.\(^3\) IBD are complex genetic disorders with multiple genes contributing to the disease pathogenesis. Until recently the focus in genetic research in IBD was mainly on CD, however, in recent years the attention also turned to UC, with six Genome Wide Association Studies (GWAS) identifying 18 UC associated loci. These studies highlighted both disease-specific loci and other loci that are shared between UC and CD.\(^4–10\)

As part of the Wellcome Trust Case Control Consortium phase 2 (WTCCC2), the UK IBD Genetics Consortium identified three novel susceptibility loci for UC comprising HNF4α, CDH1 and LAMB1 which all play a role in epithelial barrier function.\(^4\) Given the central role of the epithelium in regulating inflammatory responses, the importance of the intestinal barrier in limiting access of toxins and microbes to underlying tissues and the antimicrobial nature of the immune responses in UC, intestinal barrier dysfunction is an important factor in UC pathogenesis.\(^11\)

Replication studies are important to support the differences between true positive associations and false ones.\(^12\) Essential in a replication study is the presence of independent populations using large sample sizes with matched controls and disease phenotypes mostly comparable with those used in the initial studies.

We undertook a study in a Dutch cohort of UC patients and tested these three new associated loci (HNF4α, CDH1, LAMB1) in 821 UC patients and 1,260 controls.

MATERIALS AND METHODS

Patients and controls

Cases consisted of 838 Dutch UC patients. Cases were collected at the Academic Medical Center, Amsterdam (n = 409) the University Medical Center St. Radboud, Nijmegen (n = 206) and the University Medical Center Groningen, Groningen (n = 223) the Netherlands. Healthy controls consisted of 1,260 Dutch blood bank donors collected at the University Medical Center Groningen, Groningen. All cases and controls were of European Caucasian descent. Patients were diagnosed according to accepted clinical, endoscopic, radiological, and histological findings.\(^2\) For UC patients, phenotypes were described according to age of onset, maximum extent of disease (proctitis, left-sided, or extensive), necessity of colectomy, and the occurrence of malignancy and extra intestinal manifestations. Clinical data of the study population are presented in table 1.

In all cases, informed consent was obtained using protocols approved by the local institutional review board in all participating institutions. All patients and controls gave informed consent and DNA samples were handled anonymously.

DNA samples and SNP genotyping

All individuals in the study populations provided blood samples, DNA was extracted according to standard protocols.\(^13\) Samples that displayed undetermined genotypes for all tested SNPs were excluded from analysis (n = 17), assuming insufficient DNA quality. All excluded samples were cases, so the final number of cases was 821.

For this study the three SNPs showing genome-wide significant association in the WTCCC2 GWA study\(^4\) were selected: SNP rs6017342 (HNF4α), SNP rs1728785 (CDH1)
and SNP rs886774 (LAMB1). Probably due to an unknown polymorphism in the primer, SNP rs886774 (LAMB1) gave technical problems: genotypes of a group of individuals could not reliably be called. This SNP was replaced by a proxy SNP rs6949033 (D’ and r^2 = 1). Genotyping was performed at the Department of Genetics, University Medical Center Groningen, using Taqman technology and SNP genotyping assays for PCR obtained from Applied Biosystems (Nieuwerkerk a/d IJssel, the Netherlands) between December 2009 and March 2010. Genotyping of the SNPs was successful with a call rate > 95%.

### Statistical analysis

All genotypes obtained were tested for Hardy-Weinberg equilibrium by \( \chi^2 \)-testing. Deviation from Hardy-Weinberg equilibrium was defined when observed genotypes differed significantly from expected genotypes. Differences in allele and genotype distribution in cases and controls were tested for significance by the \( \chi^2 \)-test, and odds ratios (OR) and confidence intervals (CI) were calculated. Association between SNPs and sub-phenotypes were calculated using a within-cases analysis. The sub-phenotype analyses consisted of limited disease (proctitis) against left-sided and extensive disease; and extensive disease against proctitis and left-sided. The sub-phenotype analyses were also tested for statistical significance using the \( \chi^2 \)-test. P-values from the sub-phenotype analysis were corrected for multiple testing with Bonferroni’s correction for six analyses. To test for interaction between two loci an epistasis analysis was performed. Combined allele frequencies for paired SNPs were compared between cases and controls and tested for significance by \( \chi^2 \)-testing. All analyses were performed using Plink association analysis toolset.\(^{14}\) All significant thresholds were set at \( P < 0.05 \).
HNF4α and CDH1 are associated with ulcerative colitis in a Dutch cohort.

RESULTS
Genotyping success rate and Hardy-Weinberg equilibrium
After excluding cases with insufficient DNA quality, 97.7% of the cases and 98.0% of the controls were successfully genotyped. Controls showed no deviation from Hardy-Weinberg equilibrium for all tested SNPs.

Replication of susceptibility loci from the UC-GWA study
Allelic association analysis results are shown in table 2. SNP rs6017342 in the HNF4α locus was strongly associated with UC (p-value = $1.04 \times 10^{-11}$, OR = 1.56, CI = 1.37–1.77), which reached genome-wide significant association. Based on genotype frequency, the P-value is $6.65 \times 10^{-11}$ (OR = 1.56, CI = 1.37–1.79). Results from the sub-phenotype analyses are shown in table 3. The association could not be specified for limited or severe UC sub-phenotypes in the sub-phenotype analysis. SNP rs1728785 (CDH1) was associated to UC with a P-value of 0.01 (OR of 1.23, CI = 1.05–1.45). This locus was not associated with UC (p-value = 1.04 \times 10^{-11}, OR = 1.23, CI = 1.37–1.77). However, this association could not be specified for limited or severe UC sub-phenotypes in the sub-phenotype analyses.

No statistically significant epistasis was seen for the three SNPs. The strongest interaction was seen between rs1728785 (CDH1) and rs6017342 (HNF4α) with a P-value of 0.05.

DISCUSSION
We confirmed the association between UC and two susceptibility loci previously identified by the WTCCC2 and UK IBD Genetics Consortium: HNF4α and CDH1. The HNF4α locus was also strongly associated with UC in the WTCCC2.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr Positiona</th>
<th>Gene</th>
<th>Risk allele</th>
<th>RAF controls</th>
<th>RAF cases</th>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6017342</td>
<td>20q13.12</td>
<td>HNF4α</td>
<td>C</td>
<td>0.49</td>
<td>0.60</td>
<td>$1.04 \times 10^{-11}$</td>
<td>1.56</td>
<td>1.37–1.77</td>
</tr>
<tr>
<td>rs1728785</td>
<td>16q22.1</td>
<td>CDH1</td>
<td>C</td>
<td>0.78</td>
<td>0.81</td>
<td><strong>0.01</strong></td>
<td>1.23</td>
<td>1.05–1.44</td>
</tr>
<tr>
<td>rs6949033b</td>
<td>7q31.1</td>
<td>LAMB1</td>
<td>A</td>
<td>0.42</td>
<td>0.44</td>
<td>0.12</td>
<td>1.11</td>
<td>0.97–1.26</td>
</tr>
</tbody>
</table>

aPosition NCBI Build 36.1 coordinates
bProxy for rs886774 (D‘ and r² = 1).

Statistically significant associations in bold.

SNP, single nucleotide polymorphism; Chr, chromosome; RAF, risk allele frequency; OR, odds ratio; CI, confidence interval.
Table 3: Sub-phenotype analysis.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Minor allele</th>
<th>Sub phenotype analysis</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6949033</td>
<td>LAMB1</td>
<td>A</td>
<td>limited disease A</td>
<td>0.64</td>
<td>0.44–0.93</td>
<td>0.02</td>
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<tr>
<td>rs6949033</td>
<td>LAMB1</td>
<td>A</td>
<td>extensive disease A</td>
<td>0.69</td>
<td>0.45–0.99</td>
<td>0.20</td>
</tr>
<tr>
<td>rs1728785</td>
<td>CDH1</td>
<td>A</td>
<td>limited disease A</td>
<td>1.33</td>
<td>0.86–2.07</td>
<td>0.08</td>
</tr>
<tr>
<td>rs1728785</td>
<td>CDH1</td>
<td>A</td>
<td>extensive disease A</td>
<td>0.76</td>
<td>0.56–1.04</td>
<td>0.96</td>
</tr>
<tr>
<td>rs6017342</td>
<td>HNF4a</td>
<td>A</td>
<td>limited disease A</td>
<td>0.99</td>
<td>0.69–1.43</td>
<td>0.34</td>
</tr>
<tr>
<td>rs6017342</td>
<td>HNF4a</td>
<td>A</td>
<td>extensive disease A</td>
<td>1.12</td>
<td>0.88–1.43</td>
<td>0.34</td>
</tr>
</tbody>
</table>

aUncorrected for multiple testing.

GWA study (P-value = 8.5 × 10⁻¹⁷). The CDH1 locus was associated with a P-value of 2.8 × 10⁻⁸ in the WTCCC2 GWA study, comparable to the LAMB1 locus (P-value = 3.0 × 10⁻⁸), which could not be confirmed in the current study. The direction of the association for the associations is the same as in the WTCCC2 GWA study. However, the magnitude of association for both HNF4a and CDH1 is even larger.

The HNF4a locus showed the strongest association with UC. This locus was also the most associated locus in the WTCCC2 and UK IBD Consortium GWA study. It is extraordinary to discover a locus this strongly associated with UC after so many performed GWA studies. This can be explained by the simple fact that this SNP was not tested on previously used platforms.⁵,⁷ The HNF4a, or hepatocyte nuclear factor 4 alpha gene is the most likely candidate gene in this locus at 20q13. HNF4a is a nuclear transcription factor, controlling expression of multiple genes.¹⁵ From a functional perspective, HNF4a is important for epithelium homeostasis, cell function and cell architecture in the liver and the gastrointestinal tract.¹⁶⁻¹⁹ In the gastrointestinal tract, the protein controls the expression of several components of the cell-cell junction in the intestinal epithelium. In mice, loss of HNF4a leads to increased paracellular permeability through impairment of cell-cell junctions, indicating that HNF4a is crucial for the barrier function of the intestinal mucosa.¹⁸ Another important function of HNF4a is its role as a transcriptional regulator of ion transport: loss of HNF4a in mice initiates loss of mucosal homeostasis through a decline in mucosal ion transport. This loss of mucosal homeostasis triggers a chronic inflammatory response in the colon of the mice and worsens tissue damage in experimental colitis.²⁰,²¹ Both the impairment of the integrity of the intestinal epithelium and loss of mucosal homeostasis have been shown to be primary events in UC pathogenesis, which makes HNF4a a very attractive candidate gene for the disease. Other genes surrounding HNF4a are TTPAL, ADA, SERINC3 and PKIG. ADA (adenosine deaminase) has been associated with severe combined immunodeficiency disease.
HNF4α and CDH1 are associated with ulcerative colitis in a Dutch cohort (SCID), causing a dysfunction of both B and T lymphocytes with impaired cellular immunity and decreased production of immunoglobulins.22 However, nor ADA or the other genes are functionally as interesting as HNF4α.

The second UC associated locus we confirmed is the CDH1 locus. The CDH1 locus at 16q22 comprises three genes of which CDH1 is the most plausible candidate gene. CDH1 encodes E-cadherin, a protein that plays an important role in cell-cell adhesions.23 Several studies suggest that loss or mislocalization of E-cadherin causes IBD through disruption of cell-cell contacts and increased permeability.24, 25 Interestingly, different bacteria are able to mediate adhesion to epithelial cells and disrupt the cell-cell adhesions by means of down-regulating E-cadherin.26 In this way E-cadherin is important for invasion and adhesion of pathogens, which may contribute to IBD pathogenesis. Furthermore, CDH1 mutations are associated with multiple epithelial tumours, like gastric cancer, esophageal cancer and colorectal cancer.27–29 In these tumors, loss of E-cadherin causes increased proliferation, invasion, and metastasis.30,31 Especially the association between CDH1 mutations and colorectal cancer (CRC) is interesting, because patients with UC are more prone to develop CRC.32 The association between CDH1 and both traits gives us an indication that there might be a shared genetic background between the two diseases.

CDH3, encoding P-cadherin, is the second gene in the 16q22 locus. P-cadherin, like E-cadherin is a member of the cadherin superfamily. In contrast to E-cadherin, P-cadherin has not been related with IBD in functional studies, therefore CDH1 was selected as the most probably candidate gene in the original GWAS study.4 The third gene in this locus is a zinc finger protein, ZFP90, no specific function for this gene is known. Finemapping of this region and additional functional studies are needed to clarify which gene is actually the causative gene within the locus.

Interestingly, E-cadherin and HNF4α interact in the Wnt/β-catenin signaling pathway. Loss of HNF4α induces mislocalization of E-cadherin, which results in destabilized cell-cell junctions and increased intestinal permeability.14 These findings suggest that having defects in both genes would destabilize cell-cell junction further, increasing the risk of IBD. We did not see epistasis between the HNF4α and CDH1 risk variants in our dataset, but this could be explained by lack of statistical power.

The fact that we could not replicate the association between the LAMB1 locus and UC could be due to the lack of statistical power. Post hoc power analysis revealed that for LAMB1 the power to detect an association with an OR of 1.11 was only 32%. Other possible causes are genetic heterogeneity, or a difference in disease phenotypes between the British discovery cohort and our Dutch cohort. Cases in this study more often have extensive disease than the population in the WTCCC2 GWA study. Also, cases described in our study have a lower age at diagnosis, and undergo colectomy more often than the British cases. Differences are statistical significant (P<0.0001). Differences might have arisen because the Dutch cases were all selected at tertiary referral centers. Moreover, in the subphenotype analysis we found a trend towards association for less extensive disease. These observations suggest that our negative findings were due to more severe disease phenotypes in our cohort and that LAMB1 mostly influences risk for mild UC.

In conclusion, this is the first independent study to replicate the HNF4α and CDH1 loci as susceptibility loci for UC. The main candidate genes in these risk loci play important roles in the maintenance of the integrity of the epithelial barrier, highlighting the importance of the mucosal barrier function for UC pathogenesis.

REFERENCES

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