

## SHORT REPORT

# No evidence for involvement of *IL-4R* and *CD11B* from the IBD1 region and *STAT6* in the IBD2 region in Crohn's disease

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Linkage studies have identified the inflammatory bowel disease (IBD)1 locus on chromosome 16 and the IBD2 locus on chromosome 12 to be involved in Crohn's disease. *NOD2/CARD15* was identified as the gene of interest within the IBD1 region. However, linkage to this region could not be explained by *NOD2/CARD15* alone. Here we set out to assess the association of additional candidate genes from the IBD1 and IBD2 loci with Crohn's disease using transmission disequilibrium testing in patient–parent triads. No significant association was observed with genetic variants in the genes coding for interleukin-4 receptor gene (*IL-4R*), *CD11B* and signal transducer and activator of transcription type 6 (*STAT6*). Results for *IL-4R* were not affected by exclusion of all families carrying one of three risk alleles in *NOD2*. From this we conclude that *IL-4R* and *CD11B* in the IBD1 region and *STAT6* in the IBD2 region are not involved in Crohn's disease in this Dutch cohort.

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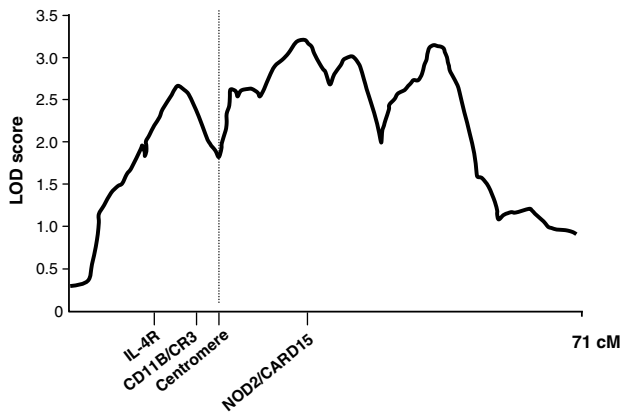
## Introduction

Genetic susceptibility to inflammatory bowel disease (IBD) depends on the contribution of multiple genes, rather than a single genetic factor.<sup>1</sup> Linkage studies revealed the IBD1 region on chromosome 16 to be involved in Crohn's disease (CD), and to a lesser extent in ulcerative colitis (UC) and the IBD2 region on chromosome 12 in UC, and to a lesser extent in CD. Both regions harbor many genes, some of which encode proteins involved in immune response, which are good candidates for CD. Recently, the *NOD2/CARD15* gene was identified as a CD susceptibility gene within the IBD1 region with a population attributable risk

of 25–30%.<sup>2</sup> However, the *NOD2/CARD15* gene cannot by itself explain the magnitude of the genetic linkage detected in previous studies. Hampe *et al*<sup>3</sup> recently provided strong evidence for the existence of another IBD gene within IBD1. The interleukin-4 receptor (*IL-4R*) gene forms an excellent candidate due to its location, the gene coincides with a strong linkage peak in IBD1 (Figure 1), and its function in a signaling pathway leading to T-cell differentiation, which is important in inflammatory responses.<sup>4,5</sup> Another putative candidate in IBD1 is the gene encoding complement receptor type 3 (*CD11B*). *CD11B* is a leukocyte surface antigen involved in granulocyte cell adhesion and is upregulated in patients with IBD.<sup>6</sup> Within the IBD2 region, the gene encoding the signal-transducer-and-activator-of-transcription (*STAT6*) is located. *STAT6* plays a role in the aforementioned *IL-4R* pathway, and therefore represents a good candidate for CD susceptibility.<sup>5,7</sup> The aim of the present study was to

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**Figure 1** Location of the linkage peaks in the pericentromeric IBD1 region on chromosome 16 (16p12.3–16q13) obtained through different CD studies (modified from 3). The approximate locations of the genes coding for IL-4R, CD11B and NOD2/CARD15 are represented on the X-axis, the position of the centromere is indicated by a dashed line. cM: centiMorgan.

**Table 1** Clinical characteristics of patients with CD

Characteristics	CD patients (n = 106)
Gender (male/female)	40/66
Age of onset (year)	22.9 ± 7.5
Location of disease	
Ileum	32 (30)
Colon	18 (17)
Ileum and colon	56 (53)
History of bowel resection	58 (55)
History of fistula	51 (48)

Results given as number of patients, percentage in brackets, or as mean ± SD.

determine whether genetic polymorphisms in candidate genes with immune regulatory functions located in IBD1 and IBD2 are associated with CD.

### Material and methods

DNA was obtained from 110 Caucasian patients with confirmed CD, of which 106 unrelated patients (66 female) were included in the analysis, as were 147 of their parents (56 complete triads, 35 dyads, 15 patients without parents). All patients originated from The Netherlands. Clinical characteristics of the patients are shown in Table 1. The transmission disequilibrium test (TDT)-PHASE package was used to test for association ([www.hgmp.mrc.uk/~fdudbrid/software/unphased](http://www.hgmp.mrc.uk/~fdudbrid/software/unphased)). This package allows inclusion of families with one or both parents missing. Applying Bonferroni's correction for multiple testing (number of tests = 4), *P*-values < 0.0127 were considered significant.

A fragment located in the 5' region of the *CD11B* gene was polymerase chain reaction (PCR)-amplified and single-strand conformation polymorphism (SSCP)-analyzed to

identify a suitable single nucleotide polymorphism (SNP) for association testing. The following primers were used: forward 5'-TGCTGGCATTACAGGCCGTGA-3'; reverse 5'-GTAGGAGGCAGAAGTCTATC-3'. Aberrant bands were analyzed by direct sequence analysis on an ABI3700 automatic sequencer using BigDye terminator chemistry.

Several SNPs were typed using Pyrosequencing® technology,<sup>8</sup> according to the protocol of the manufacturer. Primers for PCR amplification: IL-4R-I50V: forward primer 5'-biotin-CCAGATCTGTCTCACATCCGT-3', reverse primer 5'-CATGCTCGCTGGGCTTGAAG-3'; IL4R-Q576R: forward primer 5'-biotin-CGCCGAAATGTCTCCAGC-3', reverse primer 5'-CCCTGCTCCACCGCATGTA-3'; CD11B: forward primer 5'-biotin-TGCTGGCATTACAGGCCGTGA-3', reverse primer 5'-GTAGGAGGCAGAAGTCTATC-3'; STAT6: forward primer 5'-AAGGGCTGAGATTCCTCGTGT-3', reverse primer 5'-biotin-CCCAAATTTGTGTTGTCACGTA-3'; NOD2-G908R: forward primer 5'-TACTGCAGAGGGAGGAGACT-3', reverse primer 5'-biotin-CACCTGATCTCCC CAAGAAA. Sequence primers for pyrosequence analysis: IL-4R-I50V: 5'-CCGTTGTTCTCAGGG-3'; IL4R-Q576R: 5'-CCGCATGTACAACTCC-3'; CD11B: 5'-GCATATGAACAA GCTGAA-3'; STAT6: 5'-TGTACCTAGGATATGTTA-3'; NO-D2-G908R: 5'-CCTTTTCAGATTCTGGG-3'.

The NOD2 C-insertion polymorphism was typed via PCR amplification of the genomic region and subsequent gel electrophoresis on a 6.6% polyacrylamide gel.<sup>9</sup> PCR was conducted using the following primers: forward 5'-AATCCTTGAAGCTCACCATTG-3', reverse 5'-CAGGGGCC TTACCAGACT-3'. Allelic bands were visualized by silver staining. The NOD2-R702S polymorphism was analyzed by restriction fragment length polymorphism analysis using *HpaII*.<sup>9</sup> The primers used were: forward 5'-GAGCACTG GGGCCTGCTG-3', reverse 5'-GGATGGAGTGGAAAGTG CTTG-3'.

### Results

#### CD11B and STAT6

SSCP analysis of part of the 5' end of the *CD11B* gene revealed a frequent polymorphism in the promoter region, identified as an A/G variation at nucleotide position -324 (Genbank M76724; the first nucleotide 5' to the ATG start codon denoted -1). No transmission distortion of this particular *CD11B* polymorphism was observed in the patients with CD (*P* = 0.548; Table 2). For *STAT6*, we analyzed an A/G SNP (dbSNP identity: rs4559) in the 3' UTR, but here also no significant association of this polymorphism with CD was found (*P* = 0.190; Table 2).

#### IL-4R

For analysis of *IL-4R*, we used two previously reported SNPs in the coding region of the gene<sup>10</sup> (Table 2). Both SNPs concern A/G variants leading to variation at the protein level (I50V and Q576R). Although located in the same

**Table 2** Results of the TDT-phase analysis in 106 patients with CD

Gene	SNP	Transmitted	Nontransmitted	OR	P-value
IL-4R (I 50V)	A	120.2	121.4	1	0.928
	G	99.8	98.6	1.023	
IL-4R (Q576R)	A	187.9	166.7	1	0.028
	G	32.1	53.3	0.533	
IL-4R (Q576R) <sub>-NOD2</sub>	A	124.6	113.2	1	0.157
	G	23.4	34.8	0.609	
CD11B	A	143.3	150	1	0.548
	G	72.7	66	1.154	
STAT6	A	147.0	131.7	1	0.190
	G	73.0	88.3	0.740	

gene, the polymorphisms were in complete equilibrium with each other ( $P=0.706$ ). No association of the I50V variant and CD was found ( $P=0.928$ ). Analysis of Q576R revealed an OR of 1.86 for 576Q, and a  $P$ -value of 0.028. Although suggestive for association, this  $P$ -value could not be considered significant after Bonferroni's correction. To ensure that these latter findings were not obscured by mutations in *NOD2/CARD15* in the same region, we typed the three major susceptibility mutations found in this gene (see below) and repeated the IL-4R Q576R analysis excluding patients carrying one or more *NOD2/CARD15* variants ( $n=36$ ). This resulted in a nonsignificant  $P$ -value of 0.157.

#### NOD2/CARD15

Three functional variants in *NOD2* (R702W, G908R, 3020insC) were analyzed.<sup>9</sup> Allele frequency of 3020insC variant in patients was 8.1% ( $n=105$ ) with nine heterozygous and four homozygous subjects. The R702W variation was observed with a frequency of 13.2% in patients ( $n=106$ ), 26 being heterozygous and a single one homozygous. Variant G908R was not observed. Four out of 105 patients were compound heterozygotes. The proportion of patients with at least one variant *NOD2/CARD15* allele was 34.0%. Recently, it has been shown that *NOD2/CARD15* mutations are specifically involved in ileum-specific CD.<sup>11</sup> When confining our analysis to patients with ileum-specific disease ( $n=32$ ), 46.9% possessed at least one variant *NOD2/CARD15* allele.

#### Discussion

Recent findings suggest that approximately 25–30% of the genetic susceptibility in CD can be explained by mutations in *NOD2/CARD15*, a gene located in the IBD1 region.<sup>2</sup> The broad-IBD1 region on chromosome 16 is extending from 16p12.3 to 16q13. At the boundary of this region at 16p12.1, the *IL-4R* gene is located. Although this gene can be regarded as a candidate for CD, we found no significant association with two SNPs, I50V and Q576R, located in the coding region of the gene. Both SNPs were in complete equilibrium with each other, which is in accordance with previous results of Olavesen *et al.*<sup>10</sup> We re-evaluated the

association with the Q576R variant after excluding families for which the patient carried at least one variant *NOD2/CARD15* allele. For this purpose, the three most prominent variants in *NOD2/CARD15* were analyzed. At least one risk allele was found in 34% of all CD patients and in nearly 47% of patients with ileum-specific CD. Exclusion of patients with an *NOD2/CARD15* risk allele did not result in a stronger association of the IL4R-Q576R allele with the disease, suggesting that the *IL-4R* gene is not a major risk factor for CD. The same holds for another candidate gene in IBD1 on 16p11.2, the *CD11B* gene. In IBD2 on chromosome 12, no evidence was found for an association of the *STAT6* gene with CD.

In conclusion, in this Dutch cohort no evidence for association was observed between CD and alleles of the genes encoding the IL-4 receptor, CD11B or the STAT6 by TDT. However, the number of SNPs and patients analyzed in the present study is limited. Typing of additional SNPs in larger panels of patients will be needed to define putative risk haplotypes at each locus in more detail.

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