



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease

Citation for published version:

Noble, CL, Nimmo, ER, Drummond, H, Ho, G-T, Tenesa, A, Smith, L, Anderson, N, Arnott, IDR & Satsangi, J 2005, 'The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease', *Gastroenterology*, vol. 129, no. 6, pp. 1854-64.
<https://doi.org/10.1053/j.gastro.2005.09.025>

Digital Object Identifier (DOI):

[10.1053/j.gastro.2005.09.025](https://doi.org/10.1053/j.gastro.2005.09.025)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Gastroenterology

Publisher Rights Statement:

© 2005 by the American Gastroenterological Association

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



The Contribution of OCTN1/2 Variants Within the *IBD5* Locus to Disease Susceptibility and Severity in Crohn's Disease

COLIN L. NOBLE,* ELAINE R. NIMMO,* HAZEL DRUMMOND,* GWO-TZER HO,* ALBERT TENESA,† LINDA SMITH,* NORMAN ANDERSON,* IAN D. R. ARNOTT,* and JACK SATSANGI*

*Gastrointestinal Unit and †MRC Human Genetics Unit, Western General Hospital, University of Edinburgh, Edinburgh, Scotland

See editorial on page 2106.

Background & Aims: Recent data suggest that polymorphisms in the organic cation transporter (OCTN) genes OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*) represent disease-causing mutations within the *IBD5* locus (chromosome 5q31). We investigated associations with disease susceptibility, phenotype, and evidence for epistasis with *CARD15* in 679 patients with Crohn's disease (CD) or ulcerative colitis (UC). **Methods:** A total of 374 patients with CD, 305 patients with UC, and 294 healthy controls (HCs) were studied. Genotyping for single nucleotide polymorphisms IGR2096, IGR2198, and IGR2230, OCTN1 variant (*SLC22A4* 1672C→T), and OCTN2 variant (*SLC22A5* –207G→C) was performed using the Taq-Man system. **Results:** The *IBD5* OCTN1 and OCTN2 polymorphisms were in strong linkage disequilibrium (D' , >0.959). IGR2198 variant allele frequency (49.1% vs 40.8%; $P = .0046$) and homozygosity (21% vs 14.8%; $P = .044$) were associated with CD versus HCs. Variant allelic frequency of OCTN1 (53.6% vs 43%; $P = .0008$) and OCTN2 (56.1% vs 48.4%; $P = .0092$) polymorphisms and homozygosity for the OCTN1/2-TC haplotype (28.4% vs 16%; $P = .0042$) were associated with CD versus HCs. IGR2198 homozygosity and TC homozygosity were associated with stricturing/penetrating disease at follow-up ($P = .011$ and $P = .011$, respectively) and disease progression ($P = .038$ and $P = .049$, respectively) on univariate analysis and with need for surgery on multivariate analysis ($P = .016$ and $P = .004$, respectively). In the absence of the *IBD5* risk haplotype, no association of OCTN1/2 variants with CD was detected. No associations were seen with UC. **Conclusions:** The *IBD5* locus influences susceptibility, progression, and need for surgery in CD. However, the contribution of OCTN1/2 variants is not independent of the *IBD5* haplotype; a causative role for these genes remains plausible but is not yet proven. Further genetic, functional, and expression data are now required.

The inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are common causes of gastrointestinal morbidity in the Western world. The incidence of early-onset disease continues to increase in northern Europe, notably in Scotland and Scandinavia.^{1–4} Epidemiologic, molecular, and clinical studies have proven that genetic susceptibility combined with environmental interaction are central to the pathogenesis of IBD.⁵

Genome-wide scanning has identified susceptibility loci for CD on chromosomes 1,⁶ 5 (*IBD5*),^{7–9} 6 (*IBD3*; *HLA*),^{8,10} 12 (*IBD2*),¹¹ 14 (*IBD4*),^{7,12} 16 (*IBD1*),^{11,13} and 19 (*IBD6*).⁸ The most consistently replicated CD susceptibility locus is located on chromosome 16 (*IBD1*), and the susceptibility gene has been identified as the *NOD2/CARD15* gene.^{14,15} *NOD2/CARD15* contains a caspase recruitment domain (CARD) that is linked to a nucleotide-binding domain and a leucine-rich repeat region. *NOD2/CARD15* functions as an intracellular sensor of muramyl dipeptide, a highly conserved peptidoglycan motif common to many intraluminal bacteria.^{16,17} Watanabe et al suggested that *NOD2/CARD15*^{–/–} mice lose negative control of Toll-like receptor 2-mediated activation of nuclear factor κ B, potentially offering an explanation for the Th1 phenotype characteristic of CD.¹⁸ However, recently published studies do not provide support for *NOD2/CARD15* interaction with the Toll-like receptor 2 pathway, and these data emphasize the complexity of *NOD2/CARD15* activation.^{19,20}

Two single nucleotide polymorphisms (SNPs) (Gly908Arg and Arg702Trp) and a frameshift mutation (Leu1007fsinsC) induce structural changes in the leucine-rich region of *NOD2/CARD15* and have been associated with CD. Reported *NOD2/CARD15* carriage

Abbreviations used in this paper: HC, healthy control; OCTN, organic cation transporter; SNP, single nucleotide polymorphism.

© 2005 by the American Gastroenterological Association

0016-5085/05/\$30.00

doi:10.1053/j.gastro.2005.09.025

rates in CD vary between 0 and 50.0%, with highest rates seen in central European populations,^{14,21} while mutations are absent in Japanese and Chinese series.^{22,23} Lower frequencies have been reported from Finland and Scotland, suggesting heterogeneity within Europe.^{24,25} *NOD2/CARD15* polymorphisms have consistently been associated with a younger age of onset of CD, ileal disease, and fibrostenosing disease.²⁶

The *IBD5* locus on chromosome 5q31-33 was originally identified in 1999 as conferring susceptibility for CD.⁷ This finding was replicated using linkage disequilibrium mapping in the Canadian CD population and further delineated to 5q31.⁸ Fine mapping of this area has identified a single, highly conserved 250-kilobase haplotype of 11 SNPs spanning a cytokine gene cluster that is associated with CD.⁹ Further high-resolution analysis of the 5q31 region using 103 SNPs revealed 11 discrete haplotype blocks that measure tens of kilobases in length, have limited diversity, and are punctuated by sites of recombination.²⁷ Genotype-phenotype analysis has suggested an association between the *IBD5* locus, perianal disease,²⁸ and early onset of disease,²⁹ but evidence for epistasis between *IBD5* and *NOD2/CARD15* has been inconsistent.^{9,28-30}

More recently, data have suggested that mutations of 2 genes within the *IBD5* region, the organic cation transporters OCTN1 (also known as *SLC22A4*) and OCTN2 (*SLC22A5*), may be independently associated with CD.³¹ The construction of a 2-allele risk haplotype OCTN1/2-TC (*SLC22A4* exon 9 1672C→T and *SLC22A5* promoter -207G→C) was reported to be associated with CD in patients who lacked the extended *IBD5* risk haplotype. Individual allelic data were not given.

OCTN1 is a 551-amino acid protein that is strongly expressed in the kidney, trachea, bone marrow, and, to a lesser extent, small bowel and has been characterized as a carnitine transporter.³² An intronic SNP (rs2268277) in a Runt-related transcription factor 1 (*RUNX1*) binding site of OCTN1 has been associated with susceptibility to rheumatoid arthritis in the Japanese population.³³ OCTN2 is a 557-amino acid protein that is 75.8% homologous to OCTN1, and functional studies have shown it to be a high-affinity sodium carnitine transporter that is expressed in the kidney, smooth muscle, and heart tissue.³⁴ Peltekova et al suggested that the OCTN1 variant (1672C→T) alters its function in fibroblasts in vitro, with variant forms having less affinity for carnitine and a greater affinity for tetraethyl ammonium and some xenobiotics, and that the OCTN2 variant (-207G→C) disrupted a heat-shock transcription fac-

tor binding site in fibroblasts in vitro.³¹ No data demonstrating the altered function or expression of OCTN1/2 gene products in CD have yet been provided.

The provocative association data from Peltekova et al have yet to be replicated in an independent population, and epistasis with *NOD2/CARD15* has not thus far been assessed without the Canadian population. Phenotypic data from the same Canadian cohort of patients with CD have suggested a correlation between OCTN1/2 variants and ileal disease, and this correlation becomes substantially stronger when carriage of *NOD2/CARD15* variants is taken into account.³⁵ However, the phenotypic description of this cohort does not allow for analysis of disease behavior, progression, or indeed proximal small bowel disease.

In the present study, we assessed the contribution of the OCTN1 and OCTN2 polymorphisms implicated by Peltekova et al in determining genetic susceptibility in CD and UC, specifically addressing whether these OCTN1/2 variants have an association with susceptibility to IBD that is independent of other markers within the extended *IBD5* linkage interval. We also investigated whether these polymorphisms are associated with specific disease phenotype characteristics or progression in our rigorously defined IBD population and assessed gene-gene interactions with established *NOD2/CARD15* mutations.

Materials and Methods

A total of 679 patients with well-characterized IBD and 294 healthy controls (HCs) were recruited. All patients with IBD attended the clinic at Western General Hospital (Edinburgh, Scotland). This is a tertiary referral center for IBD in southeastern Scotland. The IBD group comprised 374 patients with CD and 305 patients with UC. The diagnosis of IBD adhered to the criteria of Lennard-Jones.³⁶ Age at diagnosis, location, and behavior were classified according to the Vienna classification.³⁷ Phenotypic data were collected by patient questionnaire, interview, and case note review and were composed of demographics, date of onset and diagnosis, disease location, disease behavior, progression, extraintestinal manifestations, surgical operations, smoking history, joint symptoms, family history, and ethnicity. Written informed consent was obtained from all patients. The Medicine and Oncology Subcommittee of the Lothian Local Research Ethics Committee approved the study protocol (LREC 2000/4/192).

Demographics

CD group. The demographics of the patients recruited are shown in Table 1. The duration of follow-up was defined as the time from diagnosis to the time of most

Table 1. Demographics and Clinical Features of the CD, UC, and Control Groups

	CD	UC	Controls
Total no.	374	305	294
Sex (male/female)	181/193	171/134	143/151
Median age at diagnosis, y (interquartile range)	27.8 (20.9–40.4)	34 (25–50)	39 (27–52)
Median duration of follow-up, y (interquartile range)	11.8 (6.5–20.2)	7.5 (3.35–13.4)	
non-Jewish White (%)	98.7	98.3	
Age at diagnosis, A1 (younger than 40 years)/A2 (older than 40)	72%/28%		
Location at diagnosis (%)			
Ileal disease (L1)	125 (34)	Proctitis: 105 (34.5)	
Colonic disease (L2)	139 (38)	Left-sided colitis: 116 (37)	
Ileal and colonic disease (L3)	58 (16)	Extensive colitis: 84 (27.5)	
Upper gastrointestinal disease (L4)	30 (8)		
Oral CD	6 (2)		
Perianal disease	75 (21)		
Disease behavior at diagnosis (%)			
Inflammatory (Vienna B1)	258 (74)		
Stricturing (Vienna B2)	32 (9)		
Penetrating (Vienna B3)	61 (17)		
Disease behavior at follow-up (%)			
Inflammatory (B1)	128 (34)		
Stricturing (B2)	70 (19)		
Penetrating (B3)	168 (48)		
Disease progression			
No progression from inflammatory (% of inflammatory at diagnosis)	126 (49)		
Inflammatory to stricturing or penetrating (%)	132 (51)		
Surgery for luminal complications of CD (%) ^a	237 (63)		

NOTE. Disease behavior at follow-up was defined using the Vienna classification when the patient was last clinically evaluated. Full phenotypic data were available on 94% of the patients with CD at diagnosis and 98% at follow-up. In assessing disease progression, patients were grouped as those who remained as having inflammatory (B1), nonprogressive disease and those whose disease had progressed to stricturing (B2) or penetrating (B3) disease.

^aExamination under anesthesia or drainage procedures for perianal sepsis were excluded.

recent clinic review. Disease behavior at follow-up was defined using the Vienna classification of behavior at the time point when the disease was last clinically evaluated (radiologically, endoscopically, or at surgery).^{24,37} In assessing disease progression, patients who had Vienna B1 (inflammatory) CD at diagnosis were identified and disease behavior was compared with behavior at most recent follow-up. Patients were grouped as those who remained classified as having inflammatory (B1) disease at follow-up (nonprogressive disease), and those whose disease had progressed to stricturing (B2) or penetrating (B3) disease. Ninety-seven patients (26%) in the CD group were current smokers, and 79 (21%) had a first-degree relative with a family history of IBD.

UC group. Clinical details were available regarding the entire UC group. The group consisted of 171 men and 134 women with a median age at diagnosis of 34 years (Table 1). Disease extent was recorded at diagnosis.

Control group. The control group comprised 294 healthy subjects: blood donors (n = 163) recruited from southeastern Scotland and healthy control subjects (n = 131). There were 143 men and 151 women with a median age of 39 years (interquartile range, 27–52 years).

Genotyping

Genomic DNA was extracted from peripheral venous blood by a modified salting-out technique³⁸ and resuspended in 1× Tris-EDTA buffer (10 mmol/L Tris [pH 8.0] and 1 mmol/L EDTA [pH 8.0]) at a final concentration of 100 ng/μL. To examine the relative contribution of the OCTN1/2 variants relative to the extended *IBD5* haplotype, 3 SNPs were genotyped: IGR2096 (which lies within haplotype block 4, as defined by Daly et al²⁷) (Figure 1), IGR2198 (within haplotype block 5), and IGR2230 (within haplotype block 7). The rs1050152 polymorphism of the OCTN1 gene (*SLC22A4* exon 9 1672C→T, IGR3002) and the rs26313667 (*SLC22A5* promoter −207G→C, IGR2222) polymorphism of the OCTN2 gene were typed. Details of these variants and of the TaqMan primers are provided in Supplementary Table 1 (see supplemental material online at www.mmc.med.ed.ac.uk/gi/supdata.shtml) and Figure 1.

Patients with CD and HCs were typed for polymorphisms of the *NOD2/CARD15* gene (R702W, G908R, and 1007fsinsC) using previously described methods.²⁴ All genotyping except the R702W polymorphism was performed using the TaqMan system (7900HT sequence detection system; Applied Biosystems, Foster

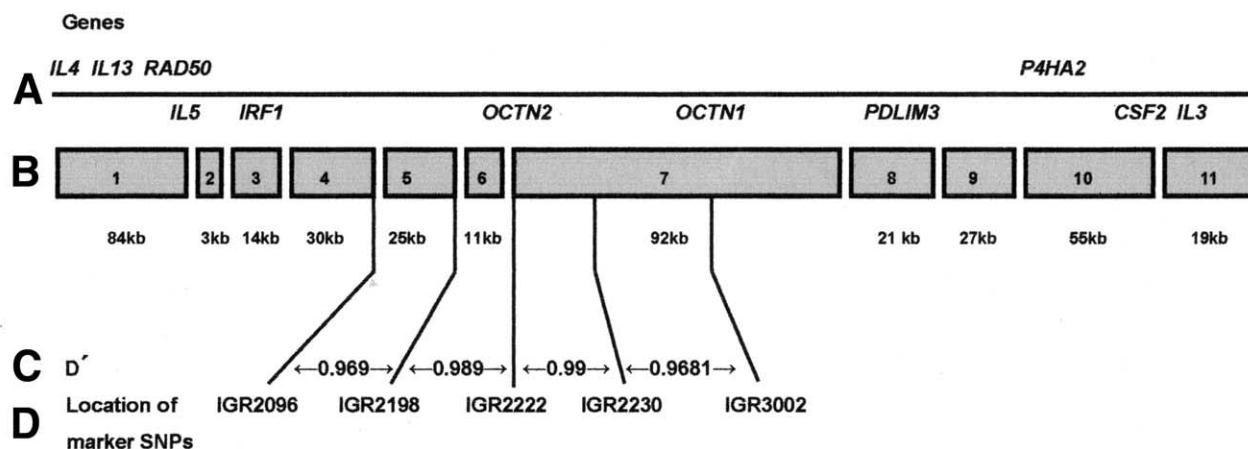


Figure 1. Haplotype structure and linkage disequilibrium across the *IBD5* region. The *IBD5* locus (5q31) with the high-resolution haplotype structure as reported by Daly et al.²⁷ (A) Candidate genes above the relevant haplotype blocks. Genes above the line are transcribed from left to right, and those below the line are transcribed from right to left. (B) Eleven blocks numbered 1–11 (between 3 and 92 kilobases), each of limited genetic diversity, are punctuated by sites of recombination. (C) D' scores are shown to demonstrate the tight linkage disequilibrium between the SNPs that were analyzed. The lowest D' (0.959) was observed between IGR2096 and the OCTN2 variant (–207G→C). (D) Location of the SNPs that were analyzed, with IGR2222 representing the OCTN2 variant (–207G→C) and IGR3002 representing the OCTN1 variant (1672C→T). IGR2078, which was used by Peltekova et al to represent the extended *IBD5* haplotype, is located in block 4.³¹

City, CA). R702W genotyping was performed by restriction fragment length polymorphism polymerase chain reaction. Restriction digestion was performed using 1 U *Msp* I at 37°C overnight and polymerase chain reaction fragments run on 4% NuSieve 3:1 agarose gels (Cambrex Bio Science, Nottingham, Ltd, Nottingham, UK). These were stained with ethidium bromide and viewed under UV light. An image was recorded digitally.

Data Analysis

Each SNP was analyzed for association with IBD overall, CD, UC, and disease phenotype. Allele frequencies and carrier frequencies were determined for all polymorphisms. Each of the variants studied was shown to be in Hardy–Weinberg equilibrium in patients and in controls. Genotype-phenotype associations were analyzed by χ^2 test using the Minitab statistical software package (Minitab Ltd, Coventry, England). Linkage disequilibrium and haplotype analysis were investigated using *cocaphase* (Rosland Franklin Centre for genomics research; <http://www.hgmp.mrc.ac.uk>). Evidence for epistasis among the OCTN1/2 allelic variants, the OCTN1/2-TC haplotype, and *NOD2/CARD15* variants was investigated by comparing allelic frequencies of the individual OCTN1/2 variants, together with homozygosity for the OCTN1/2-TC haplotype between the subgroups of patients with and without *NOD2/CARD15* variants by χ^2 analysis.

To identify significant independent variables associated with genotype, multiple logistic regression analysis was performed using the Minitab statistical software package (Minitab Ltd). The population-attributable risk percentage was defined as the excess rate of disease in individuals with a mutation compared with those without. This was estimated by the method of Schlesselman,³⁹ which estimates

population-attributable risk percentage as equal to attributable risk as a function of the prevalence in the exposed population divided by incidence of IBD in the population. To calculate this, the prevalence of CD was estimated at 100/100,000 and the frequency of all alleles in the control population was assumed to reflect that of the general population.

Results

Linkage Disequilibrium Across the *IBD5* Locus

In the CD population, strong linkage disequilibrium was observed between the SNPs within the extended *IBD5* haplotype as defined by Daly et al²⁷: IGR2096 (block 4), IGR2198 (block 5), OCTN2 (*SLC22A5* promoter –207G→C) (block 7), IGR2230 (block 7), and OCTN1 (*SLC22A4* exon 9 1672C→T) (block 7) (Figure 1). Pairwise D' values >0.959 between each SNP confirmed the difficulty of showing an independent effect of OCTN1/2 variants and *IBD5*. In the detailed analyses of the *IBD5* contribution to disease phenotype presented in this report, data are presented for the IGR2198 marker, representative of the 3-locus haplotype (IGR2096, IGR2198, and IGR2230). All data are available for each marker SNP on request.

Disease Susceptibility

Analysis of SNPs representing the extended *IBD5* haplotype (IGR2096, IGR2198, and IGR2230). Variant allelic frequencies differed significantly between patients with CD and HCs for each of the 3 *IBD5* SNPs studied

Table 2. *IBD5* Variant Allele Frequency, Carriage Rates, and Homozygote Frequency in Patients with CD and Controls

SNP/haplotype examined	Control frequency (%)	CD frequency (%)	P	Odds ratio (confidence interval)
Variant allelic frequency				
IGR2096	41.9	49.4	.03	1.42 (1.1–1.8)
IGR2198	40.8	49.1	.0046	1.40 (1.1–1.6)
OCTN2 (–207 G→C)	48.4	56.1	.0092	1.43 (1.2–1.8)
IGR2230	47.4	54.9	.011	1.35 (1.1–1.7)
OCTN1 (1672C→T)	42.9	53.6	.0008	1.48 (1.2–1.9)
Variant carriage rates				
IGR2096	173 (68.7)	264 (79)	.0034	1.75 (1.2–2.6)
IGR2198	171 (67)	258 (77)	.011	1.61 (1.2–2.3)
OCTN2 (–207 G→C)	187 (74)	280 (81)	.034	1.52 (1.0–2.2)
IGR2230	185 (73.7)	261 (80)	.047	1.5 (1.1–2.23)
OCTN1 (1672C→T)	178 (68.7)	267 (80)	.0024	1.77 (1.2–2.6)
TC haplotype	170 (69)	264 (80)	.0016	1.8 (1.3–2.7)
<i>IBD5</i> haplotype ^a	166 (63.6)	246 (73)	.016	1.5 (1.1–2.2)
Variant homozygote frequency				
IGR2096	38 (15.1)	73 (21.4)	.036	1.58 (1.0–2.4)
IGR2198	38 (14.8)	70 (21)	.044	1.56 (1.0–2.4)
OCTN2 (–207 G→C)	55 (22)	106 (31)	.0155	1.59 (1.1–2.3)
IGR2230	47 (18.6)	94 (29)	.0038	1.79 (1.2–2.6)
OCTN1 (1672C→T)	44 (17)	89 (25.6)	.011	1.69 (1.1–2.5)
TC haplotype	40 (16)	86 (28.4)	.0042	1.83 (1.2–2.8)
<i>IBD5</i> haplotype ^a	38 (14.6)	68 (20.5)	.071	1.48 (1.0–2.3)

NOTE. Three SNPs were used to define the extended *IBD5* locus (IGR2096, IGR2198, and IGR2230) (Figure 1). Each was independently associated with susceptibility to CD when allelic frequencies, carriage rates, and homozygosity were analyzed. OCTN2 variant (–207G→C) and OCTN1 variant (1672C→T) were also independently associated with susceptibility to CD when allelic frequencies, carriage rates, and homozygosity were analyzed. The 2-allele risk haplotype OCTN1/2-TC haplotype was associated with susceptibility to CD when carriage rates and homozygosity rates were analyzed.

^aA 2-allele risk haplotype using the *IBD5* marker SNPs IGR2198 and IGR2230 is also illustrated for comparison with the OCTN1/2-TC haplotype.

(IGR2096, 49.4% CD vs 41.9% HC [$P = .03$]; IGR2198, 49.1% CD vs 40.8% HC [$P = .0046$]; IGR2230, 54.9% CD vs 47.4% HC [$P = .011$]) (Table 2). Carriage rates for the *IBD5* variants were significantly higher in the patients with CD when compared with the control population (IGR2096, 79.3% CD vs 68.7% HC [$P = .0034$]; IGR2198, 77% CD vs 67% HC [$P = .011$]; IGR2230, 80% CD vs 73.7% HC [$P = .047$]). Individuals who were homozygous for the *IBD5* risk alleles at the 3 SNPs examined (IGR2096, IGR2198, and IGR2230) were more common in the CD group than in the control group (Table 2). The population-attributable risk for *IBD5* homozygosity was estimated as 15% if IGR2096 data were considered in calculation, 14.0% for IGR2198, and 14.3% for IGR2230.

No associations were observed between allelic frequency of *IBD5* variants and IBD overall (IGR2198, 45% IBD vs 40.8% HC [$P = .077$]) or UC (IGR2198, 43.7% UC vs 40.8% HC [$P = .325$]) (Supplementary Table 2; see supplemental material online at www.mmc.med.ed.ac.uk/gi/supdata.shtml).

Analysis of OCTN1/2 variants and OCTN1/2-TC haplotype. Allelic frequencies differed between patients with CD and controls for the OCTN1 variant

(*SLC22A4* exon 9 1672C→T) (53.6% CD vs 42.9% HC, $P = .0008$) and the OCTN2 variant (*SLC22A5* promoter –207G→C) (56.1% CD vs 48.4% HC, $P = .0092$) (Table 2). Carriage of the OCTN1/2-TC risk haplotype was present more frequently in patients with CD than in controls (80% CD vs 68.5% HC, $P = .0016$, OR, 1.8) (Table 2). It was clear that this difference related to homozygosity because OCTN1/2-TC homozygotes were more common in the CD group (28.4% CD vs 16.1% HC, $P = .0042$, OR, 1.83). No significant difference was observed between OCTN1/2-TC heterozygote rates ($P = .3$).

An association was observed between the OCTN1 variant and IBD when allelic frequencies were analyzed (48.9% IBD vs 42.9% HC, $P = .019$). This finding was not replicated in analysis of the OCTN2 variant (51.7% IBD vs 48.4% HC, $P = .162$), and no associations were observed between variant allelic frequencies of OCTN1 (44.7% UC vs 42.9% HC, $P = .53$) or OCTN2 (5% UC vs 48.4% HC, $P = .38$) and UC (Supplementary Table 2; see supplemental material online at www.mmc.med.ed.ac.uk/gi/supdata.shtml). No association was observed between the OCTN1/2-TC haplotype and IBD overall or with UC. There was no evidence of epistasis

Table 3. Frequency of OCTN1/2 Variant Alleles and the OCTN1/2-TC Haplotype in Patients with CD Stratified by Carriage of *NOD2/CARD15* Variants

SNP/haplotype examined	% <i>NOD2/CARD15</i> -positive patients (n = 84)	% <i>NOD2/CARD15</i> -negative patients (n = 188)	P
OCTN2 (–207 G→C) allelic frequency	55.2	56.3	.78
OCTN1 (1672C→T) allelic frequency	51.9	53.4	.69
OCTN1/2 TC haplotype carriage	72.3	71.1	.79
OCTN1/2 TC haplotype homozygosity	22.0	24.5	.574

NOTE. Analysis of evidence for epistasis between OCTN2 variant (–207G→C) and OCTN1 variant (1672C→T) and the TC haplotype of OCTN1/2 was investigated by stratifying OCTN1/2 variants by carriage of one or more of the 3 common *CARD15* variants: R702W, G908R, and 1007fsinsC. No evidence of epistasis was observed.

between the OCTN1/2 variants, the OCTN1/2-TC haplotype, and *CARD15* variants (Table 3).

The OCTN1/2 association with CD is not independent of the association with the extended *IBD5* haplotype. Previous data have suggested carriage of the OCTN1/2-TC haplotype to be an independent risk factor for CD. However, in our data set, for individuals who lacked the *IBD5* risk haplotype (homozygous with respect to the non-CD-associated alleles of IGR2096, IGR2198, and IGR2230), the OCTN1/2-TC haplotype was not associated with CD (Table 4). In the absence of variants in the marker SNP IGR2096, which is located in block 4²⁷ (Figure 1), 21.5% of the controls carried the OCTN1/2-TC haplotype compared with 30.4% in the CD group ($P = .22$). When the OCTN1/2-TC haplotype was analyzed in the absence of IGR2198 variants (block 5), 27.1% of the controls possessed the OCTN1/2-TC haplotype versus 17.1% of the patients with CD ($P = .13$). In the absence of variants in the marker SNP IGR2230, which is in the same block as OCTN1/2 (block 7), 3% of the controls possessed the

OCTN1/2-TC haplotype versus 1.6% of the patients with CD ($P = .59$).

Phenotypic Analysis

Age at diagnosis. No association was observed between the age at diagnosis in patients with IBD overall, or UC and the *IBD5* OCTN1/2 variant alleles. When allelic frequency was compared in subgroups defined by the Vienna classification for age in the patients with CD (A1, younger than 40 years; A2, older than 40 years), there was no significant difference between variant and wild-type allelic frequency for all studied SNPs.

Anatomic distribution. There was no association between the *IBD5* marker SNPs, OCTN1/2 variants, and CD when disease location was assessed by the Vienna classification (L1, terminal ileum; L2, colon; L3, ileocolonic; L4, upper gastrointestinal tract). Of note, no association was found between the presence of perianal CD

Table 4. Frequency of the OCTN1/2 TC Haplotype in Individuals Not Possessing Disease Susceptibility Risk Alleles at the Markers IGR2096, IGR2198, and IGR2230

	No. of subjects not possessing IGR2096 variants	OCTN1/2-TC haplotype frequency (%)	No. of subjects not possessing IGR2198 variants	OCTN1/2-TC haplotype frequency (%)	No. of subjects not possessing IGR2230 variants	OCTN1/2-TC haplotype frequency (%)	No. of subjects not possessing IGR2096, IGR2198, and IGR2230 variants	OCTN1/2-TC haplotype frequency (%)
Controls	79	17 (21.5)	85	23 (27.1)	66	2 (3)	63	2 (3.2)
Patients with CD	69	21 (30.4)	76	13 (17.1)	62	1 (1.6)	41	0
P		.22		.13		.59		.25

NOTE. The number of patients with CD and controls are shown in the absence of the *IBD5* risk haplotype markers IGR2096, IGR2198, and IGR2230 and all 3 SNPs combined. The location of these marker SNPs within the *IBD5* locus is illustrated in Figure 1. The frequency of the OCTN1/2-TC haplotype within each of these respective groups is illustrated together with P values. In these individuals not possessing allelic variants associated with the extended *IBD5* haplotype, the OCTN1/2-TC haplotype was not significantly associated with CD for any of the 3 markers, providing evidence against an independent effect of the OCTN1/2 haplotype on disease susceptibility. In the data set from Peltekova et al, the single marker IGR2078 (block 4) was used to define the *IBD5* risk haplotype. It is noteworthy that IGR2096, the only marker of the 3 studied in our population to show even a trend toward independent segregation from OCTN1/2, was the marker farthest from OCTN1/2 and also was closest to the single marker used by Peltekova et al.

Table 5. Phenotypic Associations of IGR2198, OCTN1/2 Variant Homozygosity, and Homozygosity for the OCTN1/2-TC Haplotype

Phenotype	Patients with CD homozygous for the variant allele/haplotype			
	IGR2198	OCTN2 (-207 G→C)	OCTN1 (1672C→T)	OCTN1/2 TC haplotype
Vienna classification of disease				
behavior at follow-up				
Inflammatory (n = 121)	14.4%	23.0%	17.4%	17.1%
Stricturing/penetrating (n = 217)	24.9%	34.8%	30.0%	29.7%
P (relative risk)	.026 (1.97)	.029 (1.78)	.011 (2.0)	.011 (2.05)
Disease progression from diagnosis to follow-up				
No progression (n = 126)	14.7%	23.4%	18.2%	17.8%
Progression (n = 132)	25.9%	31.3%	26.9%	29.1%
P (relative risk)	.038 (1.72)	.18 (1.49)	.12 (1.66)	.049 (1.63)
Surgery for luminal complications of CD				
No surgery (n = 137)	13.1%	21.9%	11.7%	12.3%
Surgery (n = 237)	23.2%	33.1%	26.6%	26.7%
P (odds ratio)	.037 (1.91)	.031 (1.77)	.0007 (2.7)	.0023 (2.2)

NOTE. An association was observed between IGR2198 variant homozygosity, OCTN2 variant homozygosity, OCTN1 variant homozygosity, and homozygosity for the OCTN1/2-TC haplotype and structuring/penetrating disease when compared with inflammatory disease at most recent follow-up. Of the 258 patients with CD who had inflammatory Vienna disease classification at diagnosis, 126 patients did not progress at follow-up and 132 patients progressed to the stricturing/penetrating group. The average duration of follow-up of these patients was 11.8 years.

and homozygosity for the OCTN1/2-TC haplotype ($P = .875$). When patients with CD were further stratified for *NOD2/CARD15* variant carriage, no associations between homozygosity for the OCTN1/2-TC haplotype and the disease location, categorized by the Vienna classification, were observed; 45% of OCTN1/2-TC homozygous patients with CD with terminal ileal disease (L1) carried no *NOD2/CARD15* variants, whereas 52% carried 1 or more *NOD2/CARD15* variants ($P = .75$). The *IBD5* OCTN1/2 variants were not associated with disease extent and severity in the UC cohort.

Disease behavior. When disease behavior at diagnosis was analyzed, no significant association between variants representing the extended *IBD5* locus, individual OCTN1/2 variants, and stricturing (B2, Vienna classification) or penetrating (B3) behavior was observed when compared with inflammatory, nonstricturing, nonpenetrating disease behavior (B1). A significant association was observed between IGR2198 homozygosity and stricturing/penetrating disease when compared with inflammatory disease at most recent follow-up (24.9% B2 and B3 vs 14.4% B1; $P = .026$; OR, 1.97) (Table 5). Significant associations were also observed between stricturing/penetrating disease when compared with inflammatory disease for OCTN1 variant homozygosity (30% B2 and B3 vs 17.4% B1; $P = .011$; OR, 2.0), OCTN2 variant homozygosity (34.8% B2 and B3 vs 23.0% B1; $P = .029$; OR, 1.78), and homozygosity for the OCTN1/2-TC haplotype (29.7% B2 and B3 vs 17.1% B1; $P = .011$; OR, 2.05). In individuals who lacked the *IBD5*

risk haplotype (homozygous with respect to the non-CD-associated allele of the IGR2198 variant), there was no association between stricturing/penetrating disease behavior at follow-up and homozygosity for the OCTN1/2-TC haplotype (11.1% inflammatory vs 14.9% stricturing/penetrating; $P = .65$).

Disease progression. Because the Vienna classification is a hierarchical system, a number of patients will move from inflammatory disease (B1) at diagnosis to either stricturing (B2) or penetrating (B3) disease during their follow-up. Of the 258 patients who had inflammatory disease at diagnosis, 126 patients remained in the inflammatory group at follow-up and 132 patients progressed to the stricturing group or the penetrating group. The median duration of follow-up of these patients was 11.8 years. Homozygosity rates of IGR2198 variants and the OCTN1/2-TC haplotype were associated with disease progression to B2 or B3 compared with those whose disease remained as B1 (Table 5). The association between the OCTN1/2-TC haplotype and disease progression was not observed in patients with CD who lacked the *IBD5* risk haplotype (18% progression vs 13% nonprogression; $P = .56$).

Requirement for surgery. As a marker of disease severity, patients who had required surgery (n = 237) for complications of luminal CD were compared with those who had had no surgery for CD (n = 137). A significant association was observed between requirement for surgery and homozygosity for variants of IGR2198,

OCTN1, OCTN2, and the OCTN1/2-TC haplotype (Table 5).

Multiple logistic regression analysis was applied, using a model that considered age at diagnosis, disease behavior, smoking status, family history, *NOD2/CARD15* carrier status, and IGR2198 homozygosity or the OCTN1/2-TC haplotype, with the outcome being surgery for luminal complications of CD. Colinearity between IGR2198 and OCTN1/2-TC homozygosity was evident and allowed for in modeling. IGR2198 homozygosity ($P = .016$; odds ratio, 2.86; confidence interval, 1.21–6.76) and the OCTN1/2-TC haplotype ($P = .004$; odds ratio, 3.52; confidence interval, 1.49–8.31) were significantly associated with the need for surgery.

Discussion

The present study has provided novel data regarding the contribution of the *IBD5* locus as a determinant of disease susceptibility and phenotype in the Scottish population, which is known to be characterized by low rates of racial admixture compared with others in Europe and North America.⁴⁰ We have shown the OCTN1 and OCTN2 polymorphisms to be in tight linkage disequilibrium ($D', >0.959$), with allelic variants of the 3 SNPs representing the extended *IBD5* haplotype (IGR2096, IGR2198, and IGR2230). Susceptibility to CD was associated with each of the 3 *IBD5* polymorphisms defining the extended *IBD5* region and the variant alleles of the OCTN1 and OCTN2 genes. Homozygosity for each of the 3 SNPs used to define the extended *IBD5* haplotype and for the OCTN1/2-TC haplotype was strongly associated with disease susceptibility. These data provide the first independent confirmation of the association with the OCTN1/2 variants studied by Peltekova et al in Canada.³¹

However, our data are not entirely consistent with the results reported by Peltekova et al in a potentially critical aspect. In the absence of allelic variants representing the extended risk haplotype for *IBD5*, our results showed no significant difference in the presence of the OCTN1/2-TC haplotype in patients with CD compared with controls. These findings lead to the important suggestion that the OCTN1/2-TC haplotype may not confer risk of CD independently of other closely linked determinants within the extended *IBD5* locus and that it is at present premature to conclude that the OCTN1/2 variants are causative in the pathogenesis of CD.

In comparing the studies reported by Peltekova et al with our own, the choice of markers used to define the *IBD5* locus is especially worthy of discussion. In the data set from Peltekova et al, the single marker IGR2078,

located in block 4 of the haplotype map²⁷ (Figure 1), was used to define the *IBD5* risk haplotype and not a marker closer to the OCTN1/2 loci (block 7), such as IGR2198 (block 5) or IGR2230 (block 7), which were used in the present study. In light of this, recombination between the IGR2078 marker in block 4 and OCTN1/2 in block 7 needs to be considered as an explanation for the apparent independence reported by Peltekova et al. In our data, the only marker for which any trend toward an independence of the OCTN1/2-TC haplotype was observed concerned the IGR2096 variants, which are also located in block 4. In the absence of variants of the markers IGR2198 and IGR2230, the OCTN1/2-TC haplotype was in fact more prevalent in the control group, further supporting the hypothesis of recombination between haplotype blocks 4 and 7.²⁷

Recent data presented in abstract form involving 3 large cohorts of patients with IBD (more than 1200 patients with CD in total) in Cambridge,⁴¹ Stockholm, Sweden (Törkvist et al, personal communication, May 2005), and the United Kingdom/Germany⁴² have all shown an association between the *IBD5* locus and CD. In each of these data sets, as in the present study, there was no independent association between the OCTN1/2-TC haplotype and CD in the absence of the extended *IBD5* risk haplotype.

The arguments as to the contribution of the OCTN1/2 variants implicated by Peltekova et al have been developed further recently and clearly require resolution. Studies involving 276 samples from healthy, ethnically diverse human populations have allowed the identification of several nonconservative SNPs of OCTN1/2 in evolutionary conserved sites in black and Chinese populations.⁴³ The investigators suggest that if these variants were shown to confer susceptibility to CD in their respective populations, this would provide strong evidence that OCTN1/2 contain the critical mutations. As yet, these studies have not been undertaken. Moreover, genetic mutations in OCTN2 cause systemic carnitine deficiency, characterized by disease of skeletal muscle, cardiac muscle, and liver but not inflammatory or intestinal disease.⁴⁴

Genetic studies to resolve the limits of the association using markers p and q telomeric to OCTN1/2 may help to clarify this issue; however, power of resolution will become a critical issue in attempting to resolve this controversy by genetic studies alone. We have calculated that 3200 individuals with CD would need to be genotyped to prove the independence of OCTN1/2 from IGR2230, which is situated between the 2 genes in haplotype block 7.²⁷ Complementary approaches such as functional and expression studies in CD are clearly re-

quired. Whereas OCTN1/2 remain strong and plausible candidates within the haplotype structure on 5q31, the case is yet to be proven beyond doubt.

Other immunoregulatory genes that have the potential to play a role in the pathogenesis of CD and are located on the *IBD5* haplotype are interleukin-3, interleukin-4, interleukin-5, and CSF2.⁹ The interleukins have a well-defined role in immune regulation, and CSF2 encodes for granulocyte-macrophage colony-stimulating factor.^{9,45}

Increasingly, in CD, it is recognized that clinical phenotype is genetically determined.⁴⁶ In our cohort, when the Vienna classification for disease behavior was analyzed at the patients' most recent follow-up assessment, there was a significant association between the *IBD5* marker SNP IGR2198, OCTN1/2 variants, the OCTN1/2-TC haplotype, and the presence of stricturing and penetrating disease. Patients with CD who were homozygous for IGR2198 variants or homozygous for the OCTN1/2-TC haplotype had a disease phenotype that was more likely to progress to stricturing or penetrating disease behavior, and multivariate analysis showed an association among IGR2198, the OCTN1/2-TC haplotype, and the requirement for surgery, a surrogate marker for severity in CD.⁴⁷

It is important to point out that all the genotype-phenotype data contained within our study are internally consistent, with the significant association of the *IBD5* OCTN1/2 variants, stricturing and penetrating disease at latest follow-up, and disease progression from inflammatory to stricturing and penetrating disease, because these patients with progressive complicated disease are more likely to require surgical intervention. Although no classification system has gained universal approval, the Vienna classification is increasingly widely used and is currently subject to reevaluation.⁴⁸ Variants within the *IBD5* locus have also been associated with a more severe disease phenotype and growth failure in an independent cohort of 200 Scottish patients diagnosed with CD at 16 years of age or younger.⁴⁹ Overall, our data show an exciting, novel genotype-phenotype association with the *IBD5* locus, and it is intriguing to propose that a genetic variant within the *IBD5* locus causes CD to become more severe (stricturing or penetrating disease) and hence require increased surgical intervention.

Previous work involving the *IBD5* locus has shown an association between the *IBD5* risk haplotype and perianal CD, but this was not replicated in our cohort.²⁸ Phenotypic data from Newman et al showed an association with ileal disease,³⁵ but no significant association was observed in our population; 32.2% of patients with ileal CD were OCTN1/2-TC homozygous and 78% car-

ried the OCTN1/2-TC haplotype compared with 22.6% of patients with nonileal CD who were OCTN1/2 homozygotes and 75.5% of whom carried the OCTN1/2-TC haplotype ($P = .075$ and $P = .63$, respectively). Differences in definitions of ileal CD may be the reason for the differences between these 2 cohorts; ileal disease in our cohort was classified strictly according to the Vienna classification (L1). Using these criteria, 34% of patients had purely terminal ileal disease; in contrast, 70.6% of patients in the Toronto cohort were classified as having ileal disease.³⁵ Newman et al did not appear to include any patients with proximal small intestinal or upper gastrointestinal disease. No data were available in this cohort regarding disease behavior or disease progression over time.

NOD2/CARD15 variants have consistently been associated with a younger age of onset of disease, ileal disease, and stricturing disease.²⁶ In the present study, no evidence of epistasis was observed between the OCTN1/2 variants and *NOD2/CARD15* variants. Genetic heterogeneity between the Scottish population and that of Canada may be responsible for observed differences in this context, and it is pertinent to note the low frequency of *CARD15* variants in the Scottish CD population (1007fsinsC, 4.7%; G908R, 1.8%; R702W, 7.1%) and the low combined population-attributable risk these variants confer, which is estimated at 11%.²⁴

In conclusion, we have determined that the *IBD5* locus is associated with disease susceptibility in our CD cohort in Scotland. This is the first independent replication of the association of the OCTN1/2 haplotype with CD outside the index population in Canada. However, a significant effect of OCTN1 and OCTN2 variants was not seen in the absence of the *IBD5* risk haplotype, and on the strength of the present data it is not possible to conclude whether or not the OCTN1/2 genes contain the disease-causing mutation or whether the association serves to only narrow the region of association within the *IBD5* locus. Finally, novel and potentially important phenotypic associations have been identified with the *IBD5* locus, which implicate this region as a determinant of disease severity in CD as well as susceptibility.

References

1. Armitage E, Drummond H, Ghosh S, Ferguson A. Incidence of juvenile-onset Crohn's disease in Scotland. *Lancet* 1999;353:1496-1497.
2. Armitage EL, Aldhous MC, Anderson N, Drummond HE, Riemersma RA, Ghosh S, Satsangi J. Incidence of juvenile-onset Crohn's disease in Scotland: association with northern latitude and affluence. *Gastroenterology* 2004;127:1051-1057.
3. Askling J, Grahnski L, Ekblom A, Finkel Y. Incidence of paediatric Crohn's disease in Stockholm, Sweden. *Lancet* 1999;354:1179.

4. Sawczenko A, Sandhu BK, Logan RF, Jenkins H, Taylor CJ, Mian S, Lynn R. Prospective survey of childhood inflammatory bowel disease in the British Isles. *Lancet* 2001;357:1093–1094.
5. Satsangi J, Morecroft J, Shah NB, Nimmo E. Genetics of inflammatory bowel disease: scientific and clinical implications. *Best Pract Res Clin Gastroenterol* 2003;17:3–18.
6. Cho JH, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci U S A* 1998;95:7502–7507.
7. Ma Y, Ohmen JD, Li Z, Bentley LG, McElree C, Pressman S, Targan SR, Fischel-Ghodsian N, Rotter JI, Yang H. A genome-wide search identifies potential new susceptibility loci for Crohn's disease. *Inflamm Bowel Dis* 1999;5:271–278.
8. Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000;66:1863–1870.
9. Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001;29:223–228.
10. Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, MacPherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nurnberg P, Mirza MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999;64:808–816.
11. Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996;14:199–202.
12. Duerr RH, Barmada MM, Zhang L, Pfutzer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 2000;66:1857–1862.
13. Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugier L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996;379:821–823.
14. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411:599–603.
15. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603–606.
16. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 2003;278:8869–8872.
17. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003;278:5509–5512.
18. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* 2004;5:800–808.
19. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005;307:731–734.
20. Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, Karin M. Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science* 2005;307:734–738.
21. Lesage S, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002;70:845–857.
22. Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, Inohara N, Nunez G, Kishi Y, Koike Y, Shimosegawa T, Shimoyama T, Hibi T. Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 2002;123:86–91.
23. Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 2003;17:1465–1470.
24. Arnott ID, Nimmo ER, Drummond HE, Fennell J, Smith BR, MacKinlay E, Morecroft J, Anderson N, Kelleher D, O'Sullivan M, McManus R, Satsangi J. NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe? *Genes Immun* 2004;5:417–425.
25. Helio T, Halme L, Lappalainen M, Fodstad H, Paavola-Sakki P, Turunen U, Farkkila M, Krusius T, Kontula K. CARD15/NOD2 gene variants are associated with familiarly occurring and complicated forms of Crohn's disease. *Gut* 2003;52:558–562.
26. Ahmad T, Tamboli CP, Jewell D, Colombel JF. Clinical relevance of advances in genetics and pharmacogenetics of IBD. *Gastroenterology* 2004;126:1533–1549.
27. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. *Nat Genet* 2001;29:229–232.
28. Armuzzi A, Ahmad T, Ling KL, de Silva A, Cullen S, van Heel D, Orchard TR, Welsh KI, Marshall SE, Jewell DP. Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. *Gut* 2003;52:1133–1139.
29. Mirza MM, Fisher SA, King K, Cuthbert AP, Hampe J, Sanderson J, Mansfield J, Donaldson P, MacPherson AJ, Forbes A, Schreiber S, Lewis CM, Mathew CG. Genetic evidence for interaction of the 5q31 cytokine locus and the CARD15 gene in Crohn disease. *Am J Hum Genet* 2003;72:1018–1022.
30. Giallourakis C, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S, Rioux JD. IBD5 is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. *Am J Hum Genet* 2003;73:205–211.
31. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Escodon D, Greenberg G, Griffiths AM, George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004;36:471–475.

32. Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, Tsuji A. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* 1997;419:107–111.
33. Tokuhito S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, Suzuki M, Nagasaki M, Ohtsuki M, Ono M, Furukawa H, Nagashima M, Yoshino S, Mabuchi A, Sekine A, Saito S, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet* 2003;35:341–348.
34. Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* 1998;273:20378–20382.
35. Newman B, Gu X, Wintle R, Cescon D, Yazdanpanah M, Liu X, Peltekova V, Van Oene M, Amos CI, Siminovitich KA. A risk haplotype in the solute carrier family 22A4/22A5 gene cluster influences phenotypic expression of Crohn's disease. *Gastroenterology* 2005;128:260–269.
36. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989;170:2–6.
37. Gasche C, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000;6:8–15.
38. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
39. Schlesselman JJ. Case control studies: design, conduct, analysis. In: Stolley PD, ed. Oxford: Oxford Press, 1982.
40. Capelli C, Redhead N, Abernethy JK, Gratrix F, Wilson JF, Moen T, Hervig T, Richards M, Stumpf MP, Underhill PA, Bradshaw P, Shaha A, Thomas MG, Bradman N, Goldstein DB. A Y chromosome census of the British Isles. *Curr Biol* 2003;13:979–984.
41. Waller S, Tremelling M, Bredin F, Padfield E, Hunter J, Middleton S, Woodward J, Parkes M. OCTN associated with Crohn's disease and ulcerative colitis but does it cause IBD? (abstr). *Gut* 2005;54:A17.
42. Onnie C, Fisher S, Hampe J, Mirza M, Forbes A, Mansfield J, Sanderson J, Lewis C, Schreiber S, Mathew C. Multiple SNPs at the IBD5 locus contribute to the risk of Crohn's disease (abstr). *Gut* 2005;54:A95.
43. Urban TJ, Giacomini KM, Risch N. Haplotype structure and ethnic-specific allele frequencies at the OCTN locus: implications for the genetics of Crohn's disease. *Inflamm Bowel Dis* 2005;11:78–79.
44. Lahjouji K, Mitchell GA, Qureshi IA. Carnitine transport by organic cation transporters and systemic carnitine deficiency. *Mol Genet Metab* 2001;73:287–297.
45. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;392:245–252.
46. Sachar DB. Genomics and phenomics in Crohn's disease. *Gastroenterology* 2002;122:1161–1162.
47. Forcione DG, Rosen MJ, Kisiel JB, Sands BE. Anti-Saccharomyces cerevisiae antibody (ASCA) positivity is associated with increased risk for early surgery in Crohn's disease. *Gut* 2004;53:1117–1122.
48. Arnott ID, Satsangi J. Crohn's disease or Crohn's diseases? *Gut* 2003;52:460–461.
49. Russell RK, Drummond H, Smith L, Anderson N, Nimmo E, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset M, Mahdi G, Satsangi J. OCTN 1/2 variants are associated with disease susceptibility and phenotype in early onset inflammatory bowel disease (IBD) (abstr). *Gastroenterology* 2005;128:A38.

Received November 11, 2004. Accepted September 7, 2005.

Address requests for reprints to: Jack Satsangi, DPhil, FRCP, Gastrointestinal Unit, Western General Hospital, Edinburgh, Scotland EH4 2XU. e-mail: j.satsangi@ed.ac.uk; fax: (44) 131-651-1085.

Supported by Wellcome Trust Programme grant 072789/Z/03/Z.