INFLAMMATORY BOWEL DISEASE

Evidence for association of OCTN genes and IBD5 with ulcerative colitis

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Background and aims: Genetic association between Crohn's disease (CD) and OCTN1 (SLC22A4) C1672T/OCTN2 (SLC22A5) G−207C variants in IBD5 has recently been reported. These genes encode solute carriers and the association was suggested to be distinct from the background IBD5 risk haplotype. There have been conflicting reports of the association between markers in the IBD5 region and ulcerative colitis (UC) and interaction (epistasis) between this locus and CARD15. Our aim was to ascertain the contribution of OCTN variants to UC and CD in a large independent UK dataset, to seek genetic evidence that the OCTN association is distinct from the IBD5 risk haplotype and to identify interactions between the IBD5 and CARD15 loci.

Methods: A total of 1104 unrelated Caucasian subjects with inflammatory bowel disease (IBD) (496 CD, 512 UC, 96 indeterminate) and 750 ethnically matched controls were genotyped for three single nucleotide polymorphisms (SNPs) in the CD associated genes (OCTN1+1672, OCTN2−207, and IGR2230), and two flanking IBD5 tagging SNPs, IGR2096 and IGR3096. Data were analysed by logistic regression methods within STATA.

Results: OCTN variants were as strongly associated with UC and IBD overall as they were with CD ($p = 0.0001$; OR 1.3 (95% confidence interval 1.1–1.5)). OCTN variants were in tight linkage disequilibrium with the extended IBD5 risk haplotype D′ 0.79 and 0.88, and $r^2 = 0.62$ and 0.72 for IGR2096 and 3096, respectively. There was no deviation from a multiplicative model of interaction between CARD15 and IBD5 on the penetrance scale.

Conclusions: The OCTN variants were associated with susceptibility to IBD overall. The effect was equally strong in UC and CD. Although OCTN variants may account for the increased risk of IBD associated with IBD5, a role for other candidate genes within this extended haplotype was not excluded. There was no statistical evidence of interaction between CARD15 and either OCTN or IBD5 variants in susceptibility to IBD.

Crohn's disease (CD) (MIM 266600) and ulcerative colitis (UC) (MIM 191390) are the two main and related forms of inflammatory bowel disease (IBD). Epidemiological evidence shows a strong genetic contribution to IBD. To date, CARD15 (NOD2) on chromosome 16 is the only IBD gene to have been confirmed, and is specific for CD.[1 2] Three relatively common CARD15 variants account for approximately 25% of the attributable risk for CD in Northern European populations.[3] No UC genes have yet been confirmed. A number of putative IBD loci have been implicated but not confirmed, including TNF-857[4 5] and DLG5 (for IBD overall),[6] and MDR (for UC).[7 8] Recent interest has focused on the role of the IBD5 region on chromosome 5q31 for association with both CD and UC,[9–14] and specifically with CD for variants within the organic cation transporter gene cluster OCTN1 and OCTN2 (otherwise known as SLC22A4 and SLC22A5, respectively).[15–17]

Linkage at IBD5 was first demonstrated by Rioux *et al* in a genome scan of 158 (predominantly CD) affected sibpairs.[18] A LOD score of 3.9 was seen at IBD5 in this study, and in a subsequent meta-analysis of genome scan linkage data from 1952 sibpairs, this locus met Lander and Kruglyak's criteria for "suggestive significance"[19] for IBD overall.[20] Evidence for an association with CD within the IBD5 linkage interval was also first reported by Rioux *et al* who have broken the region into 11 haplotype blocks, each of consistently high linkage disequilibrium (LD) separated by short recombination hotspots.[10] The finding of association with CD has been replicated in a number of independent studies.[11 12 14 21]

Genetic evidence implicating the IBD5 region in susceptibility to CD seems convincing. However, which of the genes mapping to the IBD5 interval are predisposing to IBD remains unclear due to strong LD.[9 10] Rioux *et al* identified a risk haplotype spanning the central 250 kb region in CD trios, which they suggest serves as a proxy for the association at IBD5. It appears to have conserved structure in populations with European ancestry as 11 single nucleotide polymorphisms (SNPs) were identified as unique to the haplotype and as such it can be tagged by any one of 11 SNPs referred to as "haplotype tagging" (tag) SNPs.[9 12]

Located within the IBD5 locus are the two OCTN genes, encoding transmembrane solute transporter proteins.[9 10 15] Peltekova *et al* recently reported an association between CD and a two locus risk haplotype (+1672 T, −207 C) spanning OCTN1 and OCTN2 (with heterozygous odds ratio (OR) = 2.1–2.56 and homozygous OR = 3.43–5.14). They suggested it to be distinct from the background IBD5 risk haplotype as cases with the non-risk allele at the IBD5 tag SNP IGR2078 had a higher frequency of the +1672T or −207C alleles compared with controls.[15] No other studies have replicated this finding.

Whether the effect at IBD5/OCTN1 and OCTN2 is specific for CD or whether this locus is also associated with UC is

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; LD, linkage disequilibrium; SNPs, single nucleotide polymorphisms; OR, odds ratio; TDT, transmission disequilibrium test

Table 1 Demographic and clinical characteristics of individuals with Crohn's disease (CD), ulcerative colitis (UC), indeterminate colitis (IC), and controls

	CD (n = 496)	UC (n = 512)	IC (n = 96)	Controls (n = 750)
Median age at diagnosis (y)	26	36	31	
Median duration follow up (y)	13.2	12.9	12.1	
Sex (F:M)	331:163	238:272	63:33	430:330
Smoking at diagnosis (%)				
Never	44.3	52.6	47.9	72.0%
Ex	12.9	34.5	30.2	
Current	42.8	12.9	17.9	
Jewish ancestry (%)	1.3	0.8	0.8	
Family history of IBD (%)	24.2	21.5	21.9	
Resectional surgery (%)	50.8	15.8	6.3	
Location/extent (%)	29.8 ileal	50.5 extensive		
	30.0 colonic	14.7 <splenic		
	37.3 ileocolonic	34.8 <sigmoid		
	25.6 perianal			
Behaviour (%)				
Stenosing	36.1			
Penetrating	23.4			

IBD, inflammatory bowel disease.

unclear. The majority of groups who have reported data for IBD5 have not seen evidence of an association with UC[11 14 21] although a positive transmission disequilibrium test (TDT) result was seen by Giallourakis *et al* using 187 German UC trios.[12] Only two independent groups have studied UC with the OCTN1 and OCTN2 variants, and neither found evidence of an association.[15–17]

To date, conflicting evidence has been reported in the literature regarding possible epistasis between IBD5 and CD predisposing variants in CARD15 (NOD2). This may in large part reflect the differing definitions and statistical methodologies that have been applied as well as the lack of power to detect interaction effects. Some studies have reported no evidence of interaction,[10 11 14] some have reported interaction effects in CD,[15 16 21] and some suggest interaction in individuals with UC,[12 13] although mutations in CARD15 are not thought to be associated with UC.[22] For the purposes of this paper, we have defined epistasis statistically, as a deviation from the multiplicative model on the penetrance scale.[23]

Thus our aims were to seek replication of the association between OCTN1 and OCTN2 variants with CD in a well powered study; ascertain evidence for association with UC; determine the magnitude of the effect in our British case control collection; assess whether the association was distinct from the background IBD5 risk haplotype; and look for evidence of an interaction between OCTN1/2 variants and CARD15.

MATERIALS AND METHODS

Subjects

A total of 1104 unrelated Caucasian IBD patients of north European origin resident in East Anglia, UK, were recruited, comprising 496 with CD, 512 with UC, and 96 with indeterminate colitis (IC). Diagnosis was made using standard criteria on notes review.[24] Phenotype data are summarised in table 1. CD behaviour was classified according to the Vienna classification for disease site and behaviour.[25] Patients were allocated to either a stenosing (B2) or penetrating (B3) group dependent on whichever form of behaviour was primary. Patients exhibiting neither stenosing nor penetrating behaviour remained in the inflammatory (B1) group. Disease location was coded L1 ileum (including spillover into the caecum) L2 colon, L3 ileocolon, or L4 proximal gastrointestinal tract. In addition, perianal disease was defined as the presence of perianal fistula, abscess, or ulcer but not skin tags. In view of concerns regarding the allocation of CD patients with perianal fistulating disease to the B3 penetrating group of the Vienna classification,[26] we conducted a further analysis of this group separating those with intra-abdominal fistula/abscess from patients with perianal disease.

The 750 ethnically and geographically matched healthy controls were previously recruited in East Anglia for the European Prospective Investigation of Nutrition and Cancer (EPIC). Ethics committee approval was obtained (Cambridge LREC 01/418; MREC 03/5/012), as was written informed consent from all study subjects.

With this collection of 1104 cases and 750 controls, we had 90% power to detect an effect size of 1.3, with an allele frequency of 46% and a 1% type I error, assuming a multiplicative mode of inheritance. We had 89% power for the 500 cases in the UC and CD subgroups, for a type I error of 5%, or 74% power with a type I error of 1%.

Table 2 Primer design for Taqman biallelic discrimination system

Name	Forward primer	Reverse primer	Reporter 1 (Vic)	Reporter 2 (Fam)
OCTN1+1672	GGGTAGTCTGACTGTCCTGATTG	TCTGGAAGAGTCATTCCCAAACTTTC	AAGGGTGAGGATTC	AAGGGTGAAGATTC
OCTN2-207C	GCGGCTGGCCTTACATAGG	CCGCTCTGCCTGCCA	CAGGCCCGGAACC	CAGGCCCGCAACC
IGR2230	GCAGGCAGAACAGCCATACT	GGCCACAGAACTTTCATTAAAGTAGGA	AAATACACCCTAAATGGCTAA	ACACCCTAAGTGGCTAA
IGR2096	TCTGAGACAGGAGCCACTAGAG	CACAGCATCCAGAGTGATCCT	CATGTCACTTCTCTTTAAAA	ATGTCACTTCTCTGTAAAA
IGR3096	CCTGGGAACCCAAACATCCT	TGTGTGTGATGGGACTGTTTCC	TTCTCAGCTATTCTCC	CTCAGCTGTTCTCC
CARD15 702Trp	GCTGCGGGCCAGACA	CTGAGTGCCAGACATCTGAGAAG	CCTGCTCTGGCGCC	CTGCTCCGGCGCC
CARD15 908Arg	CCACCTCAAGCTCTGGTGATC	CTGTTGACTCTTTTGGCCTTTTCAG	TCTGTTGCGCCAGAAT	CTCTGTTGCCCCAGAAT
CARD15 Leu1007fsinsC	TGTCCAATAACTGCATCACCTACCT	CTTCCAGGATGGTGTCATTCCT	TGCAGGCCCCTTGA	TGCAGGCCCTTGAA

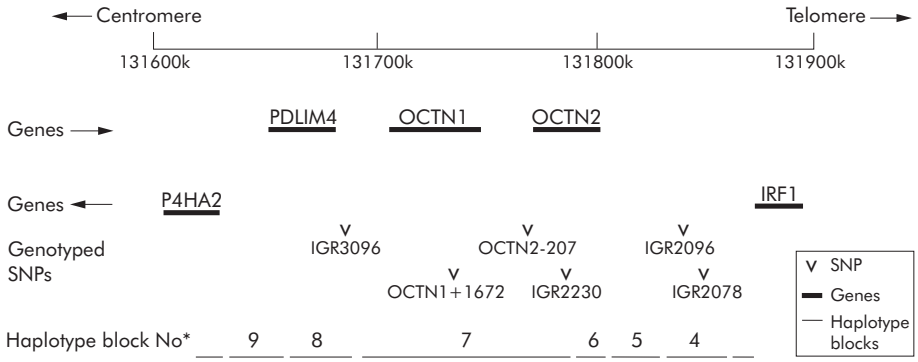

Figure 1 Schematic representation of the IBD5 locus on chromosome 5. Relative positions of single nucleotide polymorphisms (SNPs), genes, and haplotype blocks are shown.

Genotyping

Genotyping was performed using the Taqman biallelic discrimination system (Applied Biosystems) using an ABI 7900 analyser. Sequence data for primers are presented in table 2. Each 96 well plate had two negative control wells containing water only and one positive CEPH DNA control.

Marker selection

Two OCTN markers studied by Peltekova *et al* and Newman and colleagues[15 16] were genotyped: a C>T SNP in OCTN1 (OCTN1+1672; rs1050152) and a G>C transversion in the OCTN2 promoter (OCTN2−207; rs2631367) 29 kb upstream. We also genotyped a further OCTN2 SNP, IGR2230 (rs17622208). This was originally included as one of the 11 IBD5 tag SNPs by Rioux *et al* and in fact maps to intron 2 of the OCTN2 gene 12 kb (11653 bp) from the OCTN2−207 transversion (ENSEMBL release version 28.35a.1). Two IBD5 tag SNPs were also genotyped: IGR3096 (T>C, rs7705189) which is 82 kb centromeric of OCTN2−207and IGR2096 (C>A, rs12521868) which lies 79 kb telomeric to OCTN2−207 (fig 1). IGR3096 was also typed by Armuzzi *et al* while IGR2096 was studied by Giallourakis *et al*. IGR2078 (rs4705950), the IBD5 tag SNP typed by Peltekova and colleagues,[15] maps 9 kb telomeric to IGR2096, in the same haploblock.[9] IGR2078 itself was not used as a marker in our panel due to the presence of another polymorphic site within 5 bp, making it inappropriate for the Taqman assay.

In order to assess epistasis between CARD15 and IBD5, cases and controls were also genotyped for the established CD predisposing CARD15 (NOD2) variants (702Trp, 908Arg, and Leu1007fsinsC).[1 2]

Statistics

All statistical analyses, unless otherwise stated, were carried out within STATA version 8.2 (http://www.stata.com) with use of routines available from www-gene.cimr.cam.ac.uk/clayton/software/stata/.

Sex, age at diagnosis, and smoking status were assessed as possible confounders in IBD overall and in UC and CD separately, and included as nuisance parameters in the regression equations where appropriate. Each locus was analysed for association with disease and ORs calculated by logistic regression, using both a multiplicative model (loci were coded as continuous variables) and a model that assumed no particular mode of inheritance (loci coded as three level categorical genotype variables). The two models were compared by a likelihood ratio test.

Before performing subgroup analysis, we carried out case only interaction tests to check for heterogeneity of effect (interpreting deviation from a multiplicative model of epistasis on the penetrance scale as interaction) with multinomial logistic regression—that is, we verified that our subgroups of interest were significantly different before looking at them. Genotypes were coded as three level outcome variables, and disease subphenotypes (CD/UC)/smoking status/gender, were coded as binary explanatory variables.

Table 3 Results of single locus analyses at OCTN1, OCTN2, and IGR2096C>A for 1104 inflammatory bowel disease (IBD) cases and 750 controls

	Risk allele frequency (n (%))					Genotype (n (%))						
Gene (variant)	IBD	UC	CD	Controls	OR_{IBD} (95% CI)		IBD	UC	CD	Controls	OR_{IBD} (95% CI)	P_{IBD} (for association assuming multiplicative model)
OCTN1 +1672	1027 (47.3)	473 (47.4)	471 (48.0)	616 (41.0)	1.31 (1.14–1.50)	CC	284 (26.2)	131 (26.3)	124 (25.3)	261 (34.7)	1.00 (ref)	0.00010
						CT	575 (53.0)	263 (52.7)	263 (53.6)	366 (48.7)	1.44 (1.17–1.79)	
						TT	226 (20.8)	105 (21.0)	104 (21.2)	125 (16.6)	1.66 (1.26–2.19)	
OCTN2 −207	1124 (52.5)	526 (52.9)	510 (52.8)	695 (46.3)	1.31 (1.14–1.50)	GG	219 (20.5)	98 (19.7)	97 (20.1)	211 (28.1)	1.00 (ref)	0.00014
						GC	580 (54.2)	272 (54.7)	262 (54.2)	385 (51.3)	1.45 (1.15–1.82)	
						CC	272 (25.4)	127 (25.6)	124 (25.7)	155 (20.6)	1.69 [1.29–2.22)	
IGR2230	1155 (52.6)	543 (53.2)	518 (52.4)	693 (46.1)	1.31 (1.15–1.51)	CC	229 (20.8)	99 (19.4)	105 (21.3)	209 (27.8)	1.00 (ref)	0.00008
						CT	585 (53.2)	279 (54.7)	260 (52.6)	391 (52.1)	1.37 (1.09–1.71)	
						TT	285 (25.9)	132 (25.9)	129 (26.1)	151 (20.1)	1.73 (1.31–2.26)	
IGR2096	1023 (47.0)	471 (42.7)	465 (47.4)	609 (41.1)	1.29 (1.11–1.48)	CC	288 (26.4)	137 (27.2)	124 (25.2)	255 (34.4)	1.00 (ref)	0.00010
						CT	579 (53.2)	259 (51.6)	269 (54.8)	363 (49.0)	1.41 (1.14–1.75)	
						TT	222 (20.4)	107 (21.2)	98 (20.0)	123 (16.6)	1.61 (1.22–2.12)	
IGR3096 IGR3096	1106 (50.6)	591 (51.3)	495 (50.4)	655 (44.0)	1.30 (1.14–1.49)	TT	250 (22.9)	109 (21.5)	116 (23.6)	224 (30.1)	1.00 (ref)	0.00010
						TC	580 (53.1)	275 (54.3)	255 (51.9)	385 (51.7)	1.34 (1.08–1.68)	
						CC	263 (24.1)	122 (24.1)	120 (24.4)	135 (18.1)	1.74 (1.32–2.29)	

Genotype and allele frequencies are also given for the 496 individuals with Crohn's disease (CD) and the 512 individuals with ulcerative colitis (UC). Smoking and sex are included as confounders.

Haplotypes were reconstructed using snphap v1.3 separately within cases and controls (www-gene.cimr.cam.ac.uk/clayton/software/stata/).

RESULTS

All SNPs were in Hardy-Weinberg equilibrium in controls (p = 0.17–0.90). Genotype and allele frequencies for all loci studied are given in table 3. The three OCTN SNPs (OCTN2−207, OCTN1+1672, and IGR2230) were in strong LD, with D′ values ranging from 0.95 to 0.98 and r^2 from 0.76 to 0.88. LD with the flanking IBD5 tag SNPs IGR2096 and IGR3096 was therefore measured with respect to IGR2230 and, as expected, found to be strong, with D′ 0.79 and 0.88 and r^2 0.62 and 0.72, respectively.

Smoking and sex were found to have different distributions in CD and UC. As previously reported, smokers were seen to have a reduced risk of UC (OR 0.36 (95% confidence interval (CI) 0.27–0.49); $p = 3.4\times10^{-12}$) with the opposite effect seen in CD (OR 1.87 (1.48–2.37); $p = 2.15\times10^{-7}$) compared with healthy controls. Furthermore, in our sample there were more males with UC (male:female ratio 1:0.9) and more females with CD (male:female ratio 1:1.9). These confounders were accounted for in all analyses of the subphenotypes UC and CD.

All five SNPs were shown to be associated with IBD overall, using logistic regression (p = 0.0001: table 3). Allele frequencies were almost identical for IGR2230 and OCTN2−207, as they were for IGR2096, IGR3096, and OCTN1+1672. These produced an allelic OR (95% CI) of 1.3 (1.1–1.5) for the OCTN SNPs (table 3).

Using data for IGR2230, we tested if the association between IBD5 risk haplotype markers, including the OCTN SNPs, was different between UC and CD, allowing for sex and smoking status. As expected, based on the similarity of genotype frequencies in UC and CD groups (table 3), there was no statistically significant difference between them (p = 0.88). Thus OCTN variants are associated equally with UC and CD.

We were unable to distinguish the effects of the five SNPs typed in the IBD5 region by stepwise logistic regression.[27] Once IGR2230 was accounted for, the model was not improved by addition of OCTN1+1672, OCTN2−207, IGR2096, or IGR3069, (p = 0.48, 0.43, 0.94, and 0.74, respectively). This was also true when OCTN1+1672, OCTN2−207, IGR3096, or IGR2096 were placed in the model first—that is, any single locus was sufficient to explain the association at the others, meaning that association of the OCTN loci is not independent of the IBD5 association. Four locus haplotypes (OCTN1+1672, OCTN2−207, IGR2096, and IGR2230) were reconstructed separately within cases and controls and tested for an association with IBD (data not shown). There was no evidence that the combined effects of the haplotypes was greater than the effect of any single locus (p = 0.18), again confirming that the association of the OCTN loci is not independent of the IBD5 risk haplotype

We found that any single IBD5 locus could explain the association of the other IBD5 loci, and therefore only tested IGR2230 for association with subphenotypes of Crohn's disease. In contrast with the findings of Armuzzi and colleagues,[11] we found no heterogeneity of effect when CD subphenotypes of disease location (ileal, colonic, ileocolonic, perianal) were considered (p = 0.87, 0.69, 0.89, 0.78), nor when disease behaviour (inflammatory, stenosing, penetrating, p = 0.57, 0.61, 0.92) or surrogates for severity were considered (Crohn's surgery, immunomodulatory therapy, p = 0.67, 0.84). Separate analysis of all patients with proven internal fistulae, irrespective of perianal disease, also failed to show any significant heterogeneity (p = 0.53). These data are presented in supplementary data table 5 (see supplementary table 5 on the *Gut* website at http://www.gutjnl.com/supplemental).

To confirm that the association of the OCTN variants could not be distinguished from the IBD5 risk haplotype in our CD dataset, we looked at the frequency of OCTN variants in CD cases and controls who were wild-type homozygous at the IBD5 risk haplotype. Peltekova *et al* reported that 53% of CD affecteds but only 23% of controls carried OCTN1+1672T or OCTN2−207C variants while being homozygous wild-type for the IBD5 haplotype (as defined by IGR2078).[15] For the equivalent figures in our dataset, we found that 26.6% (n = 33) of CD cases and 22.0% (n = 56) of controls carried either +1672T or −207C OCTN risk alleles on a homozygous wild-type IBD5 haplotype background. Only nine CD cases who were homozygous for IGR2096 wild-type possessed both OCTN1+1672T and OCTN2−207C risk alleles.

To test for epistasis between CARD15 and the IBD5 region, cases and controls were also genotyped for the established CD predisposing CARD15 (NOD2) variants 702Trp, 908Arg, and Leu1007fsinsC.[1 2] As reported in other studies, strong association was seen with CD, particularly for Leu1007fsinsC ($p = 7\times10^{-11}$) but not UC (p = 0.27) (table 4). Forward and backward stepwise logistic regression was employed to test if a single locus could explain the association seen across all the CARD15 loci typed; all three loci 702Trp, 908Arg, and Leu1007fsinsC, were needed to explain the observed association with CD ($p = 2.79\times10^{-10}$, 0.0001, 0.0012). On reconstruction of the three CARD15

Table 4 Results of single locus analyses at 702Trp, 908Arg, and Leu1007fsinsC (snp13) for 1104 inflammatory bowel disease cases and 750 controls. Genotype and allele frequencies are also given for the 496 individuals with Crohn's disease (CD) and the 512 individuals with ulcerative colitis (UC)

	Minor allele frequency							Genotype			
	Controls		UC		CD			Controls	CD		
Gene (variant)	n	(%)	n	(%)	n	(%)		n (%)	n (%)	OR_{CD} (95%CI)	P_{CD}
702Trp	93	(6.2)	49	(4.8)	104	(10.6)	0/2	654 (87.8)	394 (80.4)	1.00 (Ref)	
							1/2	89 (11.9)	88 (17.9)	1.64 (1.2–2.3)	0.002
							2/2	2 (0.3)	8 (1.7)	6.64 (1.4–31.6)	0.006
908Arg	16	(1.1)	17	(1.7)	32	(3.2)	0/2	724 (97.8)	426 (86.8)	1.00 (Ref)	
							1/2	16 (2.2)	32 (13.2)	3.13 (1.7–5.8)	0.0001
							2/2	0 (0)	0 (0)		
Leu1007fsinsC	18	(1.1)	20	(2.0)	74	(7.5)	0/2	723 (97.6)	426 (86.8)	1.00 (Ref)	
							1/2	18 (2.4)	56 (11.4)	5.28 (3.0–9.2)	<0.0001
							2/2	0 (0)	9 (1.8)		0.0001

variants as phased haplotypes, phase was not found to be important ($p = 0.87$).

Interaction between CARD15 loci and OCTN variants was tested by multinomial logistic regression allowing for sex and smoking as confounders. As the effects of the five OCTN/IBD5 SNPs were indistinguishable, for these and subsequent analyses we only considered IGR2230. With all three CARD15 variants accounted for, there was no significant departure from a multiplicative model of interaction on the penetrance scale. There was therefore no statistical evidence for epistasis between these loci for CD ($p = 0.26$) or UC ($p = 0.15$).

Correlation between genotype and age at disease onset was tested by linear regression for both OCTN and CARD15 variants. This was not found to be significant either for OCTN variants (IGR2230; $p = 0.411$) or CARD 15 variants (702Trp, 908Arg, and Leu1007fsinsC; $p = 0.14$, 0.16, and 0.19, respectively).

DISCUSSION

In the current study we have replicated the association between variants in the OCTN genes and CD reported by Peltekova and colleagues[15] and demonstrated for the first time in a well powered study an association between OCTN variants and UC. The magnitude of this effect was the same for CD and UC, but with an OR of 1.3 (95% CI 1.1–1.5) was substantially lower than that previously reported.[15]

Our data are consistent with earlier studies that demonstrated an association between IBD5 and CD,[10 11 14 21] although these studies did not find evidence of an association with UC. However, in this regard, our findings are consistent with the report from Giallourakis *et al* who observed an association between IBD5 and UC.[12] In our dataset, allele frequencies in the large UC panel were very similar to those seen in CD (table 3) and a formal test for heterogeneity of association confirmed no statistical difference between UC and CD ($p = 0.88$). Peltekova *et al* did not find evidence for an association between OCTN markers and UC, although with 216 UC subjects their study only had 48% power so this may have represented a type 2 error. Whether the differing results between datasets with regard to UC and IBD5/OCTN reflect power, population heterogeneity, or some other factor will have to await further investigation. None the less, data from the current study, combined with the previous TDT data from Giallourakis and colleagues[12] and the meta-analysis linkage data from van Heel[20] suggest that IBD5 is a generic IBD susceptibility locus. Unsurprisingly in this context, and in contrast with the findings of Armuzzi and colleagues,[11] we found no evidence of specific subphenotype associations when CD was subdivided according to site of involvement or disease behaviour.

Both OCTN genes, encoding transmembrane solute transporter proteins, are potential candidate genes. OCTN2 functions as a carnitine transporter. Carnitine is essential for the passage of long chain fatty acids from the cytosol into the mitochondria for subsequent beta oxidation.[28] Defects within this system may lead to impaired pathogen killing by oxidation burst mediated mechanisms.[15] Persistent infection as a result of impaired eradication of luminal pathogens has been proposed as a possible mechanism causing IBD.[29] OCTN1 has lower affinity for carnitine but has been shown to have high affinity for ergothioneine, a compound exclusively synthesised in mycobacteria and fungi.[30] The biological function of OCTN1 is therefore less clear than that of OCTN2 but it is expressed in immune active cells and variants have previously been associated with rheumatoid arthritis.[15 31] Thus the OCTN genes constitute plausible candidate genes for IBD.

Despite this biological plausibility, and unlike Peltekova and colleagues,[15] we found no significant difference between the proportion of cases and controls carrying OCTN risk variants while being homozygous wild-type for the IBD5 haplotype, reflecting the tight LD in the region. This discrepancy is unlikely to reflect the different marker (IGR2078) used as the surrogate for IBD5 by Peltekova *et al*, which lies only 9 kb distal to IGR2096 with little increase in the likelihood of recombination, especially given the tight LD across this region (seen both in the current study and previously reported by Rioux *et al* in a Canadian panel which overlapped with that used by Peltekova and colleagues[10 15]). These findings, combined with the results of our stepwise logistic regression analysis, do not indicate a specific effect for the OCTN variants. Although consistent with such an effect, based on our data the IBD5 susceptibility gene could lie elsewhere in the IBD5 region. The difference between our results and those of Peltekova *et al* might reflect a number of factors, including population heterogeneity, statistical power, or type 1 error.

There is significant debate in the literature as to whether there is epistasis between the IBD5 region and CARD15 loci—that is, is the effect observed at IBD5 modified by the CARD15 locus? Reviewing previous reports it seems likely that discrepancies regarding possible epistatic effects between IBD5 and CARD15 variants have arisen due to differences in definition as well as differences in methodological approach.

None of the published groups have carried out formal interaction tests: instead, particular genotypes/subgroups have been looked at to see how the effect of a second locus is modified. Two groups previously found no evidence of interaction between IBD5 and CARD15 loci by separately analysing IBD5 risk allele frequencies after conditioning for CARD15 status[11 14] but some investigators have reported evidence suggestive of an interaction between the two loci by this method, both in UC[12 13] and in CD.[21] Adopting this approach however may mask some modifying effects at particular loci. Furthermore, whether the difference between the genotypes/subgroups is statistically significant is unknown and multiple testing corrections should be carried out.

Statistically, epistasis can be defined as deviation from a multiplicative model of interaction on the penetrance scale.[23 32] Using this definition in a formal statistical interaction test of data from our case control collection, allowing for environmental and demographic confounders, no evidence of epistasis between OCTN/IBD5 and CARD15 was seen in our dataset. Interestingly, when the CD cohort used by Peltekova *et al* was reanalysed by Newman *et al*, although the combined effects of CD risk CARD15 variants and OCTN risk variants were significantly greater than either variant alone, further examination of the relationship between OCTN and CARD15 by boot strap analysis suggested no evidence of a significant interaction.[16] This is in fact in keeping with the findings in our case control collection which show no evidence for departure from a multiplicative model, suggesting that the two loci act independently from one another to increase disease susceptibility.

Thus in the current study we have demonstrated that OCTN variants are indeed associated with CD, but to a more modest extent in our panel than reported by Peltekova *et al* and with an effect that is not distinct from the IBD5 risk haplotype. We have also documented an association between OCTN variants and UC to a degree equivalent to that seen in CD. There was no evidence of epistatic interaction between CARD15 and IBD5 in our analysis.

ELECTRONIC DATABASE INFORMATION

URLs for data presented herein are as follows:

- Clayton group at the Cambridge Institute for Medical Research for SNPHAP v1.3, http://www-gene.cimr.cam.ac.uk/clayton/software/
- Stata software: http://www.stata.com
- Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for psoriasis susceptibility)

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Supplementary table 5 can be viewed on the *Gut* website at http://www.gutjnl.com/supplemental.

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