Evidence for Association of OCTN Genes and IBD5 with Ulcerative Colitis

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KEYWORDS

Crohn’s Disease; Ulcerative Colitis; Genetics; OCTN; IBD5
ABSTRACT

**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 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:** 1104 unrelated Caucasian IBD subjects (496 CD, 512 UC, 96 indeterminate) and 750 ethnically matched controls were genotyped for 3 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 [95%CI] of 1.3 [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.
INTRODUCTION

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][10][11][12][13][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][16][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 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 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]

The 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 linkage disequilibrium (LD).[9][10] Rioux et al. identified a risk haplotype spanning the central 250 kilobases (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 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 association between CD and a two locus risk haplotype (+1672 T. −207 C) spanning OCTN1 and OCTN2 (with heterozygous odds ratio = 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 when compared to 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 unclear. The majority of groups who have reported data for IBD5 have not seen evidence of 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 to date and neither found evidence of association.[15][16][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 is distinct from the background IBD5 risk haplotype and look for evidence of interaction between OCTN1/2 variants and CARD15.

MATERIALS AND METHODS

Subjects

1104 unrelated Caucasian IBD patients of north European origin resident in East Anglia, UK were recruited comprising 496 CD, 512 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 the stenosing (B2) or penetrating (B3) groups 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 spill over into the caecum) L2 colon, L3 ileo-colon or L4 proximal GI 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) and written informed consent was obtained from all study subjects.

With this collection of 1000 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 in the 500 UC or CD sub-phenotype groups, for a type I error of 5%, or 74% power with a type I error of 1%.
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 et al. [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) 29kb 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 (11653bp) from the OCTN2-207 transversion (ENSEMBL release version 28.35a.1). Two IBD5 tag SNPs were also genotyped: IGR3096 (T>C, rs7705189) which is 82kb centromeric of OCTN2-207 and IGR2096 (C>A, rs12521868) which lies 79kb telomeric to OCTN2-207. (see figure 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 et al.[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/).

Gender, 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 odds ratios 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 3 level categorical genotype variables). The two models were compared by a likelihood ratio test.

Before doing sub-group 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, i.e. we verified that our sub-groups of interest were significantly different before looking at them. Genotypes were coded as three level outcome variables and disease sub-phenotypes (CD/UC)/smoking status/gender, were coded as binary explanatory variables.

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, \( P = 0.17\text{-}0.90 \). Genotype and allele frequencies for all loci studied are given in table 3. The three OCTN SNPs (OCTN2-207, OCTN1+1672 and IGR2230) are 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 gender 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 [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 to healthy controls. Furthermore in our sample there were more males with UC (male:female ratio of 1:0.9) and more females with CD (male:female ratio of 1:1.9). These confounders were accounted for in all analyses of the sub-phenotypes 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 to see if the association between IBD5 risk haplotype markers including the OCTN SNPs was different between UC and CD, allowing for gender and smoking status. As expected based on the similarity of genotype frequencies in UC and CD groups (table 3) there was no statistical 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, i.e. 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.IGR2230) were reconstructed separately within cases and controls and tested for 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 association of the OCTN loci is not independent of the IBD5 risk haplotype.

We found any single IBD5 locus could explain the association of the other IBD5 loci, and therefore only tested IGR2230 for association with sub-phenotypes of Crohn's disease. In contrast to the findings of Armuzzi et al.,[11] we found no heterogeneity of effect when CD sub-phenotypes of disease location (ileal, colonic, ileo-colonic, 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 when 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.
To confirm that 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 26.6% (n=33) CD cases and 22.0% (n=56) controls carried either +1672T or -207C OCTN risk alleles on a homozygous wild type IBD5 haplotype background. Only 9 CD cases who were homozygous 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, $P = 7 \times 10^{-11}$ 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, $P = 2.79 \times 10^{-10}$, $0.0001$, $0.0012$. On reconstruction of the three CARD15 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 gender 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 $P = 0.14$, $0.16$, $0.19$ respectively).

DISCUSSION

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

Our data are consistent with earlier studies that demonstrated association between IBD5 and CD,[10][11] [14] [21] although these studies did not find evidence for association with UC. However in this regard our findings are consistent with the report from Giallourakis et al. who did observe association between IBD5 and UC[12]. In our dataset the 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 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 regards to UC and IBD5 / OCTN reflects power, population heterogeneity or some other factor will have to await further investigation. Nonetheless the data from the current study, combined with the previous TDT data from Giallourakis et al.[12] and the meta-analysis linkage data from van Heel[20] do suggest that IBD5 is a generic IBD susceptibility locus.

Unsurprisingly in this context, and in contrast to the findings of Armuzzi et al.[11] we found no evidence of specific sub-phenotype associations when CD was sub-divided 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 cytosol into 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 (ET), 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 et al.,[15] we found no significant difference between the proportion of cases and controls carrying OCTN risk variants whilst being homozygous wildtype 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 9kb 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 et al.[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, i.e. 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/sub-groups 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 interaction between the two loci by this method, both in UC[12][13] and CD.[21] Adopting this approach, however, may mask some modifying effects at particular loci. Further, whether the difference between the genotypes/sub-groups 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. were re-analysed by Newman et al., although the combined effects of CD risk CARD15 variants and OCTN risk variants were significantly greater than either variant alone, a further examination of the relationship between OCTN and CARD15 by boot strap analysis suggested no evidence for 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 association between OCTN variants and UC to a degree equivalent to that seen in CD. There was no evidence for epistatic interaction between CARD15 and IBD5 in our analysis.

Competing interests: None declared

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Electronic-Database Information

URLs for data presented herein are as follows:

REFERENCES

### TABLES

#### Table 1: Demographic and clinical characteristics of individuals with Crohn disease (CD) ulcerative colitis (UC), indeterminate colitis and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD n = 496</th>
<th>UC n = 512</th>
<th>Indeterminate n = 96</th>
<th>Controls n = 750</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis</td>
<td>26 years</td>
<td>36 years</td>
<td>31 years</td>
<td></td>
</tr>
<tr>
<td>Median duration follow up</td>
<td>13.2 years</td>
<td>12.9 years</td>
<td>12.1 years</td>
<td></td>
</tr>
<tr>
<td>Smoking at diagnosis - never</td>
<td>44.3%</td>
<td>52.6%</td>
<td>47.9%</td>
<td>72.0%</td>
</tr>
<tr>
<td>ex</td>
<td>12.9%</td>
<td>34.5%</td>
<td>30.2%</td>
<td></td>
</tr>
<tr>
<td>current</td>
<td>42.8%</td>
<td>12.9%</td>
<td>17.9%</td>
<td></td>
</tr>
<tr>
<td>Jewish ancestry</td>
<td>1.3%</td>
<td>0.8%</td>
<td>0.8%</td>
<td></td>
</tr>
<tr>
<td>Family history of IBD</td>
<td>24.2%</td>
<td>21.5%</td>
<td>21.9%</td>
<td></td>
</tr>
<tr>
<td>Resectional surgery</td>
<td>50.8%</td>
<td>15.8%</td>
<td>6.3%</td>
<td></td>
</tr>
<tr>
<td>Location / extent</td>
<td>29.8% ileal</td>
<td>50.5% extensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.0% colonic</td>
<td>14.7% &lt; splenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.3% ileo-colonic</td>
<td>34.8% &lt; sigmoid</td>
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<td></td>
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<tr>
<td></td>
<td>25.6% perianal</td>
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<td></td>
<td></td>
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<tr>
<td>Behaviour</td>
<td>36.1% stenosing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.4% penetrating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Forward Primer</td>
<td>Reverse Primer</td>
<td>Reporter 1 (Vic)</td>
<td>Reporter 2 (Fam)</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>------------------</td>
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</tr>
<tr>
<td>OCTN1+1672</td>
<td>GGTAGTCTGACTGTCCCATGATTG</td>
<td>TCTGAGAGAGTTCCACTTTCTCTTCCAAACTTTTTC</td>
<td>AAGGTTGAGGAATTCC</td>
<td>AAGGTTGAAGATTCC</td>
</tr>
<tr>
<td>OCTN2-207C</td>
<td>GCGCTTGGCCCTTACATAGG</td>
<td>CCGCTTGGCCCTTACATAGG</td>
<td>CAGGCCGGAAACC</td>
<td>CAGGCCGGCAACC</td>
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<tr>
<td>IGR2230</td>
<td>GCGGCGAGAACGAGCCCATCCT</td>
<td>GGCCACGAACCTTTATTTTAAAGTTAGGA</td>
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<td>TCTGAGAGAGAGGGACACTTGCAGAAGTTCCATTACATCCT</td>
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<td>ATGTCACCTTCTCTGTAATA</td>
<td>ATGTCACCTTCTCTGTAATA</td>
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<td>IGR3096</td>
<td>CCGGCCACCAACATCCT</td>
<td>TGTGTGTAGTGGAAGTTTCTTC</td>
<td>CGCAGCTGTTTCTCC</td>
<td>CGCAGCTGTTTCTCC</td>
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<tr>
<td>CARD15 702Trp</td>
<td>GCTGCGGCGGAGACA</td>
<td>CTAGTGCACAGACATCAGGAAAG</td>
<td>CTCGCTCTGGCGGCC</td>
<td>CTCGCTCTGGCGGCC</td>
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<tr>
<td>CARD15 908Arg</td>
<td>CAACCTCAAGCTCTGGGTACCT</td>
<td>CTGTGAGACAGCTTCTCTCTTCCAAACTTTTTC</td>
<td>TGTTCGCGCTTTAGAAT</td>
<td>TGTTCGCGCTTTAGAAT</td>
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<tr>
<td>CARD15 Leu1007fsinsC</td>
<td>TGTCGCGCGGATGACTACCTACCT</td>
<td>CTTCCAGGATGTTGTCATTTCC</td>
<td>TGCAGGCCCTTTGAA</td>
<td>TGCAGGCCCTTTGAA</td>
</tr>
</tbody>
</table>
Table 3: Results of single locus analyses at OCTN1, OCTN2 and IGR2096C>A, for 1104 IBD cases and 750 controls. Genotype and allele frequencies are also given for the 496 individuals with CD, and the 512 individuals with UC. Smoking and gender are included as confounders.

<table>
<thead>
<tr>
<th>Gene (variant)</th>
<th>Risk Allele Frequency n (%)</th>
<th>OR_{IBD} (95%CI)</th>
<th>Genotype n(%)</th>
<th>OR_{IBD} (95%CI)</th>
<th>P_{IBD} (For association assuming multiplicative model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCTN1 +1672</td>
<td>1027 (47.3)</td>
<td>473 (48.0)</td>
<td>616 (41.0)</td>
<td>1.31 (1.14-1.50)</td>
<td>264 (26.2)</td>
</tr>
<tr>
<td>OCTN2 -207</td>
<td>1124 (52.5)</td>
<td>526 (52.9)</td>
<td>695 (46.3)</td>
<td>1.31 (1.14-1.50)</td>
<td>219 (20.5)</td>
</tr>
<tr>
<td>IGR2230</td>
<td>1155 (52.6)</td>
<td>543 (52.4)</td>
<td>693 (46.1)</td>
<td>1.31 (1.15-1.51)</td>
<td>272 (25.4)</td>
</tr>
<tr>
<td>IGR2096</td>
<td>1023 (47.0)</td>
<td>471 (42.7)</td>
<td>609 (41.1)</td>
<td>1.29 (1.11-1.48)</td>
<td>288 (26.4)</td>
</tr>
<tr>
<td>IGR3096</td>
<td>1,106 (50.6)</td>
<td>591 (51.3)</td>
<td>495 (50.4)</td>
<td>1.30 (1.14-1.49)</td>
<td>250 (22.9)</td>
</tr>
</tbody>
</table>
Table 4: Results of single locus analyses at 702Trp, 908Arg and Leu1007fsinsC (snp13) for 1104 IBD cases and 750 controls. Genotype and allele frequencies are also given for the 496 individuals with CD, and the 512 individuals with UC

<table>
<thead>
<tr>
<th>Gene (variant)</th>
<th>Minor Allele Frequency</th>
<th>Genotype</th>
<th>Controls n (%)</th>
<th>UC n (%)</th>
<th>CD n (%)</th>
<th>OR&lt;sub&gt;CD&lt;/sub&gt;[95%CI]</th>
<th>P&lt;sub&gt;CD&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>controls</td>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>702Trp</td>
<td>93</td>
<td>49</td>
<td>104</td>
<td>(6.2)</td>
<td>(4.8)</td>
<td>(10.6)</td>
<td></td>
</tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/2</td>
<td>654(87.8)</td>
<td>394(80.4)</td>
<td>1.00 [Ref]</td>
<td>0.002</td>
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<tr>
<td></td>
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<td></td>
<td>1/2</td>
<td>89(11.9)</td>
<td>88(17.9)</td>
<td>1.64 [1.2-2.3]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2/2</td>
<td>2(0.3)</td>
<td>8(1.7)</td>
<td>6.64 [1.4-31.6]</td>
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<tr>
<td>908Arg</td>
<td>16</td>
<td>17</td>
<td>32</td>
<td>(1.1)</td>
<td>(1.7)</td>
<td>(3.2)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0/2</td>
<td>724(97.8)</td>
<td>426(86.8)</td>
<td>1.00 [Ref]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/2</td>
<td>16(2.2)</td>
<td>32(13.2)</td>
<td>3.13 [1.7-5.8]</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2/2</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu1007fsinsC</td>
<td>18</td>
<td>20</td>
<td>74</td>
<td>(1.1)</td>
<td>(2.0)</td>
<td>(7.5)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0/2</td>
<td>723(97.6)</td>
<td>426(86.8)</td>
<td>1.00 [Ref]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1/2</td>
<td>18(2.4)</td>
<td>56(11.4)</td>
<td>5.28 [3.0-9.2]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2/2</td>
<td>0(0)</td>
<td>9(1.8)</td>
<td></td>
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</tr>
</tbody>
</table>
Figure legend

Figure 1. Schematic representation of IBD5 locus on chromosome 5.
Figure 1. Schematic representation of IBD5 locus on chromosome 5. Relative positions of SNPs (ticks) Genes (thick lines) and Haplotype blocks (thin lines) are shown.
Evidence for association of OCTN genes and IBD5 with ulcerative colitis

Sarah Waller, Mark Tremelling, Fran Bredin, Lisa Godfrey, Joanna Howson and Miles Parkes

Gut published online December 16, 2005

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