Single Nucleotide Polymorphisms in *IL4*, *OCTN1* and *OCTN2* Genes in Association with Inflammatory Bowel Disease Phenotypes in a Caucasian Population in Canterbury, New Zealand

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Abstract: *Background*: The IBD5 (MIM# 606348) chromosomal region on 5q31 contains the genes for organic cation transporters, *OCTN1* and *OCTN2*, and has been associated with susceptibility to adult onset CD. While several studies argue that *OCTN1* and *OCTN2* are the causal genes, others fail to replicate these results.

Methods: From the Canterbury Inflammatory Bowel Disease Project, 388 CD, 405 UC, and 27 indeterminate colitis (IC) participants were genotyped for functional SNPs in *OCTN1*, *OCTN2*, and *IL4* genes, in comparison with 370 controls. TaqMan® technologies were used to genotype *IL4* rs2243250, *OCTN1* rs1050152, and *OCTN2* rs2631367 according to standard methodologies. The frequencies of variant SNPs in the control population were compared with frequencies in cases, where phenotype data for IBD was classed according to the Montreal classification.

Results: For those individuals carrying only the variant allele in *IL4* or *OCTN1*, despite trends towards increased risk of developing IBD, there were not statistically significant differences. There were no other specific effects of this allele in the location of CD, behaviour of UC or CD, or any other differences between controls and patient subgroups in this population group. However, carrying the variant *OCTN2* rs2631367 allele significantly increased the risk of ileocolonic CD (OR=1.42, 95% CI=1.02-1.97, $p<0.05$).

Conclusions: Despite earlier reports suggesting that *OCTN1* and *OCTN2* variants might provide important risk factors for IBD in New Zealand, this larger population-based study failed to confirm the earlier data. SNPs in the *IL4* gene did not affect the analysis. We suggest the possibility that strong effects for these variants in these genes seen in some studies could imply gene-environment interactions.

INTRODUCTION

Inflammatory Bowel Disease (IBD), encompassing both ulcerative colitis (UC) and Crohn's disease (CD) is a chronic inflammatory disorder of the digestive tract [1]. Especially for CD, familial clustering and concordance of the disease in monozygotic twin pairs confirms a genetic susceptibility that may interact with environmental factors, including diet [2]. Linkage studies have identified several chromosomal regions that have been consistently associated with disease risk [3].

The IBD5 (MIM# 606348) chromosomal region on 5q31 has been associated with susceptibility to adult onset CD in several studies [4, 5]. A 250 kb interval, identified by hierarchical fine-mapping, has identified a risk-associated haplotype containing 11 SNPs that are strongly linked with each other, and strongly associated with CD, at least in some populations [4, 6-8]. This region contains the sodium-dependent organic cation transporters, *OCTN1* and *OCTN2* (alternatively called *SLC22A4* and *SLC22A5*) that function in the transport of various xenobiotics. Peltekova and coworkers [9] sequenced five genes in IBD5, to identify ten single nucleotide polymorphisms (SNPs), which they predicted to have functional effects on gene function. These included the polymorphism L503F in exon 9 of *OCTN1* (c.1672C/T; rs1050152) that causes an amino acid substitution, and a G to C transversion (-c.207G/C; rs 2631367) in the promoter of *OCTN2*, which is thought to disrupt a heat shock transcription factor binding element. Although these SNPs and these genes appear implicated in CD risk for certain population groups, the studies do not replicate in other populations, and the exact nature of the causal gene remains elusive and debatable [10-12]. It is noteworthy that there are also a number of important cytokine genes including interleukin 4 (*IL-4)* immediately adjacent to the 250 kb risk haplotype. The -

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590C/T SNP in this gene has been previously associated with autoimmune diseases such as type I diabetes and rheumatoid arthritis, and has been suggested as a possible causal variant in CD [13].

Gründemann *et al.* [14] have shown that the fungal metabolite, ergothionine (ET) is the main physiological substrate of *OCTN1*. Taubert *et al.* [15] demonstrated that the amino acid substitution L503F is a gain of function polymorphism, resulting in a threefold higher substrate affinity and 50% higher initial transport capacity at low levels of ET (≤ 10μmol/l) in transfected HEK293 cells.

A previous Auckland-based study suggested the possibility of an *OCTN1* and *OCTN2* haplotype associating with CD risk in New Zealand [16]. However, numbers and statistical significance were low, and we have failed to repeat this observation in our own larger Canterbury-based population.

MATERIALS AND METHODS

Study Participants

The cases in this study are a random subset of the Caucasian participants of the Canterbury Inflammatory Bowel Disease Project, which has been described in detail elsewhere [17]. The subset was identified as those who were willing to provide a second DNA sample for the new studies. All subjects consented to collection of peripheral blood for DNA extraction and genotyping in comparison with their clinical data for CD or UC, as defined using standard diagnostic criteria [18]. The Montreal Classification system allowed genotype-phenotype analysis to be performed [19].

388 CD participants, 405 UC participants, and 27 indeterminate colitis (IC) participants were genotyped for this study, as described in Table **1**. All participants self-reported European ancestry, and patients who self-reported having any Maori or other non-Caucasian ancestry are not included.

The 370 New Zealand Caucasian controls were selected at random from the electoral roll, comprising 93% of the population over eighteen years of age in Canterbury, New Zealand [20].

Applied Biosystems TaqMan®SNP Genotyping Assay. SNPs were genotyped using the TaqMan MGB diallelic discrimination system. Probes and oligonucleotides were obtained from Applied Biosystems using the Assay-by-Design product (listed in Table **2**). The reactions were prepared by using 2x TaqMan Universal Master Mix, 40x SNP Genotyping Assay Mix, DNase-free water, 10 ng genomic DNA in a final volume of 5 ul per reaction. The PCR amplification was performed using the ABI Prism 7900 HT sequence-detector machine under the following conditions: 10 min 95°C enzyme activation followed by 40 cycles at 92°C for 15 sec and 60°C for 1min (annealing/extension). The allelic discrimination results were determined after the amplification by performing an endpoint read. To estimate genotype accuracy approximately 5% of the samples were genotyped in duplicate or triplicate for each of the markers. There were no conflicting duplicate genotypes among the 70 genotypes (60 for IL4) which could be compared to a duplicate genotype.

Table 1. Summary of Clinical and Demographic Data for the Set of Caucasian IBD Patients

	CD n (%)	UC n (%)	IC n (%)
Gender			
Female	249 (64.2)	214 (52.8)	15 (55.6)
Male	139 (35.8)	191 (47.2)	12 (44.4)
Age at first diagnosis			
Below 17	39 (10.0)	26 (6.4)	0
Between 17 and 40	199 (51.3)	184 (45.4)	15 (55.6)
Above 40	150 (38.7)	195 (48.2)	12 (44.4)
CD location			
Ileal	125 (32.2)		
Colonic	169 (43.6)		
Ileocolonic	90 (23.2)		
Unknown (U + UN)	4 (1.0)		
UC location			
Proctitis		140 (34.6)	3 (11.1)
Left colon		107 (26.4)	5 (18.5)
Pancolitis		154 (38.0)	19 (70.4)
Unknown		4 (1.0)	0
Behaviour			
Non-stricturing, non-penetrating perianal disease: With	47 (21.5)		
Without	172 (78.5)		
Stricturing perianal disease: With	46 (38.0)		
Without	75 (62.0)		
Penetrating perianal disease: With	17 (35.4)		
Without	31 (64.6)		
Any relative with IBD: Yes (n=143)	74 (19.1)	65 (16.1)	5 (18.5)
Bowel resection: Yes (n=214)	142 (36.6)	70 (17.3)	2 (7.4)
Smoker at diagnosis: Yes (n=147)	97 (25.7)	49 (12.3)	2 (7.7)
Ever used immunomodulators: Yes (n=296)	203 (52.3)	86 (21.2)	8 (29.6)
Extraintestinal manifestations: Yes (n=142)	75 (19.3)	64 (15.8)	3 (11.1)

Statistical Analysis

The allelic trend test [21] and Fisher's exact genotypic test were used to compare case and control allele frequencies using the SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). An exact test was used to test for departures from Hardy-Weinberg equilibrium (HWE) in the case and the control samples [22]. Allelic odds ratios and confidence intervals for the allelic odds ratio were calculated under the

Table 2. Primer and Probe Sequences for Custom Made TaqMan SNP Genotyping Assay for IL4, OCTN1, and OCTN2

TaqMan SNP Genotyping Assay	DNA Sequence
IL4 -590C/T _forward primer	5' GACCTGTCCTTCTCAAAACACCTAA 3'
IL4 -590C/T _reverse primer	5' GGCAGAATAACAGGCAGACTCT 3'
IL4 -590C/T _VIC probe	5' CATTGTCCCCCAGTGCT 3'
IL4 -590C/T _FAM probe	5' CATTGTTCCCCAGTGCT 3'
OCTN1 Leu503Phe _forward primer	5' GGGTAGTCTGACTGTCCTGATTG 3'
OCTN1 Leu503Phe _reverse primer	5' TCTGGAAGAGTCATTCCCAAACTTTC 3'
OCTN1 Leu503Phe _VIC probe	5' AAGGGTGAGGATTC 3'
OCTN1 Leu503Phe _FAM probe	5' AAGGGTGAAGATTC 3'
OCTN2_-207G/C_forward primer	5' CCGCTCTGCCTGCCA 3'
OCTN2_-207G/C _reverse primer	5' GCGGCTGGCCTTACATAGG 3'
OCTN2_-207G/C _VIC probe	5' CAGGCCCGGAACC 3'
OCTN2_-207G/C _FAM probe	5' CAGGCCCGCAACC 3'

assumption of HWE in the cases and the control groups. Within the region, HAPLO.SCORE in R [23], was used to estimate haplotypes and to test for association of these haplotypes within the case-control population.

Results

The summary of demographic and clinical characteristics data for Caucasian IBD patients in this study is given in Table **1**. Data for *IL4*, *OCTN1*, and *OCTN2* hold the Hardy-Weinberg equilibrium (Table **3**). Table **3** also shows the genotype and allele counts between IBD patients and NZ Caucasians. Subgroup analysis is shown in Table **4**.

For those individuals with CD carrying the variant *IL4* allele, there appeared to be a decreased probability that a close relative would be affected (OR=0.61, 95% CI=0.38-0.98). However, there were no other statistically significant differences.

There was no significant difference in the genotype and allele frequencies of the c.1672T polymorphism in the *OCTN1* gene between control subjects and IBD patients (both CD and UC). Table **4** gives the odds ratio for carrying the variant allele in CD or UC as compared with the control group. There were also no statistically significant specific effects of this allele in relation to the location of CD and UC, or CD behaviour in this population group.

As concerns *OCTN2* c.-207 G/C, heterozygotes had a 1.5-fold increased risk (95% CI: 1.10-2.17, p=0.01) of developing UC as compared with individuals homozygous for the 207C allele (Table **3**). However, this did not extend to CC homozygotes (OR=1.20, 95% CI: .81-1.76, p=0.82). The allele frequencies of the variant *OCTN2* c.-207 G/C were not significantly different between the IBD patients and control subjects. However, there was a statistically significant effect of the variant allele on the ileocolonic location of CD (Table **4**). This indicates that IBD patients who carry the C allele have a significantly higher risk of ileocolonic location of CD (OR=1.42, 95% CI=1.02-1.97).

Haplotype Analysis of OCTN1 and OCTN2

Haplotype frequencies and association analyses were performed with respect to CD and UC in New Zealand IBD Caucasian patients and controls.

Each two-SNP disequilibrium block was analysed using HAPLO.SCORE and Table **5** summarises the results of HAPLO.SCORE. A score for each haplotype (Hap-score) is calculated and p-value is also calculated for the significance of each Hap-score. A positive hap-score implies that the haplotype occurs more frequently in the CD or UC case group as compared with controls. The results indicate that no significant association between these haplotypes and either CD or UC cases, p=0.86 and p=0.87 respectively. In the analysis of the two SNPs (OCTN1, and OCTN2), the haplotypes in case and control subjects occur at equal frequencies in CD or UC case as compared with control subject population (p>0.05, Table **5**). In CD subjects, the frequency of haplotype TG was uncommon (0.7%), while haplotype CG was the most common (48.5%). Haplotype CG (49.6%) was the most common and haplotype TG (0.7%) was uncommon in the UC subjects.

Haplotype Analysis of *IL4*, *OCTN1*, and *OCTN2*

Haplotype frequencies and association analyses were performed with respect to CD and UC in New Zealand IBD Caucasian patients and controls. Each three-SNP disequilibrium block was analysed using HAPLO.SCORE and Table **5** summarises the results of HAPLO.SCORE. A score for each haplotype (Hap-score) is calculated and p-value is also calculated for the significance of each Hap-score. A positive hap-score implies that the haplotype occurs more frequently in the CD or UC case group as compared with controls. The results indicate that no significant association between these haplotypes and either CD or UC cases, p=0.79 and p=0.47 respectively. In the analysis of the three SNPs (IL4, OCTN1, and OCTN2), the haplotypes in case and control subjects occur at equal frequencies in CD or UC case as compared

Table 3. Genotype and Allele Counts for IL4, OCTN1, and OCTN2 Variants in New Zealand IBD Patients and in New Zealand Caucasians

SNP	Controls	CD		UC		CD+UC		IC
IL4_2243250 CC	286 (77.3)	287 (74.9)	1.00	314 (77.7)	1.00	606 (76.3)	1.00	22 (81.5)
CT	77 (20.8)	90 (23.5)	1.16 (0.83,1.64)	85 (21.0)	1.03 (0.73,1.45)	177 (22.3)	1.08 (0.80,1.46)	5 (18.5)
TT	7 (1.9)	6 (1.6)	0.85 (0.28,2.57)	5 (1.2)	0.66 (0.21,2.09)	11 (1.4)	0.75 (0.29,1.95)	0
Genotype p-value		0.65		0.76		0.72		
HWE p-value		0.73		0.78		0.67		
T	91 (12.3)	102 (13.3)		95 (11.8)		197 (12.5)		5 (0.09)
C	649 (87.7)	664 (86.7)		713 (88.2)		1377 (87.5)		49 (0.91)
OR (95% CI)		1.10 (0.80,1.50)		0.95 (0.69,1.31)		1.02 (0.78,1.34)		
Allelic p-value		0.55		0.74		0.88		
OCTN1_1050152 CC	110 (29.7)	109 (28.5)	1.00	121 (30.0)	1.00	230 (29.2)	1.00	8 (30.8)
CT	178 (48.1)	183 (47.8)	1.02 (0.73,1.41)	209 (51.7)	1.04 (0.75,1.42)	392 (49.8)	1.05 (0.79,1.41)	15 (57.7)
TT	82 (22.2)	91 (23.8)	1.11 (0.75,1.64)	74 (18.3)	0.81 (0.55,1.21)	165 (21.0)	0.96 (0.68,1.36)	3 (11.5)
Genotype p-value		0.85		0.38		0.84		
HWE p-value		0.41		0.33		0.93		
T	342 (46.2)	365 (47.7)		357 (44.2)		722 (45.9)		21 (40.4)
C	398 (53.8)	401 (52.3)		451 (55.8)		852 (54.1)		31 (59.6)
OR (95% CI)		1.06 (0.86-1.30)		0.92 (0.75-1.13)		0.99 (0.82-1.18)		
Allelic p-value		0.58		0.42		0.88		
OCTN2_2631367 CC	106 (28.7)	98 (25.6)	1.00	88 (21.8)	1.00	186 (23.6)	1.00	5 (19.2)
CG	163 (44.0)	196 (51.2)	1.26 (0.91,1.77)	217 (53.7)	**1.54 (1.10,2.17)**	413 (52.5)	**1.44 (1.07,1.95)**	14 (53.8)
GG	101 (27.3)	89 (23.2)	0.99 (0.67,1.45)	99 (24.5)	1.20 (0.81,1.76)	188 (23.9)	1.06 (0.76,1.49)	7 (26.9)
Genotype p-value		0.14		**0.02**		**0.03**		
HWE p-value		0.64		0.13		0.16		
C	375 (50.7)	392 (51.2)		393 (48.6)		785 (49.9)		24 (46.2)
G	365 (49.3)	374 (48.8)		415 (51.4)		789 (50.1)		28 (53.8)
OR (95% CI)		1.02 (0.83,1.26)		0.92 (0.75,1.13)		0.97 (0.81,1.15)		
Allelic p-value		0.85		0.42		0.72		

with control subject population (p>0.05, Table **5**). In CD subjects, the frequency of haplotype CTG was uncommon (0.4%), while haplotype CTC was the most common (42.6%). Haplotypes CTC and CCG were the most common and haplotype CTG was uncommon in the UC subjects.

DISCUSSION

The evidence implicating the IBD5 region in risk of CD is exceptionally strong in Caucasian populations [4-6]. Fine mapping studies led to the identification of a 250kb haplotype block that associates with risk [4]. However, there may be ethnic differences, since Japanese studies [24, 25] have reported negative results for the association between these diseases and this locus.

While some groups such as Newman, *et al*. [26], Noble *et al*. [27] or Palmieri *et al*. [28] have implicated *OCTN1/2* variants within the IBD5 locus in relation both to disease susceptibility and severity in CD, such observations have not generally replicated.

Noble and colleagues in Edinburgh [27] studied 374 patients with CD, and concluded that, although the *OCTN1* and *OCTN2* variants were associated with CD risk, this association was not independent of the background risk haplotype in the region. As well as *OCTN1/2* variants this study also included a number of surrounding SNPs (IGR2096, IGR 2198

Table 4. Allelic Odds Ratios and 95% Confidence Intervals for Comparison of IL4, OCTN1, and OCTN2 Variants with IBD Status in New Zealand IBD Patients and Caucasians

	Allelic Odds Ratios and 95% Confidence Intervals for Comparison of IL4_2243250 SNP				Allelic Odds Ratios and 95% Confidence Intervals for Comparison of OCTN1_1050152 SNP				Allelic Odds ratios and 95% Confidence Intervals for Comparison of OCTN2-2631367 SNP			
	Crohns Disease		Ulcerative Colitis		Crohns Disease		Ulcerative Colitis		Crohns Disease		Ulcerative Colitis	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Female	0.82	0.56-1.20	0.98	0.65-1.47	1.06	0.82-1.36	1.23	0.95-1.60	0.92	0.72-1.18	0.79	0.61-1.03
Male	1.11	0.67-1.83	1.10	0.69-1.75	0.79	0.58-1.08	0.94	0.70-1.26	1.14	0.83-1.56	1.09	0.82-1.46
Age at first diagnosis												
0-16 years	0.82	0.42-1.61	1.31	0.51-3.38	0.74	0.46-1.18	1.38	0.78-2.45	1.25	0.78-2.00	0.90	0.51-1.58
17-40 years	0.90	0.63-1.29	1.14	0.77-1.69	0.96	0.75-1.22	1.12	0.87-1.43	0.99	0.78-1.26	0.86	0.67-1.10
>40 years	0.95	0.63-1.42	0.92	0.64-1.33	1.00	0.77-1.30	1.04	0.82-1.32	0.97	0.74-1.26	0.98	0.77-1.25
CD location												
Ileal	1.20	0.76-1.90			0.98	0.76-1.26			0.81	0.61-1.07		
Colonic	0.84	0.58-1.22			1.10	0.83-1.46			0.99	0.77-1.28		
Ileocolonic	0.80	0.50-1.28			**0.72**	**0.52-1.00**			**1.42**	**1.02-1.97**		
UC location												
Proctitis			0.88	0.59-1.32			1.12	0.83-1.52			0.80	0.61-1.05
Left colon			1.28	0.78-2.11			0.93	0.72-1.21			0.93	0.69-1.26
Pancolitis			1.05	0.70-1.58			1.27	0.96-1.67			1.02	0.79-1.33
CD Behavior												
Inflammatory	0.93	0.65-1.33			1.00	0.79-1.26			0.99	0.78-1.25		
Stricturing	0.89	0.58-1.37			0.76	0.50-1.16			0.94	0.71-1.25		
Penetrating	0.89	0.48-1.66			0.95	0.71-1.27			1.24	0.81-1.90		
Ileal/Stricturing	1.10	0.63-1.91			1.15	0.81-1.63			0.76	0.54-1.07		
Colonic/Inflammatory	0.97	0.64-1.47			1.02	0.78-1.34			0.98	0.75-1.28		
Any relative with IBD	**0.61**	**0.38-0.98**	0.76	0.45-1.28	0.84	0.59-1.19	0.74	0.51-1.07	1.13	0.80-1.60	1.20	0.83-1.74
Bowel resection	0.72	0.49-1.06	1.08	0.61-1.90	0.94	0.72-1.23	1.15	0.80-1.65	0.97	0.74-1.27	0.84	0.59-1.20
Smoker at diagnosis	0.78	0.50-1.23	0.83	0.45-1.52	0.97	0.71-1.33	1.42	0.92-2.18	0.99	0.72-1.35	0.75	0.49-1.15
Ever used immunomodulators	0.90	0.63-1.29	1.19	0.70-2.03	0.87	0.69-1.10	1.06	0.76-1.47	1.04	0.82-1.32	0.90	0.65-1.25
Any EIMs	0.65	0.40-1.05	1.05	0.59-1.88	1.01	0.71-1.43	0.78	0.54-1.13	0.92	0.65-1.31	1.17	0.81-1.70

and IGR2230) that failed to associate independently of the *OCTN1/2* variants.

The IBD5 locus or *OCTN1/2* variants have also previously been associated with specific disease locations, but not the same as in the present study. Armuzzi *et al*. [28] reported association of perianal CD with the IBD5 locus in a UK study. The IBD5 region has also been associated with markers of more severe disease such as progression to structuring or penetrating CD and need for surgery in an Edinburgh population [29]. Russell *et al*. [30] also suggested that the *OCTN* T/C haplotype was a marker for more severe CD in a pediatric population, as individuals carrying this haplotype showed lower height, weight and body mass index (BMI) when diagnosed. Data from the Italian population studied by Latiano *et al*. [31] also suggested that the IBD5 locus was related to more severe CD behaviour, although the effect may have partly been through the combination with *CARD15 (NOD2)* risk alleles rather than by IBD5 locus alone.

The enormous variability in data from different populations, even of the same ethnicity, has led to the suggestion that the IBD5 locus may be a susceptibility allele that is exceptionally susceptible to gene-gene and gene-environmental influences [10]. Because ET concentrations found in tissues and foods are in the nanomolar to micromolar range, Taubert *et al*. [14] suggested that carriers of the 503F allele accumulate higher ET concentrations in *OCTN1* expressing cells, that may constitute a possible risk factor for CD. Certainly, there have been several reports of an association with *NOD*2 [6, 7, 32], although this interaction was non-significant in our own population. The *OCTN1* and *OCTN2* genes would

Table 5a. Haplotype Analysis of three-SNPs and IBD Status in New Zealand IBD Patients and Caucasians

	Haplotype	Case Subject Frequency (%)	Control Subject Frequency (%)	Hap-Score	Haplotype-Specific Scores p Value	Global Score Statistics
Crohn's Disease	Three SNPs across region					
	IL4, OCTN1,	4.3	5.0	-0.71	0.48	$\chi^2 = 3.12$, df = 6, p value = 0.79
	OCTN2	0.4	0.7	-0.44	0.66	
	CCC	8.8	9.8	-0.22	0.82	
	CTG	39.5	38.9	-0.005	0.99	
	TCG	42.6	43.2	4e-05	0.99	
	CCG	2.3	4.1	1.46	0.15	
	CTC					
	TTC					
Ulcerative Colitis	Three SNPs across region					
	IL4, OCTN1,	41.1	43.2	-0.81	0.42	$\chi^2 = 6.59$, df = 7, p value = 0.47
	OCTN2	9.8	8.2	-0.75	0.45	
	CTC	4.2	5.0	-0.45	0.65	
	TCG	0.5	0.7	-0.20	0.84	
	CCC	2.3	2.3	-0.17	0.87	
	CTG	42.4	38.9	1.21	0.23	
	TTC	1.1	0.2	1.71	0.09	
	CCG					
	TCC					

Table 5b. Haplotype Analysis of two-SNP Haplotypes and IBD Status in New Zealand IBD Patients and Caucasians

	Haplotype	Case Subject Frequency (%)	Control Subject Frequency (%)	Hap-Score	Haplotype-Specific Scores p Value	Global Score Statistics
Crohn's Disease	Two SNPs across region					
	OCTN1, OCTN2	4.3	5.1	-0.81	0.42	$\chi^2 = 0.77$, df = 3, p value = 0.86
	CC	48.3	48.6	-0.12	0.90	
	CG	46.6	45.5	0.21	0.84	
	TG	0.8	0.7	0.43	0.67	
	TC					
Ulcerative Colitis	Two SNPs across region					
	OCTN1, OCTN2	5.2	5.2	-0.83	0.41	$\chi^2 = 0.71$, df = 3, p value = 0.87
	TC	50.6	48.6	0.05	0.96	
	CC	43.4	45.5	0.14	0.89	
	TG	0.8	0.7	0.77	0.44	
	CG					

seem to be prime candidates for the study of possible gene-diet interactions in Caucasian populations in New Zealand.

CONCLUSIONS

We conclude that these genes (*IL4, OCTN1 and OCTN2*), *per se*, are not significant risk factors for Inflammatory Bowel Disease in the New Zealand population.

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