PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(47):7525-7530
World Journal of Gastroenterology ISSN 1007-9327

• *RAPID COMMUNICATION* •

Single nucleotide polymorphisms of *OCTN1*, *OCTN2*, and *DLG5* genes in Greek patients with Crohn's disease

Maria Gazouli, Gerassimos Mantzaris, Athanassios J Archimandritis, George Nasioulas, Nicholas P Anagnou

Maria Gazouli, Nicholas P Anagnou, Department of Biology, School of Medicine, University of Athens, Athens 11725, Greece
Gerassimos Mantzaris, Department of Gastroenterology, Evagelismos Hospital, Athens 11521, Greece
Athanassios J Archimandritis, Department of Internal Medicine, Hippokration General Hospital, School of Medicine, University of Athens, Athens 11527, Greece
George Nasioulas, Molecular Biology Department Research Center HYGEIA "Antonis Papayiannis", Athens, Greece
Correspondence to: Dr N.P. Anagnou, Department of Biology, School of Medicine, University of Athens, M. Asias 75, Athens 11527, Greece. anagnou@med.uoa.gr
Telephone: +30-2107462341 Fax: +30-2107462340
Received: 2005-02-23 Accepted: 2005-06-24

Abstract

AIM: To validate novel single nucleotide polymorphisms (SNPs) in Greek patients with Crohn's disease (CD).

METHODS: A total of 120 patients with CD, 85 patients with UC, and 100 unrelated healthy controls were genotyped. Genotyping was performed by allele-specific PCR or by PCR-RFLP analysis.

RESULTS: Our results showed that the 1672T and -207C alleles were obviously over-represented in CD patients only ($P<0.01$ and $P<0.05$, respectively) compared to the control population. The G113A polymorphism was completely absent in our studied population. The odds ratio for the carriage of the TC haplotype was 2.21 for CD patients as compared with controls. Additionally, the frequency of the TC haplotype was increased in patients with ileocolitis or colitis, and was mainly associated with the fibrostenotic phenotype of the disease. Furthermore, when the TC haplotype was compared jointly with the carriage of at least one mutation of the *NOD*2/*CARD*15 gene, there was an increased risk for CD, but not for UC, compared to controls. Regarding the location of the disease, the concomitant presence of the TC haplotype and *NOD*2/*CARD*15 mutations was mainly associated with ileocolitis or ileitis.

CONCLUSION: Collectively, our results suggest that the 1672T variant of the *OCTN*1 gene and the -207C variant of the *OCTN2* gene represent risk factors for CD in the Greek population.

Key words: Crohn's disease; SNPs; *OCTN1*; *OCTN2*; *DLG5*

Gazouli M, Mantzaris G, Archimandritis AJ, Nasioulas G, Anagnou NP. Single nucleotide polymorphisms of the *OCTN1*, *OCTN2*, and *DLG5* genes in Greek patients with Crohn's disease. *World J Gastroenterol* 2005; 11(47): 7525-7530
http://www.wjgnet.com/1007-9327/11/7525.asp

INTRODUCTION

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), represent common chronic relapsing and remitting inflammatory disorders of the intestine. The pathogenesis of IBD is complex and both environmental and genetic factors contribute to its etiology. A series of genetic and epidemiologic studies have provided conclusive evidence for the presence of genetic determinants of disease susceptibility and progression[1]. Genome-wide linkage studies of IBD families that were affected by multiple factors have been remarkably successful in identifying a number of susceptibility loci, with convincing replication shown for at least 7 loci (IBD1-7)[2]. In 1996, Hugot *et al*[3]. identified the first susceptibility locus for CD adjacent to the centromere on chromosome 16 (IBD1)[3]. This has been further corroborated in several independent Caucasian populations[4-6]. In 2001, three independent CD mutations within the *NOD*2/*CARD*15 gene, mapping to chromosome 16, were discovered. These mutations are strongly associated with CD in populations of European descent[7-11].

Very recently, Peltekova *et al*[12]. reported on two novel functional single nucleotide polymorphisms (SNPs) in the carnitine/organic cation transporter (OCTN) cluster on 5q31 (designated as the IBD5 locus) that were associated with CD[12]. The cation transporters are expressed in the liver, kidney, intestine, brain, heart and placenta, and maintain physiological cation environments in the organism.

A C1672T substitution in exon 9 of the *OCTN*1 gene and a G-207C in the *OCTN*2 promoter region have been suggested as causative mutations to increase susceptibility to CD. Additionally, Stoll *et al*[13]. reported that a G113A SNP in exon 3 of the *DLG*5 gene was also associated with susceptibility to IBD. This gene is located on the long arm of chromosome 10 (10q23) and encodes a scaffolding protein involved in the maintenance of epithelial integrity. Genetic variants in *DLG*5 could therefore interfere with the epithelial barrier.

Table 1 Demographic characteristics and clinical features of 120 patients with Crohn's disease and of 85 patients with ulcerative colitis

	Crohn's disease	Ulcerative colitis
Total number	120	85
Sex (male/female)	58/62	42/43
Age of diagnosis (mean±SD yr)	29.82±14.00	33.36±14.24
Family history in first-degree relative (%)	4 (3.3 %)	5 (5.9%)
Smoking habit (%)		
Never	50 (41.7%)	48 (56.5%)
Ex-smoker	11 (9.2%)	14 (16.5%)
Current	59 (49.2%)	23 (27.1%)
Localization of disease		
Ileal	39 (32.5%)	
Colonic	11 (9.2%)	
Ileocolitis	67 (55.8%)	
Upper gastroenteric	3 (2.5%)	
Disease features		
Inflammatory	78 (65%)	
Fibrostenotic	32 (26.7%)	
Fistulizing	10 (8.3%)	
Extra-intestinal manifestations		
Arthritis	16 (13.3%)	
Erythema nodosum	5 (4.2%)	

To investigate whether the above mentioned SNPs in *OCTN*1, *OCTN*2, and *DLG*5 genes contribute to the predisposition to IBD, as well as whether the interaction of specific haplotypes of the *NOD*2/*CARD15*, *OCTN*1, *OCTN*2, and *DLG*5 genes could increase the risk for IBD in the Greek population, we genotyped 120 patients with CD, 85 patients with UC, and 100 healthy controls. Our studies documented that mutations of the *OCTN1* and *OCTN*2 genes were obviously associated with CD. Furthermore, the combination of the OCTN-TC haplotype was found to be significantly associated with ileocolitis or colitis and the fibrostenotic phenotype, while the combination of the TC haplotype with the *NOD*2/*CARD15* variants was associated with ileocolitis or ileitis.

MATERIALS AND METHODS

Subjects

Blood samples from 120 patients with CD, 85 patients with UC and 100 age- and sex-matched healthy individuals were collected at the IBD Outpatient Clinic of the Evagelismos Hospital, between September 2002 and February 2003. The vast majority of these patients had been diagnosed at our institutions (open-access visit to the IBD Outpatients Clinic or as emergency cases), but there were also some referrals by other physicians. All groups were matched with regard to sex and age, and all subjects were of Greek origin. The diagnosis of either CD or UC was based on standard clinical, endoscopic, radiological, and histological criteria[7,14]. For CD, the vast majority of the patients (102, 85%) had newly diagnosed disease that was classified according to the Vienna System. The records of CD patients were systematically reviewed for the following demographic and clinical characteristics: age, sex, smoking habits, age at diagnosis, disease localization (ileal, colonic, ileocolonic or upper gastroenteric), disease behavior (inflammatory, fibrostenotic or fistulizing), presence of extra-intestinal clinical manifestations (e.g., arthritis, erythema nodosum), and familial IBD (Table 1). Before the commencement of the study, the ethics committee at the participating centers had approved the recruitment protocols. Informed consent was obtained from all the participants.

Genotyping

DNA was isolated from blood with the NucleoSpin blood kit (Macherey-Nagel, Germany). To confirm the integrity of DNA, initially a 430-bp sequence in the human glyceraldehyde-3-phosphatate dehydrogenase gene was amplified.

The genotyping for the three casual *NOD*2/*CARD*15 variants (L1007fsinsC, R702W, and G908R) in the studied group of patients and controls has been previously performed[7].

The C1672T substitution in exon 9 of the *OCTN*1 was genotyped by a PCR amplification of specific allele assay, using two allele-specific reverse primers: octn1C, 5' TCTGACTGTCCTGATTGGAATCC 3' for the wild type allele and octn1T: 5' TCTGACTGTCCTGATTGGAATCT 3' for the mutant allele, in combination with a common forward primer octn1F: 5' AGATGAGGTTTCACTATGTTGGC 3' in two separate PCR reactions. The 3'-ends of the reverse primers were able to anneal to the regions that differed between the two alleles. The PCR profile included initial denaturation at 95 ℃ for 5 min, followed by 35 amplification cycles of denaturation at 94 ℃ for 45 s, annealing at 58 ℃ for 40 s and extension at 72 ℃ for 30 s and a final incubation at 72 ℃ for 10 min.

The mutation G-207C in the *OCTN*2 promoter region resulted in the abolishment of a restriction site for NlaIV and was genotyped by a combined PCR-RFLP method using primers 5' TCAGGTGCACTCCCGGCCCG 3' (forward) and 5' GACCAGGCAAGCCAGGCAGC 3' (reverse). The presence of a wild-type allele resulted in the generation of three fragments (42, 44, and 122 bp), whereas the RFLP profile of the -207C variant was characterized by two fragments of 42 and 167 bp, analyzed by 30 g/L agarose gel electrophoresis. The PCR conditions included initial denaturation at 95 ℃ for 5 min, followed by three cycles of denaturation at 94 ℃ for 40 s, annealing at 58 ℃ for 1 min and extension at 72 ℃ for 2 min, by two cycles of denaturation at 94 ℃ for 40 s, annealing at 56 ℃ for 1 min and extension at 72 ℃ for 2 min, and by 28 cycles of denaturation at 94 ℃ for 40 s, annealing at 54 ℃ for 1 min and extension at 72 ℃ for 2 min, and a final incubation at 72 ℃ for 10 min.

The G113A SNP in exon 3 of the *DLG*5 gene creates a restriction site for *Msp*I and was also genotyped by a combined PCR-RFLP method using a primers 5' TCACTTTCAGTTCTACCTGCTAC 3' (forward) and 5' TCTAGGAGACAGTGGTAGGG 3' (reverse). The

Table 2 Allele and genotype frequencies of C1672T SNP in *OCTN*1 gene in CD and UC patients and in healthy controls

	Alleles				Genotypes				
	C	T	T allele frequencies (%)	*P* [odds ratio (95%CI)]	CC	CT	TT	TT genotype frequencies (%)	*P* [odds ratio (95%CI)]
CD	180	60	25	0.01 [1.89 (1.16–3.07)]	70	40	10	8.33	0.095 [2.94 (0.78–10.99)]
UC	151	19	11.17	0.28 [0.71 (0.38–1.32)]	67	17	1	1.17	0.39 [0.38 (0.04–3.77)]
Controls	170	30	15		73	24	3	3	

Table 3 Allele and genotype frequencies of G-207C SNP in OCTN2 gene in CD and UC patients and in healthy controls

	Alleles				Genotypes				
	G	C	C allele frequencies (%)	*P* [odds ratio (95%CI)]	GG	GC	CC	CC genotype frequencies (%)	*P* [odds ratio (95%CI)]
CD	188	52	21.67	0.038 [1.69 (1.02–2.81)]	75	38	7	5.83	0.53 [1.49 (0.42–5.23)]
UC	152	18	10.58	0.32 [0.73 (0.39–1.37)]	69	14	2	2.35	0.53 [0.58 (0.10–3.24)]
Controls	172	28	14		76	20	4	4	

Table 4 Linkage disequilibrium (*D'* and r^2 between 1672T and -207C are indicated), and TC haplotype frequencies in patients with CD, UC and in healthy individuals

	Number of patients	*D'*	r^2	TC haplotype frequencies (%)	*P* [odds ratio (95%CI)]
CD	120	0.51	0.22	13.3	0.018 [2.21 (1.12–4.43)]
UC	85	0.5	0.23	5.9	0.81 [0.89 (0.37–2.15)]
Controls	100	0.34	0.1	6.5	

Table 5 Odds ratios for susceptibility to CD and UC of a *NOD*2/*CARD*15 mutation, and for the joint TC- NOD2/CARD15 effect

	Odds ratios		
	TC	*NOD*2/*CARD*15	Joint TC- *NOD*2/*CARD*15
CD	2.21 (1.12–4.43) *P* = 0.018	16.8 (8.6–32.7) *P* < 0.0001	9.22 (2.1–40.6) *P* = 0.0005
UC	0.89 (0.37–2.15) *P* = 0.79	3.34 (1.76–6.36) *P* = 0.0002	3.06 (0.58–16.21) *P* = 0.17

presence of a wild-type allele resulted in five fragments of 40, 51, 65, 124, and 360 bp, whereas the RFLP profile of the 113A variant was characterized by four bands of 65, 91, 124, and 360 bp. The PCR conditions included initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturation at 94 ℃ for 45 s, annealing at 58 ℃ for 40 s and extension at 72 ℃ for 30 s, and a final incubation at 72 ℃ for 10 min.

All PCR assays were performed in a 50-μL volume reaction containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 250 μmol/L dNTPs, 0.20 μmol/L of each primer, 200 ng of genomic DNA and 2.5 U of Taq DNA polymerase (Platinum Invitrogen). The specificity of PCR products was confirmed by sequencing analysis using a Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Darmstadt, Germany), and an ABI 377 automated sequencer.

Statistical analysis

Frequency and susceptibilities of mutations among the patients and controls were compared using χ^2 test. Odds ratios (OR) were calculated with the corresponding χ^2 distribution test and 95% confidence intervals (95%CI). The two-tailed $P<0.05$ were considered statistically significant. Hardy-Weinberg equilibrium was verified by the calculation of expected frequencies and numbers, and significance testing was based on the 1 df χ^2. The hypothesis that there is no linkage disequilibrium (LD) was also tested using the 1 df χ^2. Allele frequency independent estimators of LD were used: the $D' = D/D_{max}$, where D_{max} is the maximum possible D (i.e., for border frequencies p_1, p_2, q_1, q_2, the lesser of p_1q_2 or p_2q_1 if D is positive or lesser of p_1q_1 or p_2q_2 if D is negative). Inference was aided by GraphPad InStat (version 3.00, GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

We genotyped 120 patients with CD, 85 patients with UC and 100 healthy individuals in order to investigate a possible association of the genetic substitutions in the *OCTN*1, *OCTN*2 and *DLG5* genes with a susceptibility to CD in the Greek population. These mutations were reported to have significant association with CD in the Caucasian population[13,14].

The C1672T of the *OCTN*1 genotype carrier frequencies are summarized in Table 2. The distribution of genotypes was consistent with the Hardy-Weinberg equilibrium. The 1672T allele frequencies were 25%, 11.17%, and 15% in CD, UC and healthy controls, respectively. The frequency of the 1672T allele was significantly higher in CD patients compared to the controls ($P<0.05$). The 1672T allele was not found to be significantly associated with UC.

Allele and genotype frequencies of the mutations G-207C of the *OCTN*2 gene are presented in Table 3. The

distribution of genotypes was consistent with the Hardy-Weinberg equilibrium. C allele frequencies were markedly increased in only CD patients compared to the controls ($P<0.05$).

The G113A SNP of the *DLG5* gene was completely absent in the Greek IBD cases as well as in the Greek healthy population.

The C1672T and G-207C were in strong linkage disequilibrium and created a two-allele risk haplotype (TC) (Table 3). The TC haplotype was significantly overrepresented in patients with CD (13.3%) as compared to the controls (6.5%) ($P<0.05$, Table 4). Odds ratios conferred by allele 1672T, allele -207C or the TC haplotype were similar. The risk for CD was much greater in the presence of both the TC haplotype and at least one of the three main alleles of *NOD*2/*CARD*15 gene (Table 5).

A significant association was found between ileocolitis and colitis and possession of TC haplotype. Twenty-three out of the thirty carriers of the TC haplotype had ileocolitis or colitis, whereas only seven TC carriers had exclusively ileal disease ($P<0.01$). Notably, when the presence of TC haplotype was evaluated jointly with the presence of one or more of the common *NOD*2/*CARD*15 variants, a significant association was observed with ileocolitis and ileitis. Seventeen of the nineteen carriers of both TC haplotype and at least one of the *NOD*2/*CARD*15 variants had ileocolitis or ileitis, whereas only two patients presented exclusively colitis ($P<0.05$).

In CD patients, disease behavior in 32 (26.7%) was defined as fibrostenotic, in 10 (8.3%) as fistulizing and in 78 (65%) as inflammatory. A significant association was observed between the presence of the TC haplotype and fibrostenotic *vs* inflammatory phenotype of disease in our population. Twenty out of the thirty TC carriers presented a fibrostenotic phenotype since only 10 patients had inflammatory disease ($P<0.05$).

DISCUSSION

The precise etiology of CD and UC is uncertain, although it is widely accepted that IBD develops in a genetically predisposed individual following exposure to environmental stimuli[15]. The genetic basis of IBD is adequately documented, since genetic factors that affect susceptibility to IBD have been disclosed through genetic linkage and population-based association studies[9-11]. *NOD*2/*CARD*15 was the first gene which was found to be associated with IBD, specifically with CD[9,10]. Through the candidate gene approach, various genes were identified as candidate genes to predispose to IBD in some populations[16]. Very recently Peltekova *et al*[12]. reported on two functional mutations in the *OCTN* cluster on 5q31 (the IBD5 locus) that were associated with CD, while Stoll *et al*[13]. reported the association of IBD with mutations in the *DLG*5 gene.

Regarding the *OCTN*1 and *OCTN*2, it has been recently shown that mutations in these genes are associated with lower carnitine uptake rate and increased transport of xenobiotics[14,17]. It is known that carnitine deficiency could be related with a disorder of fatty acid oxidation and consequently with insufficient fatty acid β-oxidation[17]. On the other hand, there are some evidences that the inhibition of fatty acid oxidation in the epithelium of the colonic mucosa is associated with UC and inflammation[18]. Taking all these into consideration, it seems reasonable that the *OCTN* cluster might have an active role in IBD pathogenesis.

Our case-control study for *OCTN*1 and *OCTN2* genes showed that the frequency of the 1672T and -207C alleles was significantly higher in CD patients compared to UC patients and controls. Both mutations were, as expected from the previous studies on IBD5 haplotype, in strong linkage disequilibrium (LD) and created a two-allele risk haplotype, i.e. TC which in our cases had a frequency of 13.3% in CD patients compared to 6.5% in healthy individuals. Although the TC haplotype frequency that was observed was lower than that reported by Peltekova *et al*[12]. our results confirmed an association between the *OCTN* cluster and CD. The TC haplotype was not increased in UC in our population, which was in agreement with Peltekova *et al*[12]. but in contrast with several previous studies on IBD5[19,20]. It has to be pointed out that our results differed from those of a recent study in CD patients in a Japanese population, where other genetic variants have been associated with CD pathogenesis[21]. Furthermore, it is known that variants in the IBD5 haplotype appear to be very rare in the Japanese population[19].

It has been hypothesized that the third member of the *OCTN* cluster, the *OCTN*3 gene, in the *OCTN*1-*OCTN*2 interval, is also associated with IBD[22]. The *OCTN*3 gene might represent a homolog to the mouse gene Slc22a9 and several research groups were unable to identify a human counterpart or any other gene within this region[12,22].

Interestingly, the risk for CD was even greater in the presence of both TC haplotype and at least one of the *NOD*2/*CARD*15 variants, confirming the previously reported interaction between IBD5 haplotype and *NOD*2/*CARD*15[23]. Notably, in agreement with our results, very recently, Torok *et al*[24]. reported that TC haplotype was associated with an increased CD risk, which increases even more in the presence of *NOD*2/*CARD*15 mutations.

Patients with CD clinically present heterogeneous disease characteristics, including differences in disease behavior, localization and severity. Defining the relationship between *OCTN*-TC haplotype and disease phenotypic variation is not only crucial in probing the clinical diversity in disease presentation and behavior, but may also assist in defining rational treatment strategies. Concerning the disease location in the intestine, we found that the possession of the TC haplotype was associated mainly with colitis or ileocolitis, which was in agreement with previous findings that demonstrated the IBD5 association with colonic CD[24-27]. However, when the TC haplotype was combined with the presence of at least one of the *NOD*2/*CARD*15 variants, a significant association with ileitis or ileocolitis was observed, which was in agreement with the results of a recent study by Newman *et al*[28]. This observation suggests that these variants have a

biological involvement in CD pathogenesis. When disease behavior was examined, the presence of the TC haplotype was found to be associated with the fibrostenotic phenotype.

Concerning the *DLG*5 gene, which is important in maintaining the epithelial structure, the 113A variant was completely absent in our studied population. Our observations concerning the *DLG*5 gene were strongly in contrast with previous data reported by Stoll *et al*[13]. but were in full agreement with the studies by Torok *et al*[24]. and Noble *et al*[29]. in German and Scottish populations, respectively. These findings are not unexpected in a polygenic disease model, and imply significant differences in the genetic background for CD susceptibility among the different populations.

Collectively, our study confirms recent findings suggesting that the mutations in *OCTN*1 and *OCTN*2 genes are associated with CD[12]. Additionally, our results indicate that the carriage of the *OCTN*-TC haplotype is significantly associated with ileocolitis or colitis and the fibrostenotic phenotype, but the TC haplotype combined with the presence of *NOD*2/*CARD*15 variants, associates with ileocolitis or ileitis. However, further studies involving a larger number of cases and controls in a worldwide scale are needed to elucidate the complex biological mechanisms underlying IBD susceptibility.

REFERENCES

1 **Bonen DK**, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003; **124**: 521-536

2 **Ahmad T**, Tamboli CP, Jewell D, Colombel JF. Clinical relevance of advances in genetics and pharmacogenetics of IBD. *Gastroenterology* 2004; **126**: 1533-1549

3 **Hugot JP**, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie 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

4 **Cavanaugh J**. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: **Crohn disease and chromosome 16.** *Am J Hum Genet* 2001; **68**: 1165-1171

5 **Zouali H**, Chamaillard M, Lesage S, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, Montague S, Gassull M, Christensen S, Finkel Y, Gower-Rousseau C, Modigliani R, Macry J, Selinger-Leneman H, Thomas G, Hugot JP. Genetic refinement and physical mapping of a chromosome 16q candidate region for inflammatory bowel disease. *Eur J Hum Genet* 2001; **9**: 731-742

6 **Brant SR**, Panhuysen CI, Bailey-Wilson JE, Rohal PM, Lee S, Mann J, Ravenhill G, Kirschner BS, Hanauer SB, Cho JH, Bayless TM. Linkage heterogeneity for the IBD1 locus in Crohn's disease pedigrees by disease onset and severity. *Gastroenterology* 2000; **119**: 1483-1490

7 **Gazouli M**, Zacharatos P, Mantzaris GJ, Barbatis C, Ikonomopoulos I, Archimandritis AJ, Lukas JC, Papalambros E, Gorgoulis V. Association of NOD2/CARD15 variants with Crohn' s disease in a Greek population. *Eur J Gastroenterol Hepatol* 2004; **16**: 1177-1182

8 **Gazouli M**, Mantzaris G, Kotsinas A, Zacharatos P, Papalambros E, Archimandritis A, Ikonomopoulos J, Gorgoulis VG. Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. *World J Gastroenterol* 2005; **11**: 681-685

9 **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, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606

10 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard 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

11 **Hampe J**, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001; **357**: 1925-1928

12 **Peltekova VD**, Wintle **RF, Rubin LA, Amos CI, Huang Q, Gu** X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36:** 471-475

13 **Stoll M**, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, Rosenstiel P, Albrecht M, Croucher PJ, Seegert D, Nikolaus S, Hampe J, Lengauer T, Pierrou S, Foelsch UR, Mathew CG, Lagerstrom-Fermer M, Schreiber S. Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet* 2004; **36**: 476-480

14 **Podolsky DK**. Inflammatory bowel disease 2. *N Engl J Med* 1991; **325**: 928-937

15 **Orchard TR**, Satsangi J, Van Heel D, Jewell DP. Genetics of inflammatory bowel disease: a reappraisal. *Scand J Immunol* 2000; **51**: 10-17

16 **Wild GE**, Rioux JD. Genome scan analyses and positional cloning strategy in IBD: successes and limitations. *Best Pract Res Clin Gastroenterol* 2004; **18**: 541-553

17 **Lahjouji K**, Mitchell GA, Qureshi IA. Carnitine transport by organic cation transporters and systemic carnitine deficiency. *Mol Genet Metab* 2001; **73**: 287-297

18 **Roediger WE**, Nance S. Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. *Br J Exp Pathol* 1986; **67**: 773-782

19 **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**

20 **McGovern DP**, Van Heel DA, Negoro K, Ahmad T, Jewell DP. Further evidence of IBD5/CARD15 (NOD2) epista**sis in the** susceptibility to ulcerative colitis. *Am J Hum Genet* 2003; **73**: 1465-1466

21 **Yamazaki K**, Takazoe M, Tanaka T, Ichimori T, Saito S, Iida A, Onouchi Y, Hata A, Nakamura Y. Association analysis of SLC22A4, SLC22A5 and DLG5 in Japanese patients with Crohn disease. *J Hum Genet* 2004; **49**: 664-668

22 **Lamhonwah AM**, Skaug J, Scherer SW, Tein I. A third human carnitine/organic cation transporter (OCTN3) as a candidate for the 5q31 Crohn's disease locus (IBD5). *Biochem Biophys Res Commun* 2003; **301**: 98-101

23 **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

24 **Török HP**, Glas J, Tonenchi L, Lohse P, Müller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brünnler G, Jagiello P, Epplen JT, Griga T, Klein W, Sc**hiemann U, Folwaczny M, Ochsenkühn T, Folwaczny** C. Polymorphisms in the DLG5 and OCTN cation transporter

genes in Crohn's disease. *Gut* 2005; **54**: 1421-1427

25 **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

26 **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

27 **Crawford NP**, Colliver DW, Funke AA, Young MN, Kelley S, Cobbs GA, Petras RE, Galandiuk S. Characterization of genotype-phenotype relationships and stratification by the CARD15 variant genotype for inflammatory bowel disease susceptibility loci using multiple short tandem repeat genetic markers. *Hum Mutat* 2005; **25**: 156-166

28 **Newman B**, Gu X, Wintle R, Cescon D, Yazdanpanah M, Liu X, Peltekova V, Van Oene M, Amos CI, Siminovitch 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

29 **Noble CL,** Nimmo ER, Drummond H, Smith L, Arnott ID, Satsangi J. DLG5 variants do not influence susceptibility to inflammatory bowel disease in the Scottish population. *Gut* 2005; **54**: 1416-1420

Science Editor Kumar M and Guo SY **Language Editor** Elsevier HK