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

World J Gastroenterol 2006 September 14; 12(34): 5550-5553
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease

Judit Bene, Lili Magyari, Gábor Talián, Katalin Komlósi, Beáta Gasztonyi, Beáta Tari, Ágnes Várkonyi, Gyula Mózsik, Béla Melegh

Judit Bene, MTA PTE Clinical Genetics Research Group of Hungarian Academy of Sciences at the University of Pécs, Pécs, Hungary
Judit Bene, Lili Magyari, Gábor Talián, Katalin Komlósi, Béla Melegh, Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary
Beáta Tari, Ágnes Várkonyi, Department of Pediatrics and Child Health Center, University of Szeged, Szeged, Hungary
Beáta Gasztonyi, Gyula Mózsik, 1st Department of Medicine, School of Medicine, University of Pécs, Pécs, Hungary
Supported by the grant of Hungarian Science Foundation No. OTKA T 49589
Correspondence to: Dr. Béla Melegh, Professor of Medical Genetics and Pediatrics, Department of Medical Genetics and Child Development, University of Pécs, H-7624 Pécs, Szigeti 12, Hungary. bela.melegh@aok.pte.hu
Telephone: +36-72-536427 Fax: +36-72-536427
Received: 2006-06-13 Accepted: 2006-07-07

Abstract

AIM: To investigate the frequency of the common NOD2/CARD15 susceptibility variants and two functional polymorphisms of OCTN cation transporter genes in Hungarian pediatric patients with Crohn's disease (CD).

METHODS: A cohort of 19 unrelated pediatric and 55 unrelated adult patients with Crohn's disease and 49 healthy controls were studied. Genotyping of the three common CD-associated CARD15 variants (Arg702Trp, Gly908Arg and 1007finsC changes) with the SLC22A4 1672C→T, and SLC22A5 -207G→C mutations was performed by direct sequencing of the specific regions of these genes.

RESULTS: At least one CARD15 mutation was present in 52.6% of the children and in 34.5% of the adults compared to 14.3% in controls. Surprisingly, strongly different mutation profile was detected in the pediatric *versus* adult patients. While the G908R and 1007finsC variants were 18.4% and 21.1% in the pediatric group, they were 1.82% and 11.8% in the adults, and were 1.02% and 3.06% in the controls, respectively. The R702W allele was increased approximately two-fold in the adult subjects, while in the pediatric group it was only approximately 64% of the controls (9.09% in the adults, 2.63% in pediatric patients, and 4.08% in the controls). No accumulation of the OCTN variants was observed in any patient group *versus* the controls.

CONCLUSION: The frequency of the NOD2/CARD15 susceptibility variants in the Hungarian pediatric CD population is high and the profile differs from the adult CD patients, whereas the results for SLC22A4 and SLC22A5 mutation screening do not confirm the assumption that the carriage of these genotypes means an obligatory susceptibility to CD.

Key words: OCTN1; OCTN2; NOD2/CARD15; Crohn's disease

Bene J, Magyari L, Talián G, Komlósi K, Gasztonyi B, Tari B, Várkonyi Á, Mózsik G, Melegh B. Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. *World J Gastroenterol* 2006; 12(34): 5550-5553

http://www.wjgnet.com/1007-9327/12/5550.asp

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Although the peak onset of the disease typically occurs in the second and third decades of life[1], the incidence of pediatric cases has been strongly increasing recently[2]. Despite the comprehensive research that has been made to discover the background of the disease, the etiology is still unknown. Besides the environmental effects, it is supposed that genetic susceptibility plays a crucial role in the development of the disease[3,4].

Genome-wide linkage analyses have resulted in identification of several loci of potential CD susceptibility genes[5-11]. CARD15 (NOD2) gene, which is located at the pericentromeric region of chromosome 16, was the first gene to be identified as CD gene[12-14]. NOD2 is an intracellular protein expressed in peripheral blood monocytes, Paneth and intestinal epithelial cells; and it is important for inflammatory signal transduction *via* activation of the transcription factor, nuclear factor kappa-B (NF-κB)[15]. Several studies on Caucasian populations have reported an association between CARD mutations and CD. Three coding variants (R702W, G908R

and 1007finsC) have been identified as independent risk factors for development of CD.

Recently two polymorphisms in the carnitine/organic cation transporter gene cluster (SLC22A4 and SLC22A5, encoding OCTN1 and OCTN2, respectively) have been found to confer risk for CD[16]. The aim of the present study was to investigate the prevalence of these two functional variants of the SLC22A4 and SLC22A5 genes and the three CARD15 mutations in Hungarian pediatric population with CD.

MATERIALS AND METHODS

Patients

We examined 19 pediatric (14 males and 5 females; mean age: 13.4 years) and 55 adult (27 male and 28 female with real maturity onset disease; mean age: 42.3 years) patients with CD. This cohort was compared with 49 age- and sex-matched healthy controls (28 males and 21 females; mean age: 14.4 years). Both the pediatric and adult CD patients exhibited different clinical manifestations, therefore they represented mixed clinical CD populations. The diagnosis was confirmed by clinical, radiological, endoscopic and histological findings. Informed consent was obtained from each participant of the study and the study design was approved by the Local Ethics Committee.

Methods

Genomic DNA from the patients and the controls was isolated from peripheral blood using standard desalting procedure.

The presence of the NOD2 and OCTN variants was detected by direct sequencing using the primers designed in our laboratory. The primers' sequences for the PCR amplification as well as for the sequencing and annealing temperatures are listed in Table 1. The PCR was carried out in a final volume of 50 μL containing 200 μmol/L of each dNTP, 2 units of Taq polymerase, 5 μL of reaction buffer [100 mmol/L Tris HCl (pH 9.0), 500 mmol/L KCl, 15 mmol/L $MgCl_2$, 0.2 μmol/L of each primer and 1 μg of DNA to be amplified. The amplification was performed for a total of 35 cycles in an MJ Research PTC-200 thermal cycler. The amplification conditions were: pre-denaturation at 95℃ for 2 min, denaturation at 95℃ for 30 s, annealing for 30 s at the temperatures listed in Table 1 for the different SNP, primer extension at 72℃ for 30 s, and the final extension at 72℃ for 5 min. For DNA sequencing, a BigDye Terminator labeling was used and the analysis was performed in an ABI 3100 automatic sequencer.

Statistical analysis

Chi-square test (cross-table analysis) was used to analyze the possible associations with mutations in comparison of either the susceptibility variants and/or the normal haplotypes. $P < 0.05$ was considered statistically significant.

RESULTS

The allele frequencies are shown in Table 2. A total of 52.6% of pediatric patients with Crohn's disease carried at least one NOD2 mutation compared to 34.5% of adult patients and to 14.3% of the controls (pediatric patients *vs* controls $P < 0.05$).

While the T allele frequency, leading to heterozygous and homozygous R702W mutation, was increased approximately two-fold in the adult CD population (9.09%) compared to the controls (4.08%), it was only 2.63% in the pediatric CD patients (Table 2; $P < 0.05$ comparing the pediatric susceptibility and/or normal variants *versus*

Table 1 Primer sequences and annealing temperatures for genotypings

	SNP	Primers	Tannealing (℃)
NOD2/ CARD15	R702W	F: GAGCCGCACAACCTTCAGATC	50
		R: ACTTGAGGTGCCCAACATTCAG	
	G908R	F: GTTCATGTCTAGAACACATATCAGG	50
		R: GTTCAAAGACCTTCAGAACTGG	
	1007finsC	F: CCTTGAAGCTCACCATTGTATC	50
		R: GATCCTCAAAATTCTGCCATTC	
OCTN1	C1672T	F: AGAGAGTCCTCCTATCTGATTG	54
		R: TCCTAGCTATTCTTCCATGC	
OCTN2	G-207C	F: AGTCCCGCTGCCTTCCTAAG	58
		R: GTCACCTCGTCGTAGTCCCG	

Table 2 Comparison of the alleles of OCTN cation transporters and NOD2/CARD15 genes in pediatric and adult Crohn's disease patients with controls

		Pediatric patients *n* = 19 (%)	Adult patients *n* = 55 (%)	Controls children *n* = 49 (%)
CARD15 genotype				
R702W	CC	18 (94.7)	46 (83.6)	46 (93.9)
	CT	1 (5.3)	8 (14.6)	2 (4.1)
	TT	-	1 (1.8)	1 (2.0)
	T allele frequency (%)	2.63	9.09	4.08
G908R	GG	14 (73.7)	53 (96.4)	48 (98.0)
	GC	3 (15.8)	2 (3.6)	1 (2.0)
	CC	2 (10.5)	-	-
	C allele frequency (%)	18.4	1.82	1.02
1007finsC	- -	13 (68.5)	44 (80.0)	46 (93.9)
	- insC	4 (21.0)	9 (16.4)	3 (6.1)
	insC insC	2 (10.5)	2 (3.6)	-
	Cins allele frequency (%)	21.1%	11.8%	3.06%
SLC22A4 genotype				
C1672T	CC	4 (21.0)	18 (32.7)	12 (24.5)
	CT	11 (58.0)	30 (54.5)	25 (51.0)
	TT	4 (21.0)	7 (12.8)	12 (24.5)
	T allele frequency (%)	50.0	40.0	50.0
SLC22A5 genotype				
G-207C	GG	3 (15.8)	14 (25.5)	10 (20.4)
	GC	7 (36.8)	31 (56.4)	26 (53.1)
	CC	9 (47.4)	10 (18.1)	13 (26.5)
	C allele frequency (%)	65.8	46.4	53.1

the same values of the adult patients or the controls). By contrast, the C allele frequency, encoding the G908R variant, was found highly elevated (18.4%) in pediatric patients, and was only 1.82% in adult CD patients, and 1.02% in the controls ($P < 0.05$). For the 1007finsC variant, a significantly increased prevalence was found both in pediatric (21.1%) and in adult CD (11.8%) patients as compared with the controls (3.06%) ($P < 0.05$).

There were no significant differences in the allele frequencies of SLC22A4 C1672T and SLC22A5 G-207C mutations when compared either the results of the pediatric or the adult CD populations to the results of the controls (Table 2).

DISCUSSION

The carriage rate for the three common CD-associated CARD15 mutations was reported 31% in a pediatric CD population in North America[17], 60% in Germany[18], 51.5% in the Israeli Jewish patients[19] and 40.7% in an Italian cohort[20]. In two studies, the cytosine insertion mutation 3020insC was significantly more common in the pediatric CD population[18,21], whereas among the Jewish patients G908R missense mutation was the most frequent variant[19,22].

An association of the three CARD15 mutations R702W, G908R and 1007finsC with CD has been confirmed in several studies[17,18,23], although different allele frequencies have been observed. While the allele frequencies of the three mutations were almost the same (8.3%, 8.3% and 7.4%) in the Italian pediatric patients[20], the G908R variant was the most frequent among Jewish children[19] and 1007finsC was more common in Germany[18] and in the USA[21]. An earlier onset of disease was found in the presence of a CARD15 mutation in three additional studies[23-25]. These findings suggest that CARD15 mutations may be more frequent in pediatric CD.

To our surprise, in our study groups, two mutations, G908R and 1007finsC, were significantly more frequent in the pediatric population with the allele frequencies of 18.42% in children *versus* 1.02% in controls and of 21.05% in children *versus* 3.06% in controls, respectively. The genotyping results for the adult population are in agreement with previous Hungarian findings[26,27].

The OCTN1 and OCTN2 transporters mediate the transport of carnitine and a wide range of organic cations[28-30] and have an important role in the energy supply of epithelial cells. Recently, it has become clear that the OCTN1 also transports the ergothioneine[31], and the affinity parameters make it almost unquestionable that the carnitine transport function is secondary. A C1672T missense substitution in exon 9 of the SLC22A4 gene results in marked changes in OCTN1 transporter activity, whereas G-207C transversion in the SLC22A5 promoter region causes OCTN2 promoter function impairment.

By resequencing the five genes in the IBD5 interval, which harbors the cytokine gene cluster, and, therefore, is an attractive candidate region for IBD, Peltekova *et al*[16] identified 2 novel polymorphisms in the SLC22A4 and SLC22A5 genes. These two mutations (SLC22A4 C1672T and SLC22A5 G-207C) form a two-allele risk haplotype (OCTN-TC) which was associated with CD and showed significant interactions with CD-associated CARD15 mutations. This observation has been repeatedly confirmed[32-34], although, in the absence of the IBD5 risk haplotype, no association of OCTN1/2 variants with CD was reported in two studies[33,34]. While a Belgian group[35] found that the OCTN did not play a role in the susceptibility to CD, the two functional variants in the SLC22A4 and SLC22A5 genes were completely absent in Japanese[36].

In our study, which is probably the first in the international literature for pediatric population, we could not find accumulation of any of the susceptibility haplotypes either in the pediatric or in the adult CD subjects, thereby not supporting the susceptibility role of the above haplotypes in the development of CD.

In conclusion, we observed an accumulation of CARD15 mutations in pediatric cases, whereas the results for SLC22A4 and SLC22A5 mutation screening do not confirm the assumption that the carriage of these genotypes means significant susceptibility to CD. However, for genotype-phenotype correlations, further studies are needed with larger study populations.

ACKNOWLEDGMENTS

We thank Judit Oksai and Edit Papp for their help in the technical management of the study.

REFERENCES

1 **Oliva-Hemker M**, Fiocchi C. Etiopathogenesis of inflammatory bowel disease: the importance of the pediatric perspective. *Inflamm Bowel Dis* 2002; **8**: 112-128
2 **Baldassano RN**, Piccoli DA. Inflammatory bowel disease in pediatric and adolescent patients. *Gastroenterol Clin North Am* 1999; **28**: 445-458
3 **Gent AE**, Hellier MD, Grace RH, Swarbrick ET, Coggon D. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* 1994; **343**: 766-767
4 **Hampe J**, Heymann K, Krawczak M, Schreiber S. Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure. *Int J Colorectal Dis* 2003; **18**: 413-417
5 **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
6 **Satsangi J**, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996; **14**: 199-202
7 **Cho JH**, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998; **95**: 7502-7507
8 **Duerr RH**, Barmada MM, Zhang L, Pfützer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 2000; **66**: 1857-1862
9 **Hampe J**, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nürnberg P, Mirza

MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999; **64**: 808-816

10 **Rioux JD**, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000; **66**: 1863-1870

11 **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

12 **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

13 **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

14 **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

15 **Schreiber S**, Nikolaus S, Hampe J. Activation of nuclear factor kappa B inflammatory bowel disease. *Gut* 1998; **42**: 477-484

16 **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

17 **Tomer G**, Ceballos C, Concepcion E, Benkov KJ. NOD2/CARD15 variants are associated with lower weight at diagnosis in children with Crohn's disease. *Am J Gastroenterol* 2003; **98**: 2479-2484

18 **Sun L**, Roesler J, Rösen-Wolff A, Winkler U, Koch R, Thürigen A, Henker J. CARD15 genotype and phenotype analysis in 55 pediatric patients with Crohn disease from Saxony, Germany. *J Pediatr Gastroenterol Nutr* 2003; **37**: 492-497

19 **Weiss B**, Shamir R, Bujanover Y, Waterman M, Hartman C, Fradkin A, Berkowitz D, Weintraub I, Eliakim R, Karban A. NOD2/CARD15 mutation analysis and genotype-phenotype correlation in Jewish pediatric patients compared with adults with Crohn's disease. *J Pediatr* 2004; **145**: 208-212

20 **Ferraris A**, Knafelz D, Torres B, Fortina P, Castro M, Dallapiccola B. Analysis of CARD15 gene variants in Italian pediatric patients with inflammatory bowel diseases. *J Pediatr* 2005; **147**: 272-273

21 **Kugathasan S**, Collins N, Maresso K, Hoffmann RG, Stephens M, Werlin SL, Rudolph C, Broeckel U. CARD15 gene mutations and risk for early surgery in pediatric-onset Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 1003-1009

22 **Wine E**, Reif SS, Leshinsky-Silver E, Weiss B, Shaoul RR, Shamir R, Wasserman D, Lerner A, Boaz M, Levine A. Pediatric Crohn's disease and growth retardation: the role of genotype, phenotype, and disease severity. *Pediatrics* 2004; **114**: 1281-1286

23 **Lesage S**, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857

24 **Brant SR**, Picco MF, Achkar JP, Bayless TM, Kane SV, Brzezinski A, Nouvet FJ, Bonen D, Karban A, Dassopoulos T, Karaliukas R, Beaty TH, Hanauer SB, Duerr RH, Cho JH. Defining complex contributions of NOD2/CARD15 gene mutations, age at onset, and tobacco use on Crohn's disease phenotypes. *Inflamm Bowel Dis* 2003; **9**: 281-289

25 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866

26 **Büning C**, Molnar T, Nagy F, Lonovics J, Weltrich R, Bochow B, Genschel J, Schmidt H, Lochs H. NOD2/CARD15 gene polymorphism in patients with inflammatory bowel disease: is Hungary different? *World J Gastroenterol* 2005; **11**: 407-411

27 **Lakatos PL**, Lakatos L, Szalay F, Willheim-Polli C, Osterreicher C, Tulassay Z, Molnar T, Reinisch W, Papp J, Mozsik G, Ferenci P. Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: phenotype-genotype correlations. *World J Gastroenterol* 2005; **11**: 1489-1495

28 **Wu X**, George RL, Huang W, Wang H, Conway SJ, Leibach FH, Ganapathy V. Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim Biophys Acta* 2000; **1466**: 315-327

29 **Wu X**, Prasad PD, Leibach FH, Ganapathy V. cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family. *Biochem Biophys Res Commun* 1998; **246**: 589-595

30 **Tamai I**, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* 1998; **273**: 20378-20382

31 **Gründemann D**, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E. Discovery of the ergothioneine transporter. *Proc Natl Acad Sci U S A* 2005; **102**: 5256-5261

32 **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

33 **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, Schiemann 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

34 **Noble CL**, Nimmo ER, Drummond H, Ho GT, Tenesa A, Smith L, Anderson N, Arnott ID, Satsangi J. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. *Gastroenterology* 2005; **129**: 1854-1864

35 **Vermeire S**, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, Joossens S, Ferrante M, Henckaerts L, Bueno de Mesquita M, Vlietinck R, Rutgeerts P. Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 2005; **129**: 1845-1853

36 **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

S- Editor Liu Y **L- Editor** Kumar M **E- Editor** Bai SH