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ORIGINAL ARTICLE

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Association analysis of *SLC22A4*, *SLC22A5* and *DLG5* in Japanese patients with Crohn disease

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Abstract Crohn disease (CD) is an inflammatory bowel disease characterized by chronic transmural, segmental, and typically granulomatous inflammation of the gut. Recently, two novel candidate gene loci associated with CD, *SLC22A4* and *SLC22A5* on chromosome 5 known as *IBD5* and *DLG5* on chromosome 10, were identified through association analysis of Caucasian CD patients. We validated these candidate genes in Japanese patients with CD and found a weak but possible association with both *SLC22A4* ($P = 0.028$) and *DLG5* ($P = 0.023$). However, the reported genetic variants that were indicated to be causative in the Caucasian population were completely absent in or were not associated with Japanese CD patients. These findings imply significant differences in genetic background with CD susceptibility among different ethnic groups and further indicate some difficulty of population-based studies.

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Introduction

Inflammatory bowel diseases (IBDs), which are usually classified into two clinical entities—Crohn disease (CD; MIM 266600) and ulcerative colitis (UC)—are chronic conditions characterized by remitting and relapsing inflammation of the small and/or large intestines. Familial aggregation and twin studies indicate a presence of genetic factors susceptible to this condition. Genome-wide linkage analyses have localized genes conferring susceptibility to IBD to several possible candidate loci on chromosomes 1, 3, 5, 6, 7, 10, 12, 14, 16, 19, and 22 (Hugot et al. 1996; Satsangi et al. 1996; Cho et al. 1998; Duerr et al. 2000; Hampe et al. 1999; Rioux et al. 2000, 2001).

Among the several candidate loci, susceptible genes at two distinct loci were recently identified through the evidences of strong association with the CD phenotype. In the *IBD5* locus on chromosome 5, single-nucleotide polymorphisms (SNPs) in two candidate genes, *SLC22A4* and *SLC22A5*, both of which encode organic cation transporters, revealed significant associations with CD (Peltekova et al. 2004). A C1672T substitution in exon 9 of the *SLC22A4* gene and a G-207C in the *SLC22A5* promoter region were indicated as functional and causative mutations to increase susceptibility to CD. The other gene identified from chromosome 10 was the *DLG5* gene, encoding a scaffolding protein involved in the maintenance of epithelial integrity. Risk-associated variants, including a G113A substitution in exon 3 of the *DLG5* gene, constructed two distinct haplotypes with a replicable distortion in transmission (Stoll et al. 2004).

To investigate a possible role of these candidate gene loci, one corresponding to *SLC22A4* and *SLC22A5* and another corresponding to *DLG5*, in the pathogenesis of

CD in Japanese, we examined SNPs of these three genes in a large number of clinical samples. We here report an absence of DNA substitutions or lack of association for the candidate-causative SNPs, which were indicated in the previous reports, in the Japanese CD patients. However, we observed a weak association of other genetic substitutions in these genes of Japanese patients with CD. Our results indicate that the reported substitutions in the three genes are unlikely to be causative to Japanese CD patients, but the candidacy of these two loci for Japanese CD cannot be totally excluded.

Materials and methods

Subjects and DNAs

Japanese blood samples were obtained with written informed consent from 484 CD patients at the Social Insurance Central General Hospital and from 345 unaffected control individuals belonging to the Osaka-Midosuji Rotary Club. All CD cases were diagnosed at the Inflammatory Bowel Unit of the Social Insurance Hospital by clinical, radiological, endoscopic, and histological findings according to the Lennard-Jones' criteria (Lennard-Jones 1989). Patients with indeterminate colitis were excluded. DNAs were prepared from these samples according to standard protocols.

DNA sequencing

To search genetic variations in these candidate loci including the five reported variants, C1672T in exon 9 of *SLC22A4* and G-207C in the *SLC22A5* promoter, as well as G113A in exon 3, C4136A in exon 23 and 35delA in intron 26 of *DLG5*, we carried out direct sequencing of those regions in 48 individuals with confirmed diagnosis of CD by means of the BigDye Terminator RR Mix (Applied Biosystems, USA) with ABI 3700 sequencers using the primers listed in Table 1.

Markers

SNPs in the *SLC22A4* and *SLC22A5* genes were screened according to methods described previously (Saito et al. 2002). We selected 17 SNPs, including six in *SLC22A4* (SLC22A4 1–6), three in *SLC22A5* (SLC22A5 1–3), and eight in *DLG5* (DLG5 1–8) (Table 2). Information for each SNP in the *SLC22A4*, *SLC22A5*, and *DLG5* chosen for this study was obtained from the Japanese SNP (JSNP) database (http://snp.ims.u-tokyo.ac.jp) (Hirakawa et al. 2002; Haga et al. 2002).

SNP analysis and genotyping

We amplified multiple genomic fragments using 20 ng of genomic DNA for each polymerase chain reaction (PCR), as described previously (Ohnishi et al. 2001). We genotyped all participants for a total of 17 SNPs indicated in Table 2 by means of the Invader assay (Mein et al. 2000). The C4136A in exon 23 and 35delA in intron 26 (deletion of an adenine at the 35th nucleotide in intron 26) of *DLG5* were examined by direct sequencing using the same primers.

Statistical analysis

Genotype distributions and allele frequencies of each SNP were compared, respectively, between the cases and the controls, as described elsewhere (Yamada et al. 2001). Haplotype frequencies were estimated using the expectation-maximization algorithm (Ott 1977).

Results

To examine a possible association of genetic substitutions in the two candidate loci—one including *SLC22A4* and *SLC22A5* and the other at *DLG5*—with susceptibility to CD in the Japanese population, we first examined DNA sequences from 48 CD patients in 438–976 bp genomic regions, including the five major genetic

Table 1 List of primers in this study

	Position	Primer		Product size
		Forward	Reverse	
Amplification for mutation analysis and genotyping				
SLC22A4	Ex9	AACTCTGGTAGGCAAAGAACTC	GTCCTACTTACCATTTCACTTTC	438
SLC22A5	Promoter	CTAGGATCGTTAATCGTGAAG	CTGAGCAGGAAGAAGATGAG	866
DLG5	Ex3	TCACTTTCAGTTCTACCTGCTAC	TCTAGGAGACAGTGGTAGGG	641
	Ex23	GAGACAGGATGCTCACAGCTTC	AACTCCTGAAGACCTGGTGTG	527
	Ex26	CTGATCGTGTTCCTTCTGTGCTG	AGGTCTCAAGGCTACATCTCCTC	976
Sequencing for mutation analysis and genotyping				
SLC22A4	Ex9	CATGCACAATGTCATCTGCC	ATAGGAGGACTCTCTGGGCAC	
SLC22A5	Promoter	GGACTCGGACCCCAAGGCCTC	AAGAAGATGAGGCGCTGGAAG	
DLG5	Ex3	ACTTTCAGTTCTACCTGCTACCG		
	Ex23	ATGCTCACAGCTTCCTGAGGTC	AAGACCTGGTGTGCGGCCTG	
	Ex26	CCTTCTGTGCTGTGGTCCAG	CGTTATGCCTTCTGACCCATC	

Table 2 List of genotyped single-nucleotide polymorphisms (SNPs) in *SLC22A4*, *SLC22A5* and *DLG5*

SNP No.	Contig No.	Contig position	Location	Position	Substitution	Major allele	Minor allele	IMS-JST ID	dbSNP ID
SLC22A4									
SLC22A4_1	NT_034772.5	34051988	Intron 1	6274		A	G	IMS-JST150334	rs3792874
SLC22A4_2	NT_034772.5	34052322	Intron 1	6608		C	T	IMS-JST150336	rs3792876
SLC22A4_3	NT_034772.5	34052415	Intron 1	6701		A	G	IMS-JST150337	rs3792877
SLC22A4_4	NT_034772.5	34062488	Intron 1	16774		G	A	IMS-JST190202	rs3828671
SLC22A4_5	NT_034772.5	34063419	Intron 2	450		T	C	IMS-JST000452	rs270608
SLC22A4_6	NT_034772.5	34066274	Intron 3	1801		A	G	IMS-JST150344	rs3792884
SLC22A5									
SLC22A5_1	NT_034772.5	34129422	Intron 2	237	L269L	T	C	IMS-JST175234	rs270608
SLC22A5_2	NT_034772.5	34136187	Exon 4	155		G	A	IMS-JST101643	rs274558
SLC22A5_3	NT_034772.5	34144702	Intron 9	187		T	C	IMS-JST001553	rs2074610
DLG5									
DLG5_1	NT_008583.16	28131940	Intron 15	56		C	T	IMS-JST111768	rs3758463
DLG5_2	NT_008583.16	28131859	Intron 15	137		C	T	IMS-JST111767	rs3758462
DLG5_3	NT_008583.16	28123048	Intron 21	8948		G	C	IMS-JST013817	rs1248625
DLG5_4	NT_008583.16	28116810	Intron 26	862		C	T	IMS-JST040839	rs2289311
DLG5_5	NT_008583.16	28107184	Intron 28	181		C	A	IMS-JST013818	rs2241831
DLG5_6	NT_008583.16	28106275	Intron 29	700		C	T	IMS-JST013820	rs2241833
DLG5_7	NT_008583.16	28103306	Exon 32	151		G	A	IMS-JST025913	rs1058202
DLG5_8	NT_008583.16	28102795	Exon 32	662		G	A	IMS-JST025916	rs2165047

variants—C1672T in exon 9 of *SLC22A4* and G-207C in the *SLC22A5* promoter, G113A in exon 3, C4136A in exon 23, and 35delA in intron 26 of *DLG5*—that were reported to have significant associations with CD in the Caucasian population (Table 1). Among these five genetic variations reported previously, we found that the three SNPs, C1672T, G-207C, and G113A, were completely absent in the Japanese CD cases. Since the C4136A and 35delA variations were observed in the Japanese population, we carried out genotyping of 484 Japanese CD patients for these variations and found no association of these two reported substitutions to CD in the Japanese population (Table 3).

To further verify whether these three genes can be excluded as candidates for Japanese CD, we performed case-control association studies by means of genotyping of 17 JSNPs located within the three genes at the two loci as shown in Table 2. The analyses using allelic, recessive, and dominant models for CD patients versus controls disclosed an association of two SNPs, one at SLC22A4_2 ($P = 0.028$) by dominant model and the other at DLG5_2 ($P = 0.023$) by recessive model, although the associations observed here were much weaker than those for the five genetic variations observed in Caucasian CD cases (Table 4). In addition, we constructed the haplotype structure using the 19 genotyped variations and examined its association with CD but found no significant association with CD (data not shown). Our studies have indicated that the five reported variants are unlikely to be disease causative, but we have not excluded a possibility that these genes may play some role in susceptibility to CD in the Japanese population.

Discussion

Genetic factors that affect susceptibility to CD have been disclosed through genetic linkage and population-based association studies although it is very far from complete understanding of the subject. *CARD15* was found to be associated with IBD by means of genome-wide sib-pair

Table 3 Association of major genetic variants in *DLG5* with Crohn disease (CD) in the case-control study

SNP No.	Case	Control	Allele 1[a] versus 2		Genotype 11 versus others		Genotype 11 + 12 versus others	
			χ^2 (*P*-value)	OR (95% CI)	χ^2 (*P*-value)	OR (95% CI)	χ^2 (*P*-value)	OR (95% CI)
4136C → A in exon 23								
1–1	334	221						
1–2	129	109	2.08	1.21	2.48	1.27	0.057	1.10
2–2	14	11	(0.15)	(0.93–1.56)	(0.12)	(0.94–1.71)	(0.81)	(0.49–2.46)
Sum	477	341						
35delA in intron 26								
1–1	31	18						
1–2	173	115	1.52	1.16	0.56	1.25	1.31	1.18
2–2	273	210	(0.22)	(0.92–1.46)	(0.46)	(0.69–2.28)	(0.25)	(0.89–1.57)
Sum	477	343						

[a]Allele 1 indicated as risk allele

Table 4 Association of *SLC22A4* and *DLG5* with Crohn disease (CD) in the case-control study

SNP No.	Case	Control	Allele 1[a] versus 2		Genotype 11 versus others		Genotype 11 + 12 versus others	
			χ^2 (*P*-value)	OR (95% CI)	χ^2 (*P*-value)	OR (95% CI)	χ^2 (*P*-value)	OR (95% CI)
SLC22A4_2								
1–1	49	37						
1–2	227	133	2.33	1.18	0.08	0.94	4.82	1.36
2–2	207	174	(0.13)	(0.95–1.45)	(0.78)	(0.60–1.47)	(0.028)*	(1.03–1.80)
Sum	483	344						
DLG5_2								
1–1	323	201						
1–2	140	126	3.33	1.25	5.14	1.39	0.09	0.89
2–2	19	12	(0.068)	(0.98–1.60)	(0.023)*	(1.05–1.86)	(0.76)	(0.43–1.87)
Sum	482	339						

[a]Allele 1 indicated as risk allele
*$P < 0.05$

analysis (Hampe et al. 2001; Hugot et al. 2001; Ogura et al. 2001). Through the candidate gene approach, various genes, such as mucin 3 (*MUC3*), tumor necrosis factor (*TNF*), and *HLA class II*, were identified as candidate genes susceptible to IBD in some populations (Nakajima et al. 1995; Kyo et al. 1999, 2001; Negoro et al. 1999). In addition, recent studies identified three candidate susceptibility genes at two loci, one was *SLC22A4* and *SLC22A5* on chromosome 5 corresponding to *IBD5* (Peltekova et al. 2004), and the other was *DLG5* on chromosome 10 (Stoll et al. 2004).

Our case-control study for *SLC22A4*, *SLCA22A5*, and *DLG5* showed no evidence of association between SNPs in the *SLC22A5* gene and CD and that there might be some associations with SNPs in the two gene loci, *SLC22A4* and *DLG5*, to the disease, if any. In addition, it is notable that the SNPs showing weak and possible associations in our study were different from ones reported previously; three variations, C1672T in exon 9 of *SLC22A4*, G-207C in the *SLC22A5* promoter region, and G113A in exon 3 of *DLG5*, that showed the strong associations in Caucasian CD were completely absent in Japanese. The two remaining candidate variants, C4136A of exon 23 and 35 delA in intron 26 of *DLG5*, were found to be polymorphic in Japanese, but no association between these SNPs and Japanese CD was observed.

Interestingly, the genetic variants that showed the strong association in Caucasian but were completely absent in Japanese CD were indicated to interact with other genetic variants of *CARD15* that was also indicated to be a candidate susceptible gene to CD. Three major polymorphisms in the *CARD15* gene—R702W, G908R, and 1007fs—were confirmed to be independently associated with susceptibility to Caucasian patients with CD (Ahmad et al. 2002; Cuthbert et al. 2002; Lesage et al. 2002). However, our extensive DNA sequence analysis of this gene in more than 400 Japanese CD patients failed to identify such genetic variations except for a single case, indicating no involvement of *CARD15* in pathogenesis of Japanese CD (Yamazaki et al. 2002). Ethnic differences in the genetic variations among Caucasian, Asian, and African populations were also shown by others (Bonen et al. 2002; Inoue et al. 2002; Croucher et al. 2003).

We failed to confirm the association of the five candidate genetic variations in the *SLC22A4*, *SLCA22A5*, and *DLG5* genes in the previous reports to be susceptible to Japanese CD. However, we found a weak association of SNPs in the two genes, *SLC22A4* and *DLG5*, with Japanese CD. The results indicate a possibility that the five SNPs in the previous reports may not be causative, but the SNPs that we found to have possible association with or specific genetic substitutions having linkage disequilibrium with these SNPs in the region may play some role in Japanese CD. Nonetheless, combining the data that there is no association of *CARD15* with Japanese CD, it is apparent that there should be a presence of ethnic differences in susceptibility to CD. Further studies including both large-scale genomic and environmental analysis involving a large number of cases and controls are warranted to identify genes susceptible to CD on a worldwide scale, and such studies would eventually shed more light on the etiology of IBD.

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References

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 (2002) The molecular classification of the clinical manifestations of Crohn's disease. Gastroenterology 122:854–866

Bonen DK, Nicolae DL, Moran T, Turkyilmaz MA, Ramos R, Karaliukas R, Brant SR, Duerr RH, Kirschner BS, Hanauer SB, Cho JH (2002) Racial differences in *NOD2* variation: characterization of *NOD2* in African-Americans with Crohn's disease. Gastroenterology 122(Suppl):A29

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 (1998) 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 U S A 95:7502–7507

Croucher PJ, Mascheretti S, Hampe J, Huse K, Frenzel H, Stoll M, Lu T, Nikolaus S, Yang SK, Krawczak M, Kim WH, Schreiber S (2003) Haplotype structure and association to Crohn's disease of *CARD15* mutations in two ethnically divergent populations. Eur J Hum Genet 11:6–16

Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, Mascheretti S, Sanderson J, Forbes A, Mansfield J, Schreiber S, Lewis CM, Mathew CG (2002) The contribution of *NOD2* gene mutations to the risk and site of disease in inflammatory bowel disease. Gastroenterology 122:867–874

Duerr RH, Barmada MM, Zhang L, Pfutzer R, Weeks DE (2000) High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. Am J Hum Genet 66:1857–1862

Haga H, Yamada R, Ohnishi Y, Nakamura Y, Tanaka T (2002) Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190,562 genetic variations in the human genome. Single-nucleotide polymorphism. J Hum Genet 47:605–610

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

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 (2001) Association between insertion mutation in *NOD2* gene and Crohn's disease in German and British populations. Lancet 357:1925–1928

Hirakawa M, Tanaka T, Hashimoto Y, Kuroda M, Takagi T, Nakamura Y (2002) JSNP: a database of common gene variations in the Japanese population. Nucleic Acids Res 30:158–162

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 (1996) Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 379:821–823

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard 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 (2001) Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411:599–603

Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, Inohara N, Nunez G, Kishi Y, Koike Y, Shimosegawa T, Shimoyama T, Hibi T (2002) Lack of common *NOD2* variants in Japanese patients with Crohn's disease. Gastroenterology 23:86–91

Kyo K, Parkes M, Takei Y, Nishimori H, Vyas P, Satsangi J, Simmons J, Nagawa H, Baba S, Jewell D, Muto T, Lathrop GM, Nakamura Y (1999) Association of ulcerative colitis with rare VNTR alleles of the human intestinal mucin gene, *MUC3*. Hum Mol Genet 8:307–311

Kyo K, Muto T, Nagawa H, Lathrop GM, Nakamura Y (2001) Associations of distinct variants of the intestinal mucin gene *MUC3A* with ulcerative colitis and Crohn's disease. J Hum Genet 46:5–20

Lennard-Jones JE (1989) Classification of inflammatory bowel disease. Scand J Gastroenterol 170(Suppl):2–6

Lesage S, Zouali H, Cezard 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 (2002) *CARD15*/*NOD2* mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. Am J Hum Genet 70:845–857

Mein CA, Barratt BJ, Dunn MG, Siegmund T, Smith AN, Esposito L, Nutland S, Stevens HE, Wilson AJ, Phillips MS, Jarvis N, Law S, de Arruda M, Todd JA (2000) Evaluation of single nucleotide polymorphism typing with invader on PCR amplicons and its automation. Genome Res 10:330–343

Nakajima A, Matsuhashi N, Kodama T, Yazaki Y, Takazoe M, Kimura A (1995) HLA-linked susceptibility and resistance genes in Crohn's disease. Gastroenterology 109:1462–1467

Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, Toyota T (1999) Crohn's disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. Gastroenterology 117:1062–1068

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, Nunez G, Cho JH (2001) A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. Nature 411:603–606

Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y (2001) A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477

Ott J (1977) Counting methods (EM algorithm) in human pedigree analysis: linkage and segregation analysis. Ann Hum Genet 40:443–454

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 (2004) Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 36:471–475

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 (2000) Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. Am J Hum Genet 66:1863–1870

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 (2001) Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 29:223–228

Saito S, Iida A, Sekine A, Ogawa C, Kawauchi S, Higuchi S, Nakamura Y (2002) Catalog of 238 variations among six human genes encoding solute carriers (*hSLCs*) in the Japanese population. J Hum Genet 47:576–584

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP (1996) Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 14:199–202

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 (2004) Genetic variation in *DLG5* is associated with inflammatory bowel disease. Nat Genet 36:476–480

Yamada R, Tanaka T, Unoki M, Nagai T, Sawada T, Ohnishi Y, Tsunoda T, Yukioka M, Maeda A, Suzuki K, Tateishi H, Ochi T, Nakamura Y, Yamamoto K (2001) Association between a single-nucleotide polymorphism in the promoter of the human interleukin-3 gene and rheumatoid arthritis in Japanese patients, and maximum-likelihood estimation of combinatorial effect that two genetic loci have on susceptibility to the disease. Am J Hum Genet 68:674–685

Yamazaki K, Takazoe M, Tanaka T, Ichimori T, Nakamura Y (2002) Absence of mutation in the *NOD2*/*CARD15* gene among 483 Japanese patients with Crohn's disease. J Hum Genet 47:469–472