dbSNP database. The allele frequency of the SNP in the present Japanese population was 10.5%, about half that reported in the database (22.5%). Among SNPs identified in this study, the minor allele frequency of SNP04, which was associated with CAL in male patients, was the highest in the Japanese. In contrast, allele frequency was only 0.7% in Caucasians. The Japanese nationwide surveys of KD incidence have documented that male children are more often affected and have a higher risk for cardiac complications.[7] Unlike adults, no differences in lifestyle or environment are conceivable in early childhood. Thus, it seems rational to postulate that one of the genes influencing KD susceptibility and disease severity is located on the X chromosome. Although it is clear that larger numbers of patients with CAL will need to be analyzed to obtain a conclusive result, the finding that SNP04 is very rare in Caucasians is interesting when the low incidence rate of KD is considered. It is unknown whether this SNP by itself affects the transcription, splicing or stability of CD40L mRNA. It is possible that the observed association with SNP04 is due to a genetic variation at a different locus that is in linkage disequilibrium with this marker.

CU-rich *cis*-acting elements known to regulate mRNA stability[22,23] exist in the 3'-UTR of the gene. Although a total of five SNPs were identified in the 3'-UTR (Figure 1), none of these polymorphisms are located within the element. In the 5'-flanking region, there are three NFAT transcription factor-binding sites, the most distal one being about 760 bp upstream of the transcription start site.[21] 'Again, no variation likely to be involved in transcriptional factor binding was identified despite extensive screening.

Eight haplotypes were determined by genotyping seven SNPs in male subjects. A CA repeat near the CU-rich element was found to be highly polymorphic. Although no positive association was found with the haplotypes and the CA repeat, the new information about polymorphisms in this study provide a valuable tool for the future studies of other diseases. Validation of our current findings in larger cohorts of KD patients will define the importance of CD40L SNP04 in disease susceptibility and outcome.

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References

1 Kawasaki T: Pediatric acute febrile mucocutaneous lymph node syndrome with characteristic desquamation of fingers and toes: my clinical observation of 50 cases (in Japanese). *Jpn J Allergy* 1967; 16: 178–222.
2 Kato H, Koike S, Yamamoto M, Ito Y, Yano E: Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. *J Pediatr* 1975; 86: 892–898.
3 Taubert KA, Rowley AH, Shulman ST: Seven-year national survey of Kawasaki disease and acute rheumatic fever. *Pediatric Infect Dis J* 1994; 13: 704–708.
4 Fujita Y, Nakamura Y, Sakata K *et al*: Kawasaki disease in families. *Pediatrics* 1989; 84: 666–669.
5 Uehara R, Yashiro M, Nakamura Y, Yanagawa H: Kawasaki disease in parents and children. *Acta Paediatr* 2003; 92: 694–697.
6 Dean AG, Melish ME, Hicks R, Palumbo NE: An epidemic of Kawasaki syndrome in Hawaii. *J Pediatr* 1982; 100: 552–557.
7 Yanagawa H, Nakamura Y, Yashiro M *et al*: Incidence survey of Kawasaki disease in 1997 and 1998 in Japan. *Pediatrics* 2001; 107: E33.
8 Wang CL, Wu YT, Liu CA *et al*: Expression of CD40 ligand on CD4+ T-cells and platelets correlated to the coronary artery lesion and disease progress in Kawasaki disease. *Pediatrics* 2003; 111: E140–E147.
9 Biancone L, Cantaluppi V, Camussi G: CD40–CD154 interaction in experimental and human disease (review). *Int J Mol Med* 1999; 3: 343–353.
10 Yellin MJ, Brett J, Matsushima A *et al*: Functional interactions of T cells with endothelial cells: the role of CD40L–CD40-mediated signals. *J Exp Med.* 1995; 182: 1857–1864.
11 Henn V, Slupsky JR, Grafe M *et al*: CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 1998; 391: 591–594.
12 Asano T, Ogawa S: Expression of IL-8 in Kawasaki disease. *Clin Exp Immunol* 2000; 122: 514–519.
13 Terai M, Jibiki T, Harada A *et al*: Dramatic decrease of circulating levels of monocyte chemoattractant protein-1 in Kawasaki disease after gamma globulin treatment. *J Leukoc Biol* 1999; 65: 566–572.
14 Asano T, Ogawa S: Expression of monocyte chemoattractant protein-1 in Kawasaki disease: the anti-inflammatory effect of gamma globulin therapy. *Scand J Immunol* 2000; 51: 98–103.
15 Nash MC, Shah V, Dillon MJ: Soluble cell adhesion molecules and von Wilebrand factor in children with Kawasaki disease. *Clin Exp Immunol* 1995; 101: 13–17.
16 Resarch Committee on Kawasaki Disease, Ministry of Health and Welfare: *Report of Subcommittee on Standardization of Diagnostic Criteria and Reporting of Coronary Artery Lesions in Kawasaki Disease* 1984.
17 Fukushima Y, Ohashi H, Wakui K, Nishida T, Oh-ishi T: A rapid method for starting a culture for the establishment of Epstein–Barr virus-transformed human lymphoblastoid cell lines. *Jpn J Hum Genet* 1992; 37: 149–150.
18 Sambrook and Russel: *Molecular Cloning A Laboratory Manual*, 3rd Edn. Cold Spring Harbor Laboratory Press: New York, pp 6.4–6.28.
19 Cordell HJ, Kawaguchi Y, Todd JA, Farral M: An extension of the Maximum Lod Score method to X-linked loci. *Ann Hum Genet* 1995; 59: 435–449.
20 Nyholt DR: All LODs are not created equal. *Am J Hum Genet.* 2000; 67: 282–288.
21 Lobo F, Xu S, Lee C, Fuleihan L: Transcriptional activity of the distal CD40 ligand promoter. *Biochem Biophys Res Commun* 2000; 279: 245–250.
22 Kosinski P, Laughlin J, Singh K, Covey L: A complex containing poly pyrimidine tract-binding protein is involved in regulating the stability of CD40 ligand (CD154) mRNA. *J immunol* 2003; 170: 979–988.
23 Hamilton BJ, Genin A, Cron RQ, Rigby WF: Delineation of a novel pathway that regulates CD154 (CD40 ligand) expression. *Mol Cell Biol* 2003; 23: 510–525.
24 Allen RC, Armitage RJ, Conley ME *et al*: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993; 259: 990–993.

25 Aruffo A, Farrington M, Hollenbaugh D *et al*: .The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 1993; **72**: 291–300.
26 DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G: CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. *Nature* 1993; **361**: 541–543.
27 Sabeti P, Usen S, Farhadian S *et al*: CD40L association with protection from severe malaria. *Genes Immun* 2002; **3**: 286–291.
28 Miller R, Miller P, Kwok P: Regions of low single-nucleotide polymorphism incidence in human and orangutan Xq: deserts and recent coalescences. *Genomics* 2001; **71**: 78–88.
29 Sabeti PC, Reich DE, Higgins JM *et al*: Detecting recent positive selection in the human genome from haplotype structure. *Nature* 2002; **419**: 832–837.

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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.

Keywords Crohn disease · Single-nucleotide polymorphism (SNP) · *DLG5* · *SLC22A4* · *SLC22A5* · *OCTN1* · *OCTN2* · Japanese population

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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

特集

胎児染色体異常の診断およびスクリーニング

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要旨

母体の年齢や病歴，あるいは母体血清マーカーや超音波検査により胎児の染色体異常のリスクが高いと考慮される場合，妊娠中に羊水細胞や絨毛組織を採取して出生前に胎児染色体の分析が施行されている．画像診断により胎児の明らかな先天異常が確認されている症例は別として，胎児スクリーニングという側面があり，慎重な遺伝カウンセリングに基づいて提供されるべき検査である．

Key Words
高齢妊娠
母体血清マーカー検査
超音波検査
項部皮膚肥厚
脈絡叢嚢胞

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はじめに

妊娠中に胎児の染色体検査が考慮される症例は大きく分けて2種類に分類される．まず第一に，超音波検査などの画像診断により明らかな胎児の異常所見が認められて，精査目的で検査が実施される場合，第二には，いわゆる胎児の先天異常スクリーニングによりハイリスクと推定される場合である．

1988年に日本産科婦人科学会は，表に示すような先天異常の胎児診断に関する会告を呈示した[1]．とくに「妊娠初期絨毛検査に関して」とはいうものの，現在の周産期医療の現場で実施されている羊水検査や，胎児血採取などの侵襲的な胎児検査に広く共通して用いられる適応基準である．このうち1)，2)，3）については，いわゆる胎児染色体異常のハイリスク妊婦のケースであり，7）については母体血清マーカー検査や，胎児超音波検査による新たな適応

表　先天異常の胎児診断（文献1）より引用）
とくに妊娠初期絨毛検査に関する見解

1）夫婦のいずれかが染色体異常の保因者
2）染色体異常児を分娩した既往を有するもの
3）高齢妊娠
4）重篤な伴性（X連鎖）劣性遺伝性疾患の保因者
5）重篤で胎児診断が可能な先天性代謝異常症の保因者
6）重篤でDNA診断が可能な遺伝性疾患の保因者
7）その他重篤な胎児異常のおそれがある場合

判定基準が含まれる．いずれにしても，明らかに胎児の異常が確認されている場合を除き，胎児スクリーニングという側面があり，慎重な遺伝カウンセリングが要求される．

高齢妊娠

一般的な妊娠初期の羊水検査において，もっとも多い検査適応である．通常，検査実施に伴う流産などの発生率と，母体年齢別胎児染色体異常発生率を比較して，35歳以上の妊婦に検査が考慮される．依然として，主治医より年齢のみを理由として検査を推奨されて来院する妊婦は後を絶たないが，周産期領域においても遺伝カウンセリングが普及しつつあり，当院では，検査前遺伝カウンセリングを受けた後に，検査は受けず妊娠継続することを決断する妊婦が増加している．また，後述するような超音波検査によるリスク判定も追加して，侵襲的な検査の実施を再考する妊婦も多い（図1）[2]．

なお，最近では生殖補助医療技術の恩恵により，不妊症カップルの高齢妊娠，さらに多胎妊娠といった症例が少なくない．とくに多胎妊娠の場合には，検査に伴う流産もさることながら，一児のみに染色体異常が認められた場合にどのような対応を希望するかが問題であり，検査前に十分な配慮が必要である．

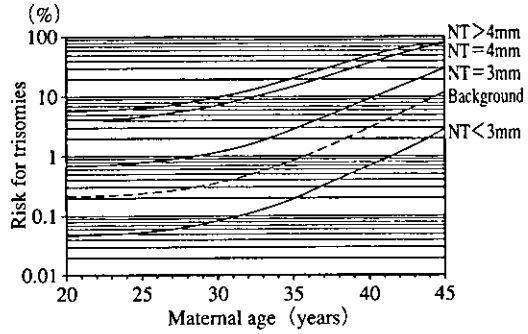

図1　母体年齢およびNTと21トリソミーのリスク
（文献2）より引用）

保因者・既往歴

自然流産を反復する不育症や，先天異常児妊娠の既往をもつカップルの場合，夫婦のいずれかに均衡型染色体構造異常が判明する場合がある．流産か先天異常か，あるいは夫婦のどちらが保因者かによって次妊娠時の胎児染色体異常発生率は異なるため，妊娠前から十分な文献的データに基づく遺伝カウンセリングが望ましい[3]．なお，両親の染色体構造異常が微細な場合，絨毛組織や羊水細胞を用いた通常のGバンド法による染色体分析のみでは十分な解析結果が得られない場合がある．当院では，SKY法なども追加して，視覚的に理解しやすい検査結果を提供できるように対応している[4]．

一方，前児に染色体数的異常（21トリソミーや18トリソミーなど）を認めた場合にも，次妊娠における胎児染色体異常の発生率が高いことは広く知られている．この場合にも，最近では母体年齢や血清マーカー検査，超音波検査による総合的なリスク判定が普及してきている．既往歴のあるカップルの不安は計り知れず，安易に「前児が染色体異常だったから今回もハイリスクです．」といった説明をするのではなく，さまざまな非侵襲的手法により，胎児の染色体異常率を推定できることを情報提供する必要がある．

母体血清マーカー検査

母体血清マーカー検査は平成6年より導入され，母体採血のみで結果が得られるという手軽さから急速に普及して社会的問題となった．妊婦の意思を考慮せず十分な遺伝カウンセリングもないままに検査のみが先行し，ハイリスクと判定された多くの妊婦が妊娠継続を憂慮するといった事態に陥った．平成11年に厚生科学審議会先端医療技術評価部会・出生前診断に関する

専門委員会が「母体血清マーカー検査に関する見解（報告）」を示し，妊婦に積極的に推奨すべきものではない，との結論に至っている．

厚生科学研究「遺伝カウンセリング体制の構築に関する研究」分担研究課題：周産期遺伝カウンセリングシステム構築に関する研究―産科診療における遺伝カウンセリング―（佐合ら）の平成13年度研究報告によれば，母体血清マーカー検査数は最近減少傾向となっている[5]．平成10年は21,708件であったが，見解が出された後の平成11年は18,312件，平成12年は15,927件と年々著明に減少し，検査解析を行う施設も7施設から5施設に減少している．

超音波検査

超音波検査は，内科医の聴診器と同様に日々の産科臨床で手軽に用いられている．最近では，超音波検査による胎児異常のスクリーニングも一般的になってきた[6]．重篤で生命予後の不良な胎児を早期に診断して，母体の精神的・肉体的な負担を軽減したり，胎内での治療や早期療育によって児のよりよい予後が期待されることは望ましい恩恵である．しかし，スクリーニング検査には偽陽性例が必ず存在し[7]，妊婦に不要な心痛を与えかねない．

以下に述べる胎児項部皮膚肥厚（nuchal translucency，以下NTと略す）や，脈絡叢嚢胞（choroid plexus cyst，以下CPCと略す）は，胎児染色体異常の一つのマーカーであるが，この所見自体が疾患なのではない．母体血清マーカー検査と同じく単にリスクの算定が可能なだけであり，本来は遺伝カウンセリングとともに提供される検査手技である．その逆に，偽陰性も存在するわけであり，これらのスクリーニングで胎児異常のすべてが診断できたかのような結果説明は避けなくてはならない．

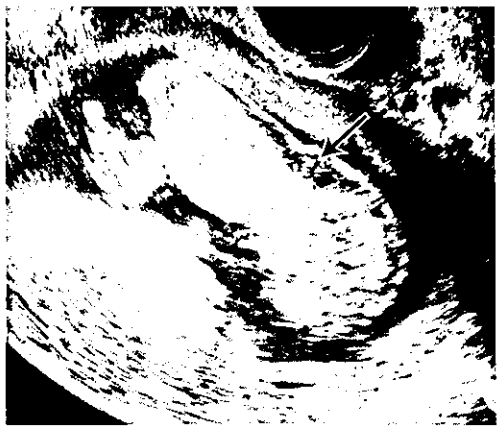

図2　項部皮膚肥厚：nuchal translucency（妊娠12週）（文献6）より引用）

1．胎児項部皮膚肥厚（nuchal translucency）

NT（図2）は，妊娠10～14週に認められる染色体異常の超音波検査マーカーである[6]．胎児の後頭部から背部にかけて認められる低輝度エコーの領域であり，NTの大半は疾患ではなく，一つの染色体異常のサインで，この所見のみで妊娠継続をあきらめるというのは早計である．通常，妊娠12～13週以降は軽減して妊娠15～16週頃には消失することが多いが，なかには頸部嚢胞状リンパ管種cystic hygromaや，胎児水腫などに進行する症例，あるいは心奇形が明らかとなる場合もある．

最近では，本スクリーニングにより陽性と判定されて胎児染色体検査目的で紹介されてくる症例が増加している．しかし，測定方法が適切でなく紹介時には消失（？）している症例も少なくない．妊娠14週を過ぎると再確認もできなくなるため，早めの患者紹介が望まれる．なお，NTに関しては欧米で研究が進み，計測方法や染色体異常の発症頻度（図1）についてはさまざまな報告がある．日本でも早急に多施設共同研究を行って，統一した計測方法，日本人女性におけるリスク算定基準づくりが急務である．

2．脈絡叢嚢胞（choroid plexus cyst）脈絡CPC（図3）も，染色体異常の超音波検査マー

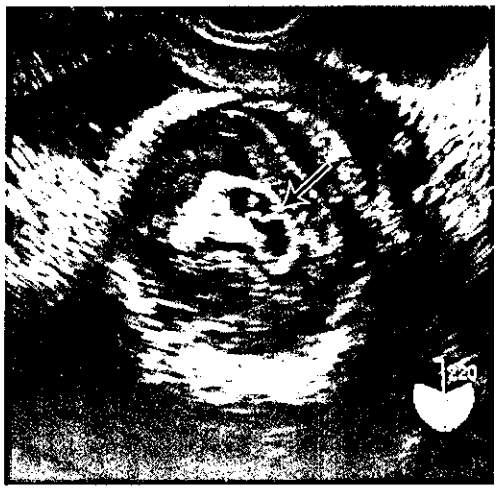

図3　脈絡叢嚢胞：choroid plexus cyst（妊娠19週）（文献6）より引用）

カーであり，側脳室内に高輝度エコーとして観察される脈絡叢のなかに出現する嚢胞である[6]．妊娠15～16週頃から認められるようになり，通常妊娠20数週で消失する一つの所見であり，中枢神経系の異常ではない．

欧米の報告では，CPCの発症頻度は0.18～3.64%であり，それほどまれな所見とはいえないが，現在のところ，わが国では妊娠初期にCPCとして診断される症例はまだ少なく，むしろ水頭症と疑われて紹介されることが多い．染色体異常のなかでも，とくに18トリソミーのマーカーとして有用であり，早期診断が望ましいが，胎児頭部の所見であるため妊婦の不安も大きく，CPCの説明は慎重に行わなければならない．あわてて所見の説明をする前に，まずは18トリソミーを疑う発育遅延，手指の異常，心奇形，小脳低形成などの病的な異常所見を伴っていないかどうか，超音波検査により注意深く観察する．CPCのみが単独で認められる症例では，染色体異常のリスクはあまり高くなく，Guptaらは1/150であると報告している[8]．ただし，これはあくまでもほかに異常所見がない，と診断できた場合であり，検査実施者の技術レベルによっては，そのリスク値は異なることが想定され，胎児染色体検査の必要性を一律に判断することはできない．

おわりに

母体血清マーカー検査や超音波検査は，いわゆる胎児のスクリーニングであり，安易な検査実施は避けねばならない．しかし，年齢や既往歴，過剰な不安から侵襲的な胎児染色体検査を即断する前に，これらのスクリーニングを導入することにより，妊婦に安心感とともに，胎児染色体検査の必要性を熟考する機会と時間を与えることも可能である．時間的にも余裕をもって（できれば妊娠前から），適切な遺伝カウンセリングに基づいて，これらの検査やスクリーニングが実施されることが望まれる．

●文　献

1）日本産科婦人科学会会告：先天異常の胎児診断，とくに妊娠初期絨毛検査に関する見解．日本産科婦人科学会誌 40（1），1988
2）Snijders RJ et al.: First-trimester ultrasound screening for chromosomal defects. Ultrasound Obstet Gynecol 7: 216-226, 1996
3）三春範夫：遺伝カウンセリング・ロールプレイ不妊・不育．第13回遺伝医学セミナーテキスト，日本人類遺伝学会遺伝医学セミナー実行委員会，117-118, 2003
4）鈴森　薫：流産・死産・先天異常の原因検索と遺伝カウンセリングに有用なSpectral Karyotyping（SKY）法について．産婦人科の世界 55: 31-38, 2003
5）佐合治彦：厚生科学研究「遺伝カウンセリング体制の構築に関する研究」分担研究課題：周産期遺伝カウンセリングシステム構築に関する研究—産科診療における遺伝カウンセリング—．平成13年度厚生科学研究報告書，629-641, 2002
6）種村光代・他：妊娠初期の超音波スクリーニング．産婦人科の実際 51（8）：1099-1106, 2002
7）Boyd PA et al.: 6-year experience of prenatal diagnosis in an unselected population in Oxford, UK.

Lancet 352:1577-1581, 1998
8) Gupta JK et al.:Clinical significance of fetal choroid plexus cysts. Lancet 346:724-729, 1995

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