J Hum Genet (2007) 52:575–583
DOI 10.1007/s10038-007-0156-z

ORIGINAL ARTICLE

Association analysis of genetic variants in *IL23R*, *ATG16L1* and 5p13.1 loci with Crohn's disease in Japanese patients

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Received: 4 April 2007 / Accepted: 29 April 2007 / Published online: 30 May 2007

Abstract Inflammatory bowel diseases, Crohn's disease (CD) and ulcerative colitis are characterised by chronic transmural, segmental and typically granulomatous inflammation of the gut. Each has a peak age of onset in the second to fourth decades of life and prevalence has been increasing significantly in both Western countries and Japan over the last decade, while their pathogenesis remains largely unknown. Recently, positive association of CD with the variants in interleukin 23 receptor (*IL23R*), autophagy-related 16-like 1 (*ATG16L1*) genes and chromosome 5p13.1 locus was reported through genome-wide association studies which are now recognised as a robust tool for the identification of susceptibility genes for complex diseases. To examine an association of reported susceptible variants in the three loci with Japanese CD patients, a total of 484 CD patients and 439 controls were genotyped. No evidence of positive association for any of these loci with CD was found in the Japanese population, even after clinically stratified subgroups of CD were used. Our result revealed a distinct ethnic difference of genetic background of CD that we reported previously in other genes between Japanese and Caucasian populations. Further genetic studies are required to confirm our findings with ethnically divergent populations.

Keywords Crohn's disease · Susceptibility · Autophagy-related 16-like 1 (*ATG16L1*) · Interleukin 23 receptor (*IL23R*) · Chromosome 5p13.1 · Japanese population · Genome-wide association (GWA) study

The authors declare that they have no competing interests.

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Introduction

Inflammatory bowel diseases (IBDs) that are usually classified into two clinical entities, Crohn's disease (CD; MIM 266600) and ulcerative colitis (UC; MIM 191390), are chronic conditions characterised by remitting and relapsing inflammation of the small and/or large intestines. The combined prevalence of the two diseases in the West and in Asia has been increasing significantly over the last decade; the prevalence rate of UC and of CD in the Japanese population was estimated to be 6.31 and 0.88 per 100,000 in 1985, but the rate increased to 18.12 and 5.85 per 100,000 in 2006 (Hilmi et al. 2006). A similar increasing trend in the annual incidence was observed in Korea, Singapore and China (Law et al. 1998; Yang et al. 2000; Leong et al. 2004). Although these values in Asian

countries were still relatively low when compared to Western countries (Loftus 2004), IBDs are now thought to be common diseases in Asia.

In spite of a significant number of studies to identify the fundamental pathophysiologic processes, the etiology of IBDs still remains largely unknown. IBDs were thought to be multifactorial diseases. In fact, aggregate effects of genetic, environmental and other processes are found to induce an abnormal response of the mucosal immune system (Podolsky 2002). The role of genetic factors in the etiology of IBDs was suggested by familial aggregation through twin studies (Vermeire and Rutgeerts 2005).

Linkage analyses followed by fine-mapping or genome-wide association (GWA) analyses have identified susceptible variants to CD in several genes, including *CARD15* (*NOD2*) (Hugot et al. 2001; Ogura et al. 2001), *DLG5* (Stoll et al. 2004), *SLC22A4* and *SLC22A5* (Peltekova et al. 2004), *CARD4* (also known as *NOD1*) (McGovern et al. 2006) and *TNFSF15* (Yamazaki et al. 2005). Recently, three independent groups have newly reported susceptible genes by way of genome-wide linkage disequilibrium-based association studies. The first group determined ten single nucleotide polymorphisms (SNPs) in the *IL23R* (interleukin 23 receptor) gene as significant markers (Duerr et al. 2006). The *IL23R* gene is located on chromosome 1p31 and forms a receptor for IL23 (interleukin 23), together with the beta 1 subunit of IL12 (*IL12RB1*) (Parham et al. 2002). The allele A of rs11209026 (c.1142G>A, p.Arg381Gln) is found to be protective against CD development in the two ethnic cohorts, European and Jewish. The second gene identified was the *ATG16L1* (autophagy-related 16-like 1) (Hampe et al. 2007) located on chromosome 2. Although a recent study has revealed that a mouse orthologue ATG16l is localised to the autophagic isolation membrane during autophagosome formation (Mizushima et al. 2003), the function of human *ATG16L1* remains uncertain. The allele G of rs2241880 (c.898G>A, p.Thr300Ala) conferred susceptibility to CD and was found to interact with the *CARD15* risk genotype. The last group identified a susceptible 250-kb region on chromosome 5p13.1 (Libioulle et al. 2007). Several genetic variants were found to influence the expression of the *PGER4* (prostaglandin E receptor 4) gene, which resides closest to the associated region.

To investigate a possible role of these candidate genes, *IL23R* and *ATG16L1* and 5p13.1 loci, in the pathogenesis of CD development of Japanese patients, we assessed the distribution of 29 selected markers and examined the genotype–phenotype analysis. In addition, we performed haplotype analysis and found an ethnical divergence between the European and Japanese populations.

Methods

Human subjects and phenotypic analysis

Japanese blood samples were obtained with written informed consent from 484 CD patients at the Social Insurance Chuo General Hospital and from 439 unaffected control individuals belonging to the Osaka-Midosuji Rotary Club. The study protocol was approved by the local ethics board. 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) and patients with indeterminate colitis were excluded. Extensive clinical characterisation was available in 482 CD patients. The clinical characteristics of CD patients were assessed at the time of diagnosis and were categorised using the Vienna classification (Gasche et al. 2000). In addition to that, the past medical history of surgical operation was obtained from the clinical records of 418 patients. The demographics of the CD patients and controls are shown in Table 1.

SNP analysis and genotyping

We selected a total of 29 SNPs for genotyping; 12 in *ATG16L1*, which was reported to constitute susceptible

Table 1 Demographic and clinical features of the Crohn's disease and the control groups

		Crohn's disease (*n*=482)	Controls (*n*=439)
Sex (M/F/unknown)		351/129/2	360/79
Age at disease onset (years) (median [range])		22.4 [7–55]	53.1 [18–93]
Age at disease onset (*n*)[a]	<40 years	460 (95.8%)	
	≥40 years	20 (4.2%)	
Disease location	Ileal disease (L1)	191 (39.6%)	
	Colonic disease (L2)	70 (14.5%)	
	Ileal and colonic disease (L3)	196 (40.7%)	
	Upper G1 disease (L4)	22 (4.6%)	
	Uncertain	3 (0.6%)	
Disease behaviour	Inflammatory (B1)	126 (26.1%)	
	Stricturing (B2)	244 (50.6%)	
	Penetrating (B3)	107 (22.2%)	
	Uncertain	5 (1.0%)	
Need for surgery (*n*)[a]		210 (69.3%)	

[a] Information about age at onset was available in 480 patients and about surgery in 418 patients

haplotypes, 10 in *IL23R* and 7 in 5p13.1, with which positive association was shown (Duerr et al. 2006; Libioulle et al. 2007; Hampe et al. 2007). TaqMan assay (Applied Biosystems) was applied for genotyping 18 SNPs. Nine SNPs, four of *ATG16L1* and five of 5p13.1 locus, were genotyped by the Invader assay (Ohnishi et al. 2001). The genotyping of rs1441090 was performed by sequencing and rs11465804 was by polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) analysis. The primers and typing methods used are described in Table 2.

Statistical analysis

The markers were evaluated for deviation from the Hardy-Weinberg equilibrium in the controls before inclusion in the analysis ($P>0.05$). Allele frequencies were analysed by χ^2 tests and the p-values were corrected by 1,000 random permutations for each SNP. Haplotype frequencies and linkage disequilibrium (LD) were estimated and visualised using Haploview 3.32 (available at http://www.broad.mit.edu/mpg/haploview/) (Barrett et al. 2005). Plots of the relative D' levels between each locus in the Japanese population were calculated from the genotype data of the case and control populations and those in the Caucasian population were from the HapMap data. LD structure of the *ATG16L1* locus in the Caucasian population was constructed with 11 SNPs instead of 12, as rs2241879 has been eliminated in the HapMap data. p values for haplotype association analysis were obtained after 1,000 permutation tests.

Results

Case–control analysis of *IL23R*, *ATG16L1* and 5p13.1 loci with Japanese CD patients

As shown in Table 3, all 29 SNPs located in the three candidate loci did not show any positive association with Japanese CD cases. Among the ten SNPs of *IL23R* examined, two SNPs, rs11465804 and rs11209026, were absent both in the Japanese CD cases and controls; the latter was identified during the GWA study and both SNPs were in strong LD. Another SNP named rs4613763 in the 5p13.1 locus was also absent in Japanese population; only allele T existed.

Genotype–phenotypic analysis of three candidate loci with Japanese CD patients

Duerr et al. 2006 focussed on ileal CD to minimise pathogenic heterogeneity and identified *IL23R* as a susceptible gene. That prompted us to analyse ten SNPs of *IL23R* with ileal CD as cases and we found that one SNP, rs11209032, was marginally associated; however, the significance was not confirmed after permutation tests (Table 4). Other sub-phenotypes of CD (ileocolitis and colitis) did not demonstrate any significant effects (data not shown). Likewise, all 12 SNPs of *ATG16L1*, including rs2241880 and seven SNPs on 5p13.1, were not significant when any sub-phenotype was used as cases (data not shown).

Haplotype analysis of *ATG16L1*, *IL23R* and 5p13.1 loci

The haplotype frequency of each locus was estimated with the genotyping data of 29 markers. As shown in Tables 5, 6 and 7, haplotype association analysis of the three loci to CD susceptibility did not show any positive result. In the *IL23R* locus, a slight increase of major haplotype (TTACCAAG) frequency in Japanese CD patients to controls (CD 41.3% vs. controls 36.1%) was observed but was not deemed significant. Furthermore, the frequency of the haplotype of three loci also has a significantly different structure between the Japanese and Caucasian populations when compared to Caucasian frequency data. In order to recognise the difference between genetic and racial background in these regions visually, we characterised the LD structure of the three loci in both populations. Great divergence was clearly demonstrated (Fig. 1) and this might partly explain the ethnic difference of CD susceptibility.

Discussion

In this paper, we analysed a total of 29 candidate variants of the three loci, *IL23R*, *ATG16L1* and 5p13.1, recently reported to confer susceptibility with CD in the Japanese population. The three variants described to be responsible for the disease were absent in the Japanese population; two variants in *IL23R*, only allele G of rs11209026 and allele T of rs11465804, and one variant in 5p13.1 locus, only allele T of rs4613763 existed. All of the remaining 26 genetic markers have failed to show any positive association to Japanese CD. Duerr et al. 2006 performed GWA studies with clinically stratified CD subjects, ileal CD, to minimise pathogenic heterogeneity. That enabled us to analyse in the same way stratified samples employed as cases, however, no significant association were obtained. As shown in Fig. 1, the analysis of LD structure showed us evident ethnic differences in all of the three loci. Our previous finding that most of the susceptible genes identified in cohorts of European and Jewish ancestry were not adaptable for Japanese CD patients (Yamazaki et al. 2002; Yamazaki et al. 2004) but were again reproduced in the present study.

Table 2 List of genotyping methods for each single nucleotide polymorphism (SNP) and primer sequences

SNP	Location	Position[a]	Amino acid substitution	Methods	Primers[b]
IL23R (*n*=10)					
rs1004819	Intron 5	+3,633		TaqMan	
rs7517847	Intron 6	+8,931		TaqMan	
rs10489629	Intron 7	+2,936		TaqMan	
rs2201841	Intron 7	+8,789		TaqMan	
rs11465804	Intron 8	+41		PCR-RFLP	F: ATATGCAGCCGTTCTTTTGG
					R: GTATGATGGGTTAAAATGGGCAAGT
rs11209026	Exon 8	1,142	Arg381Gln	TaqMan	
rs1343151	Intron 9	+13,165		TaqMan	
rs10889677	Exon 11	2,199	UTR	TaqMan	
rs11209032	Intergenic			TaqMan	
rs1495965	Intergenic			TaqMan	
ATG16L1 (*n*=12)					
rs2083575	Intergenic			TaqMan	
rs12471449	Intergenic			TaqMan	
rs11685932	Intron 2	+1,481		TaqMan	
rs6431660	Intron 2	+2,393		TaqMan	
rs1441090	Intron 2	+3,201		Sequencing	F: ATATGCAGCCGTTCTTTTGG
					R: GGAGGGCAGGCTTATTATGG
rs3792110	Intron 6	+1,925		Invader assay	F: TGTTGCTGTTTATCCCAGCG
					R: CAGGACATTGTCAAAGGCTCAG
rs2289472	Intron 7	+542		Invader assay	F: CCTTCAAGAGTGGGGATTGG
					R: CTCTGCATCACTGACACCTG
rs2241880	Exon 9	898	Thr300Ala	TaqMan	
rs2241879	Intron 9	+44		invader assay	F: GCATGTGCTGGCTCTCTTTC
					R: CCAAAAGGTGGAAAGGCTTG
rs3792106	Intron 11	+919		invader assay	F: CTGCTTCCTCCAAGCCAGTC
					R: GACGGACACCACAGGCAG
rs4663396	Intron 12	+852		TaqMan	
rs6748547	Intron 17	+763		TaqMan	
5p13.1 (*n*=7)					
rs348601	Intergenic			Invader assay	F: ACTGGTTGGAGACCCACTGC
					R: AAACTGGCACAAGACCACCC
rs1002922	Intergenic			Invader assay	F: GCTGTTGCTGCTCCACAAAC
					R: AACACTGTAGCTGCTGGTCTGG
rs4613763	Intergenic			Invader assay	F: GCCAGCCTGAGTCTGAAGTG
					R: TGTCCTGCCTGATCTGTTGC
rs10512734	Intergenic			TaqMan	
rs1373692	Intergenic			TaqMan	
rs4495224	Intergenic			Invader assay	F: TGGACATATACACAGGTGGTGC
					R: GGAGAGGAGGTGAAGTCCTTG
rs7720838	Intergenic			Invader assay	F: ATGAACCATCTGCCCTTGAG
					R: ATGTGGGATGTGATGCATTG

[a] Position is relative to the ATG start site and the reference. The first nucleotide of the exon1 start site is designated as position 1 based on the reference sequence GenBank NM_144701.2 for *IL23R* and AY398617.1 for *ATG16L1*

[b] The underlined bases in the primer differ from the original sequences and serve to introduce a restriction site

Table 3 Genotype distribution for *IL23R*, *ATG16L*1 and 5p13.1 loci markers

Marker	A allele[a]	Number of CD genotypes				Number of control genotypes				χ^2	*p* value	p_c value[b]
		AA	Aa	aa	Sum	AA	Aa	aa	Sum			
IL23R												
rs1004819	T	148 (30.7%)	253 (52.5%)	81 (16.8%)	482	142 (32.3%)	205 (46.7%)	92 (21.0%)	439	0.29	0.59	0.98
rs7517847	T	167 (34.5%)	241 (49.8%)	76 (15.7%)	484	138 (31.5%)	213 (48.6%)	87 (19.9%)	438	2.41	0.12	0.42
rs10489629	A	241 (49.8%)	209 (43.2%)	34 (7.0%)	484	229 (52.3%)	167 (38.1%)	42 (9.6%)	438	0.00	0.99	1.00
rs2201841	C	236 (48.8%)	213 (44.0%)	35 (7.2%)	484	220 (50.5%)	170 (39.0%)	46 (10.6%)	436	0.14	0.70	1.00
rs11465804	T	473 (100%)	0 (0.0%)	0 (0%)	473	438 (100%)	0 (0%)	0 (0%)	438	-	-	-
rs11209026	G	469 (100%)	0 (0%)	0 (0%)	469	430 (100%)	0 (0%)	0 (0%)	430	-	-	-
rs1343151	C	384 (79.5%)	98 (20.3%)	1 (0.2%)	483	359 (82.0%)	74 (16.9%)	5 (1.1%)	438	0.30	0.59	0.98
rs10889677	A	232 (47.9%)	219 (45.2%)	33 (6.8%)	484	220 (50.1%)	173 (39.4%)	46 (10.5%)	439	0.12	0.73	1.00
rs11209032	A	111 (23.1%)	244 (50.7%)	126 (26.2%)	481	89 (20.3%)	209 (47.7%)	140 (32.0%)	438	3.35	0.07	0.26
rs1495965	G	115 (23.8%)	246 (50.8%)	123 (25.4%)	484	98 (22.4%)	209 (47.8%)	130 (29.7%)	437	1.48	0.22	0.62
ATG16L1												
rs2083575	A	459 (95.2%)	23 (4.8%)	0 (0.0%)	482	413 (94.5%)	23 (5.3%)	1 (0.2%)	437	0.41	0.52	0.99
rs12471449	C	413 (85.5%)	66 (13.7%)	4 (0.8%)	483	362 (82.8%)	72 (16.5%)	3 (0.7%)	437	0.97	0.33	0.91
rs11685932	A	47 (9.8%)	213 (44.5%)	219 (45.7%)	479	54 (12.3%)	206 (47.0%)	178 (40.6%)	438	2.95	0.09	0.45
rs6431660	G	23 (4.8%)	184 (38.2%)	275 (57.1%)	482	33 (7.6%)	166 (38.0%)	238 (54.5%)	437	1.76	0.18	0.72
rs1441090	C	426 (88.9%)	49 (10.2%)	4 (0.8%)	479	379 (87.7%)	50 (11.6%)	3 (0.7%)	432	0.22	0.64	1.00
rs3792110	A	168 (35.1%)	241 (50.4%)	69 (14.4%)	478	150 (34.6%)	208 (47.9%)	76 (17.5%)	434	0.63	0.43	0.97
rs2289472	A	23 (4.9%)	178 (38.0%)	268 (57.1%)	469	32 (7.6%)	156 (37.1%)	233 (55.3%)	421	1.20	0.27	0.87
rs2241880	G	23 (4.8%)	184 (38.3%)	274 (57.0%)	481	32 (7.3%)	167 (38.2%)	238 (54.5%)	437	1.55	0.21	0.77
rs2241879	T	23 (4.8%)	185 (38.2%)	276 (57.0%)	484	33 (7.5%)	167 (38.0%)	239 (54.4%)	439	1.75	0.19	0.73
rs3792106	G	39 (8.1%)	214 (44.5%)	228 (47.4%)	481	36 (8.3%)	187 (43.1%)	211 (48.6%)	434	0.06	0.81	1.00
rs4663396	C	328 (67.9%)	147 (30.4%)	8 (1.7%)	483	305 (69.8%)	117 (26.8%)	15 (3.4%)	437	0.00	0.98	1.00
rs6748547	G	433 (89.8%)	49 (10.2%)	0 (0.0%)	482	400 (91.5%)	37 (8.5%)	0 (0.0%)	437	0.74	0.39	0.95
5p13.1												
rs348601	T	15 (3.1%)	127 (26.3%)	341 (70.6%)	483	7 (1.6%)	116 (26.4%)	316 (72.0%)	439	0.73	0.39	0.76
rs1002922	T	40 (8.3%)	215 (44.5%)	228 (47.2%)	483	39 (8.9%)	194 (44.2%)	206 (46.9%)	439	0.04	0.84	1.00
rs4613763	C	0 (0%)	0 (0%)	472 (100%)	472	0 (0%)	0 (0%)	439 (100%)	439	–	–	–
rs10512734	A	42 (8.7%)	212 (43.9%)	229 (47.4%)	483	39 (9.0%)	192 (44.2%)	203 (46.8%)	434	0.05	0.83	1.00
rs1373692	C	16 (3.3%)	135 (27.9%)	333 (68.8%)	484	7 (1.6%)	129 (29.4%)	303 (69.0%)	439	0.31	0.58	0.95
rs4495224	A	42 (8.7%)	208 (43.1%)	233 (48.2%)	483	38 (8.7%)	189 (43.1%)	212 (48.3%)	439	0.00	0.98	1.00
rs7720838	G	289 (60.0%)	169 (35.1%)	24 (5.0%)	482	272 (62.1%)	142 (32.4%)	24 (5.5%)	438	0.18	0.67	0.97

[a] Associated alleles were based on previous papers and annotated as ''A'' in this table; *ATG16L1* markers were formed as Haplotype 1 (ACAGCAAGTGCG) in Table 3 by Hampe et al. 2007; *IL23R* markers were overtransmitted alleles in non-Jewish CD and Jewish CD, indicated in Table 2 by Duerr et al. 2006

[b] P_c value was calculated by permutation tests after performing 1,000 random permutations for each tested SNP for multiple testing adjustment

Table 4 Genotype distribution for *IL23R* between ileal CD patients and controls in Japanese

Marker	Number of ileal CD genotypes				χ^2	*p* value	p_c value [b]
	AA[a]	Aa	aa	Sum			
rs1004819	62 (32.5%)	102 (53.4%)	27 (14.1%)	191	1.30	0.25	0.70
rs7517847	71 (37.2%)	91 (47.6%)	29 (15.2%)	191	2.91	0.09	0.32
rs10489629	102 (53.4%)	76 (39.8%)	13 (6.8%)	191	0.50	0.48	0.95
rs2201841	101 (52.9%)	77 (40.3%)	13 (6.8%)	191	1.22	0.27	0.75
rs1343151	159 (83.7%)	31 (16.3%)	0 (0.0%)	190	0.65	0.42	0.92
rs10889677	98 (51.3%)	80 (41.9%)	13 (6.8%)	191	0.76	0.38	0.88
rs11209032	45 (23.7%)	102 (53.7%)	43 (22.6%)	190	4.30	0.04	0.16
rs1495965	46 (24.1%)	102 (53.4%)	43 (22.5%)	191	2.11	0.15	0.51

[a] Associated alleles were shown according to the notation in Table 3. Genotyping data of rs11465804 and rs11209026 were omitted

[b] p_c values were calculated by permutation tests after performing 1,000 random permutations for each tested SNP

Table 5 Result of a haplotype analysis of eight SNPs at the *IL23R* locus in Japanese CD patients and controls

Haplotype	Frequency[a]	Case, control ratios	*p* value[b]	HapMap CEU frequency
TTACCAAG	0.389	0.413, 0.361	0.11	0.199
CGGTCCGA	0.143	0.142, 0.144	1.00	0.117
TTACCAGA	0.121	0.117, 0.126	1.00	0.017
CGACCAGA	0.117	0.113, 0.121	1.00	–
CGGTTCGA	0.096	0.099, 0.092	1.00	0.281

[a] Haplotypes with estimated frequencies >0.03 are shown

[b] *p* values for haplotype association analysis were obtained from case–control samples with 1,000 permutation tests

Table 6 Result of a haplotype analysis of 12 SNPs at the *ATG16L1* locus in Japanese CD patients and controls

Haplotype	Frequency[a]	Case, control ratios	*p* value[b]	Frequency in Caucasian[c]
ACGACTGACACG	0.538	0.547, 0.531	1.00	0.285
ACAGCAAGTGCG	0.222	0.212, 0.234	0.96	0.533
AGAATAGACATG	0.044	0.044, 0.045	1.00	0.066

[a] Haplotypes with estimated frequencies >0.03 are shown

[b] *p* values for haplotype association analysis were obtained from case–control samples with 1,000 permutation tests

[c] Each of the haplotype frequencies in CEU was referred from Table 3 in the control population by Hampe et al. (2007)

Since *CARD15* was identified as the first gene conferring susceptibility to CD in 2001 (Hugot et al. 2001; Ogura et al. 2001), a significant number of studies of its replication and newly identified responsible genes followed. Three major polymorphisms in the *CARD15* gene, R702W, G908R and 1007fs, were confirmed to be associated with susceptibility to Caucasian CD patients by independent groups (Ahmad et al. 2002; Lesage et al. 2002), even though the frequencies of these variants were quite different between ethnically divergent populations. Among CD patients of European ancestry, *CARD15* variants were more significantly associated in the Central European population (Hugot et al. 2001; Ahmad et al. 2002; Lesage et al. 2002) than in the North European population (Paavola-Sakki et al. 2003; Arnott et al. 2004; Medici et al. 2006). The absence of the variants were widely known in Asia; Japanese (Yamazaki et al. 2002), Korean (Croucher et al. 2003) and Chinese populations (Leong et al. 2003). Susceptible genes identified next were *DLG5* (Stoll et al. 2004), *SLC22A4* and *SLC22A5* (Peltekova et al. 2004). The association has been studied with various ethnic populations and the results shown were also extremely heterogeneous (Yamazaki et al. 2004; Friedrichs and Stoll 2006; Silverberg 2006).

Table 7 Result of a haplotype analysis of six SNPs on the 5p13.1 locus in Japanese CD patients and controls

Haplotype	Frequency[a]	Case, control ratios	*p* value[b]	HapMap CEU frequency
GCGTCG	0.666	0.668, 0.664	1.00	0.266
GTATAT	0.118	0.115, 0.122	1.00	0.045
ATAGAT	0.086	0.093, 0.078	0.90	0.449
ATAGAG	0.055	0.054, 0.056	1.00	0.038

[a] Haplotypes with estimated frequencies >0.03 are shown

[b] *p* values for haplotype association analysis were obtained from case–control samples with 1,000 permutation tests

The *IL23R*, *ATG16L1* and 5p13.1 loci were identified by GWA studies and the results were confirmed with large independent Caucasian samples. The biological, technical and statistical foundations have been laid to apply GWA

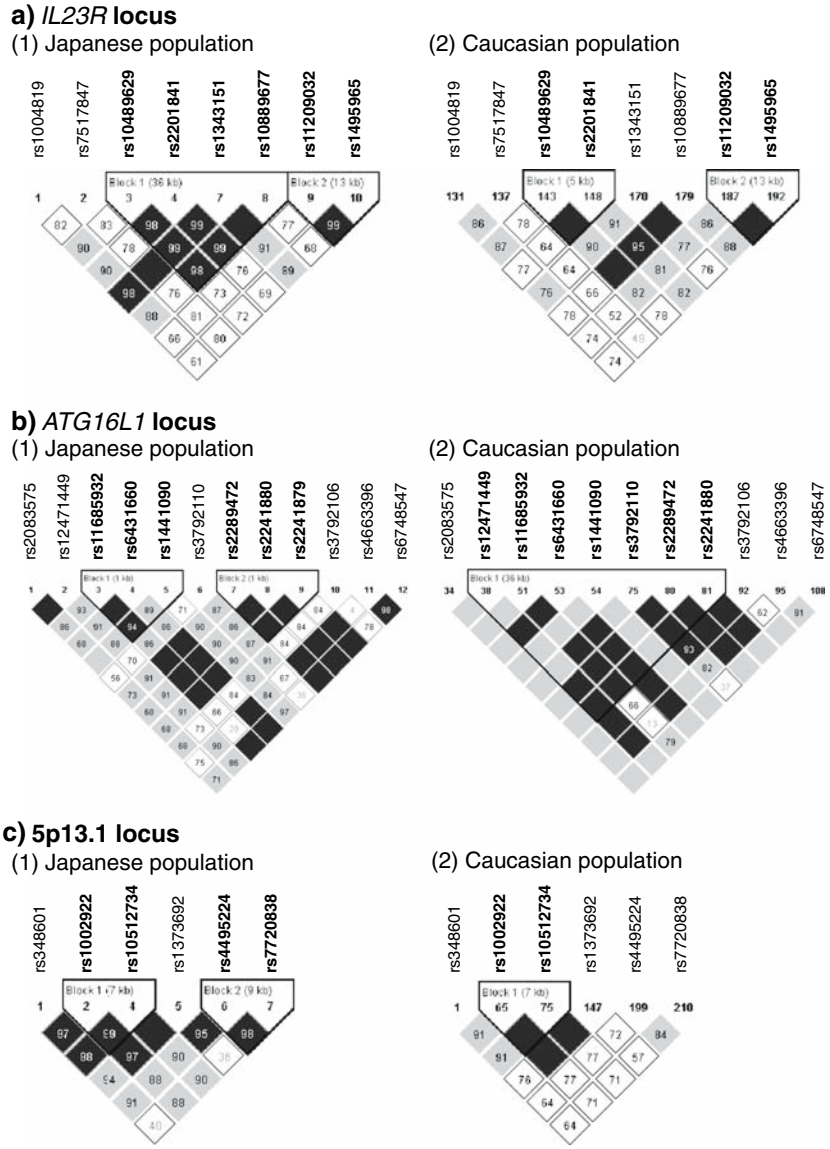

Fig. 1a–c The structure of linkage equilibrium (D') among Japanese and Caucasian populations. The D' scores in the Japanese population were estimated from the genotyping case and control data across each of **a** *IL23R*, **b** *ATG16L1* and **c** 5p13.1 loci and in the Caucasian population was as generated by Haploview from the Caucasian HapMap data. Registration of rs2241879 in the *ATG16L1* locus was eliminated in the HapMap data

studies as a critical tool for the identification of susceptibility genes for complex diseases and they have produced more robust results. These original comprehensive analyses provided the information of replication in previously reported regions. Libioulle et al. 2007 have identified the 5p13.1 locus to confer CD susceptibility and, at the same time, confirmed previously reported regions, *IL23R* and *ATG16L1*. Furthermore, by a group that identified *IL23R* as a susceptible gene to CD, GWA studies reported replicated positive association with *ATG16L1* (Rioux et al. 2007). These findings supported the theory that *IL23R* and *ATG16L1* were common susceptible genes to CD in Caucasian populations. Although it was generally accepted that the clinical profiles of CD are similar between Caucasians and Asians (Hilmi et al. 2006), common susceptible variant(s) to CD has not been reported so far. Great ethnical diversity of susceptible genes to CD between Japanese and European ancestries seems to exist.

In conclusion, we failed to confirm the association between the candidate genetic variations in the *IL23R*, *ATG16L1* genes and the 5p13.1 locus in Japanese CD. Our result suggested that these candidate genes were not common variants to CD among the Japanese and Caucasian populations. In consideration of the increased prevalence of IBDs in Asian, systematic screening should be carried out as GWA studies among various populations with different ethnical backgrounds and it will lead to elucidate the contribution of susceptibility genes to IBD.

Acknowledgments We thank Ayumi Kemori and Rie Funahashi and the other members of the Laboratory for Gastrointestinal Diseases for their assistance. This work was supported by a grant from the Japanese Millennium Project and in part by a ''Grant-in-Aid for

Young Scientists (B)'' (grant no. 18790484) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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