Fine Mapping of a Psoriasis-Susceptibility Locus within the HLA Class II Region by using Microsatellite Markers in an Association Study of Japanese Cases and Controls

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***Background*: The association between psoriasis and the HLA antigens encoded by the Major Histocompatibility Complex (MHC) genomic region is well known, but the role of the HLA class II region in susceptibility to psoriasis is unclear.**
Objective: The purpose of this study is to map the psoriasis-susceptibility locus within the HLA class II region.
***Methods*: Three hundred seventy five unrelated Japanese patients with psoriasis vulgaris and 375 unrelated Japanese healthy controls were studied by an association analysis using 15 polymorphic microsatellite markers.**
***Results*: Statistically significant differences were found at three microsatellite loci. The most significant association of psoriasis vulgaris with the microsatellite markers was found with *DRA_CA* (*pc*=0.0000135), the second with *DQCARII* (*pc*=0.0000840) and the third with *G5_11525* (*pc*=0.0240). These significant microsatellite markers are in close vicinity to the *DQA2*, *DQB1*, *DRB1*, *DRA* and *BTNL2* genes.**
***Conclusion*: The results suggest that there are psoriasis susceptibility genes located within the HLA class II region and therefore strongly support previous findings of a positive association between psoriasis and certain alleles of the *DQB1* and *DRB1* genes.**

Key words: Psoriasis, HLA class II, microsatellite marker

INTRODUCTION

Psoriasis is a common, genetically determined, inflammatory and proliferative disease of the skin [1]. The prevalence of psoriasis is between 1 and 3% in Caucasians [2], 0.1% in Far Eastern populations [3], 0.3% in Chinese [4] and 0.02 to 0.1% in Japanese [5]. The exact cause of psoriasis is unknown, but it is thought to be a multifactorial disease triggered by both genetic and environmental factors such as trauma, drugs, infection, alcohol, smoking and stress [1].

The infiltration of epidermis and dermis of psoriatic lesions by the immune T cells is well known and has been reviewed in detail [6, 7]. Essentially, both $CD4^+$ and $CD8^+$ T cells infiltrate the psoriatic lesions with the $CD8^+$ T cells located mainly in the epidermis and $CD4^+$ T cells located mainly in the dermis. Although, it is still unclear which of the two T cell subsets is more important in the development of psoriasis, it appears that $CD4^+$ T cells are necessary for providing critical inductive and helper signals while the $CD8^+$ T cells are the principal effecter agents in the pathogenesis of psoriasis. For example, psoriasis is initiated and exacerbated in HIV positive individuals with relative few circulating $CD4^+$ T cells [8], whereas the remedial effect of anti-CD4 monoclonal antibody on psoriasis [9] suggests that $CD4^+$ T cells are indispensable for inducing and maintaining the disease. In addition, psoriatic lesions in the skin of psoriatic patients grafted to SCID mice were induced by the addition of purified $CD4^+$ T cells, but not with purified $CD8^+$ T cells. However, $CD8^+$ T cells were observed to proliferate in the epidermis and the grafted psoriatic skin of this SCID mouse [10].

Classical HLA class II molecules -DR, -DQ and -DP expressed on the surface of macrophages, B cells and dendritic cells present peptide fragments (HLA class II-antigen complex) to CD4 receptors on the

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helper T cells leading to T cell activation [11]. Many autoimmune and immune related diseases, including psoriasis, have been strongly associated with HLA class II alleles [12]. For example, the HLA DR7 antigen was associated with Japanese psoriasis patients [13], the HLA-DQA1*0104 and -DQA1*0201 alleles were positively associated while the HLA-DQA1*0501 allele was negatively associated with Chinese Han psoriatic patients [14] and the HLA-DRB1*0701/2, -DQA1*0201, -DQB1*0303 extended haplotype was associated with type I psoriasis in Europeans [15]. However, a number of studies have not supported the association between psoriasis and the HLA class II region [16-18] and indicated that the psoriasis susceptibility locus, called PSORS1, was within the HLA class I region [16-19].

Microsatellite markers can provide an economical and rapid alternative to SNP analysis of HLA class II genes for fine mapping the psoriasis susceptibility locus within the HLA class II region in a similar way that they were applied to the analysis of HLA class I region by using patients and controls in an association study [19]. Moreover, there is an extensive distribution of high-density polymorphic microsatellite markers within the HLA genomic region for fine mapping studies of the HLA class II region and identification of the psoriasis susceptibility locus to be achieved successfully [20-22]. Since the HLA class II molecules are likely to have both genetic and immunogenic effects on psoriasis, we therefore performed a detailed genetic study of the association between the HLA class II genomic region and psoriasis for 375 psoriasis patients and 375 healthy controls by using 15 polymorphic markers distributed evenly from the centromeric *RING1* gene to telomeric *BTNL2* gene of the class II region. Here, we report on the results of the study and the identification of two psoriasis susceptibility loci near the *BTNL2*, *DRA* and *DRB1* genes and the *DQA2* and *DQB1* genes, respectively.

MATERIALS AND METHODS

1. Patients and controls

Three hundred seventy five unrelated Japanese patients with psoriasis vulgaris and 375 sex- and age-matched unrelated Japanese healthy controls were investigated in this study. The patients consisted of 285 males and 90 females with the psoriasis onset mean age of 38.9 years (SD = 16.9). Each patient was diagnosed and treated at the Departments of Dermatology, Tokai University School of Medicine (Kanagawa, Japan), Fukuoka University School of Medicine (Fukuoka, Japan), Tohoku University Graduate School of Medicine (Miyagi, Japan), Kurume University School of Medicine (Fukuoka, Japan), Juntendo University School of Medicine (Tokyo, Japan), Aichi Medical University School of Medicine (Aichi, Japan), and Showa University School of Medicine (Tokyo, Japan). Written informed consent was obtained from all the patients and healthy controls by explaining the details of this study prior to collection of peripheral blood. The medical ethical committee of Tokai university school of medicine approved all described studies (No. 01-11).

2. Polymorphic microsatellite markers

During the course of our large-scale genomic sequencing of the human HLA region, 1,549 microsatellite loci ranging from di- to penta-nucleotide repeats were identified within the 3.6 Mb region that extends from the centromeric *HSET* gene to the telomeric *HLA-F* gene [22-24]. Among these microsatellites, 147 were characterized for their polymorphism in the Japanese population, and 68 were found to be informative [20-22, 24-27]. In this study, we selected a total of 15 highly polymorphic microsatellite markers (Table 1) located at regular intervals in the HLA class II region (1.1 Mb) to use for high-resolution mapping.

3. Genotyping for microsatellite alleles

The PCR primers used for amplification of the 15 microsatellites in the HLA class II region are shown in Table 1. PCR was performed as follows; fluorescent-dye conjugated PCR primers were unilaterally labeled at the 5' -end with the fluorescent reagent, 6-FAM (Proligo Kyoto Japan). The PCR reaction mixture contained 1 ng of genomic DNA, 2 µl of dNTP (2.5 mM each), 2 µl of 10 × buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$) and 20 pmol of forward and reverse primers as well as 0.5 U AmpliTaqGold DNA polymerase (Applied Biosystems Japan Co.) in a total volume of 20 µl. The initial cycle was for 9 min at 95˚C, 1 min at X˚C (Annealing temperature is different for some markers as shown in Table 1) and 1 min at 72 ˚C. Amplification was carried out in an automated thermal cycler (Applied Biosystems Japan Co.) for 40 cycles consisting of 45 sec at 96˚C, 45 sec at X ˚C and 1 min at 72˚C, with a final extension of 5 min at 72˚C. Each PCR product was diluted 1:2 with water, mixed with formamide-containing loading buffer, denatured for 5 min at 96˚C and then separated on capillary gels with a size standard marker of GeneScan500LIZ (Applied Biosystems Japan Co.) using an ABI PRISM 3730 automated sequencer and analyzed by using Genotyper software (Applied Biosystems Japan Co.). Fragment sizes were determined using the MultiTyper developed by Applied Biosystems Japan.

4. Statistical analysis of the data

The significance of the distribution of alleles between the patients and the controls was tested by Fisher's exact probability test (*p*-value test) using the 2 by 2 and the 2 by m contingency tables. To control for the effect of linkage disequilibrium between loci, the Odds Ratio (OR) of the risk to psoriasis vulgaris was calculated from the 2 by 2 contingency table. The smallest *p*-value was selected and corrected for multiple comparisons by the Bonferroni adjustment (corrected *p*-value: *pc*-value). The exact *p*-value test of Hardy-Weinberg proportion for multiple alleles was simulated by the Markov chain method within the GENEPOP software package [28, 29]. The exact *p*-value was estimated by the simulations under the following parameters; the dememorization number = 1,000, the number of batches = 400 and the iteration per batch = 8,000.

Table 1 Microsatellite markers used in this association study.

Microsatellite	Localization	Primer Sequence	Annealing temperature (℃)	Reference
M2_2_9	Telomeric (0 kb)/*RING2*	Forward: TGAAGACTGAGTCTGTGACC	53	[21]
	Centromeric (0 kb)/*RING2*	Reverse: ACCCATGACACAGAAAGTGC		
D6S0512i	Telomeric (6 kb)/*COL11A2*	Forward: CTAGGCACTGTGGCTACTGTAC	61	[27]
	Centromeric (69 kb)/*DPB1*	Reverse: ATTTGGATCATGAGTCAAACAC		
M2_2_22	Telomeric (0 kb)/*DOA*	Forward: GGAGACACATTCAAACCATAGC	57	[21]
	Centromeric (14 kb)/*RING3*	Reverse: CAATTGGTGACATACATCAACTTG		
M2_2_24	Telomeric (2 kb)/*DMB*	Forward: GAATGGATGCTGCATGAGG	57	[21]
	Centromeric (73 kb)/*PSMB9*	Reverse: AAGTGTTGAAGGAACTCCCTGC		
M2_4_25	Telomeric (48 kb)/*DMB*	Forward: TCACTCATGGTTGCTTTTCC	57	[21]
	Centromeric (27 kb)/*PSMB9*	Reverse: GAATGATAGGAGTCCATTGTGG		
D6S2820	Telomeric (4 kb)/*TAP1*	Forward: AGATCCTGGCTTGATGATGC	62	[20]
	Centromeric (6 kb)/*PSMB8*	Reverse: TTGCAGTGAGCCCAGATC		
D6S2818	Telomeric (43 kb)/*DOB*	Forward: ATGAAGTGAGCTGTGATCGC	59	[20]
	Centromeric (6 kb)/*DQB2*	Reverse: AGAGCTGCAGTGCTGTATTG		
D6S1100i	Telomeric (7 kb)/*DQB2*	Forward: GAACCCAGCATTCTGGAG	61	[27]
	Centromeric (0 kb)/*DQA2*	Reverse: TGTATGACTCTGGTTATAATGCC		
G5-11525	Telomeric (46 kb)/*DQA2*	Forward: GACAGCTCTTCTTAACCTGC	60	[20]
	Centromeric (36 kb)/*DQB1*	Reverse: GGTAAAATTCCTGACTGGCC		
DQCARII	Telomeric (9 kb)/*DQB1*	Forward: GGGCAGCATTTGTAGATTTC	60	[20]
	Centromeric (117 kb)/*DRB1*	Reverse: GGCAAGAATCCAGCATTTTGG		
D6S0588i	Telomeric (23 kb)/*DQB1*	Forward: AACTCACTTGAATAAACTGCAC	57	[27]
	Centromeric (103 kb)/*DRB1*	Reverse: AGAATACTCTGGAGCTATGGAG		
DRA_CA	Telomeric (4 kb)/*DRA*	Forward: ACATTATGTTCTGTTGCATG	57	[20]
	Centromeric (28 kb)/*BTNL2*	Reverse: TACTTTCCTAATTCTCCTCC		
2-10	Telomeric (20 kb)/*BTNL2*	Forward: AGGTGACCTGGACCTTACTG	57	[27]
	Centromeric (kb)/	Reverse: ACACTATGCTAGTCTGTGCC		
D6S2913	Telomeric (36 kb)/*BTNL2*	Forward: CTAGATGACGAGTTAGTGGG	59	[20]
	Centromeric (kb)/	Reverse: CCGGTGTATCATTGATGGAC		
D6S0051i	Telomeric (89 kb)/*BTNL2*	Forward: CATGAAGCTCTGTCGAAAG	57	[27]
	Centromeric (kb)/	Reverse: GTAGAGGCTGACCAGAAACTAC		

Table 2 Exact test of the Hardy-Weinberg proportion of microsatellites.

Marker	Case			Control		
	Deficiency	Excess	Probability	Deficiency	Excess	Probability
M2_2_9	0.02	0.98	0.10	0.29	0.72	0.86
D6S0512i	0.72	0.29	0.81	0.27	0.73	0.11
M2_2_22	0.97	0.02	0.84	0.48	0.52	0.07
M2_2_24	0.48	0.52	0.45	0.35	0.67	0.59
M2_4_25	0.47	0.53	0.38	0.52	0.46	0.41
D6S2820	0.21	0.88	0.34	0.30	0.85	0.43
D6S2818	0.41	0.59	0.24	0.34	0.66	0.71
D6S1100i	0.53	0.44	0.26	0.61	0.37	0.96
G5-11525	0.25	0.75	0.40	0.68	0.32	0.15
DQCARII	0.58	0.41	0.03	0.19	0.82	0.21
D6S0588i	0.64	0.37	0.09	0.20	0.81	0.41
DRA_CA	0.92	0.08	0.26	0.22	0.78	0.13
2-10	0.28	0.73	0.28	0.55	0.48	0.31
D6S2913	0.52	0.49	0.28	0.41	0.58	0.99
D6S0051i	0.30	0.69	0.18	0.12	0.87	0.32

RESULS

The allele frequencies of 15 polymorphic microsatellite markers within the HLA class II region were determined for 375 psoriasis patients and 375 unrelated healthy controls. Table 1 shows the microsatellite marker names, the number of alleles, the most common or significant allele frequency for the cases and controls, the Odds Ratios (with 95% confidence intervals) and the results of Fisher's exact test using the 2 by 2 and the 2 by m contingency tables for the most significant differences between patients and controls.

The results (deficiency, excess and probability) of the exact test of Hardy-Weinberg proportion are listed in Table 2 and were determined for the 15 microsatellite markers by the Markov chain method in order

Table 3 Statistically significant alleles associated with psoriasis vulgaris.

Marker	No. of alleles	Allele	Heterozygosity	Fisher's exact test		Allele Frequency		Odds Ratio	Fisher's exact test	
				exact 2 by 2	*pc*-value	Case	Control	(95% confidence interval)	exact 2 by m	*pc*-value
M2_2_9	7	214	0.612	0.026	1	0.052	0.029	1.83 (1.08-3.09)	0.30	1
D6S0512i	9	238	0.647	0.039	1	0.031	0.054	0.56 (0.33-0.94)	0.14	1
M2_2_22	12	211	0.804	0.026	1	0.056	0.086	0.63 (0.42-0.94)	0.072	1
		215		0.045	1	0.189	0.150	1.33 (1.01-1.74)		
M2_2_24	5	432	0.251	0.014	1	0.071	0.109	0.63 (0.44-0.90)	0.024	0.360
M2_4_25	7	200	0.674	0.0032	0.464	0.552	0.475	1.37 (1.11-1.67)	0.044	0.660
D6S2820	2	198	0.213	0.0063	0.522	0.840	0.889	0.65 (0.48-0.88)	0.0063	0.0945
		194		0.0063	0.522	0.160	0.111	1.53 (1.13-2.06)		
D6S2818	6	136	0.703	0.131	1	0.228	0.264	0.83 (0.65-1.05)	0.45	1
D6S1100i	12	104	0.490	0.0034	0.493	0.112	0.164	0.64 (0.47-0.86)	0.023	0.345
G5-11525	9	215	0.688	0.0030	0.435	0.088	0.137	0.60 (0.43-0.84)	0.0016	0.0240
		237		0.0070	1	0.028	0.058	0.47 (0.28-0.80)		
		209		0.011	1	0.460	0.527	1.31 (1.07-1.61)		
DQCARII	14	134	0.822	0.0011	0.1595	0.280	0.207	1.48 (1.17-1.88)	0.0000056	0.0000840
		136		0.0014	0.203	0.075	0.036	0.47 (0.29-0.74)		
		130		0.015	1	0.061	0.034	0.53 (0.33-0.87)		
		128		0.030	1	0.147	0.109	0.71 (0.52-0.97)		
D6S0588i	9	449	0.674	0.053	1	0.111	0.145	1.36 (1.00-1.84)	0.23	1
DRA_CA	7	148	0.795	0.0010	0.145	0.132	0.196	0.62 (0.47-0.82)	0.0000090	0.0000135
		150		0.0030	0.435	0.349	0.275	1.41 (1.13-1.76)		
		138		0.030	1	0.291	0.240	1.30 (1.03-1.64)		
		156		0.039	1	0.031	0.054	0.56 (0.33-0.94)		
		139		0.048	1	0.115	0.151	0.73 (0.54-0.99)		
2-10	15	148	0.790	0.0025	0.3625	0.035	0.071	0.48 (0.30-0.76)	0.0060	0.0900
		168		0.015	1	0.030	0.056	0.51 (0.31-0.86)		
		162		0.025	1	0.070	0.043	1.69 (1.08-2.64)		
D6S2913	11	224	0.638	0.056	1	0.378	0.427	0.81 (0.66-1.00)	0.54	1
D6S0051i	20	369	0.834	0.031	1	0.061	0.091	0.64 (0.44-0.95)	0.24	1

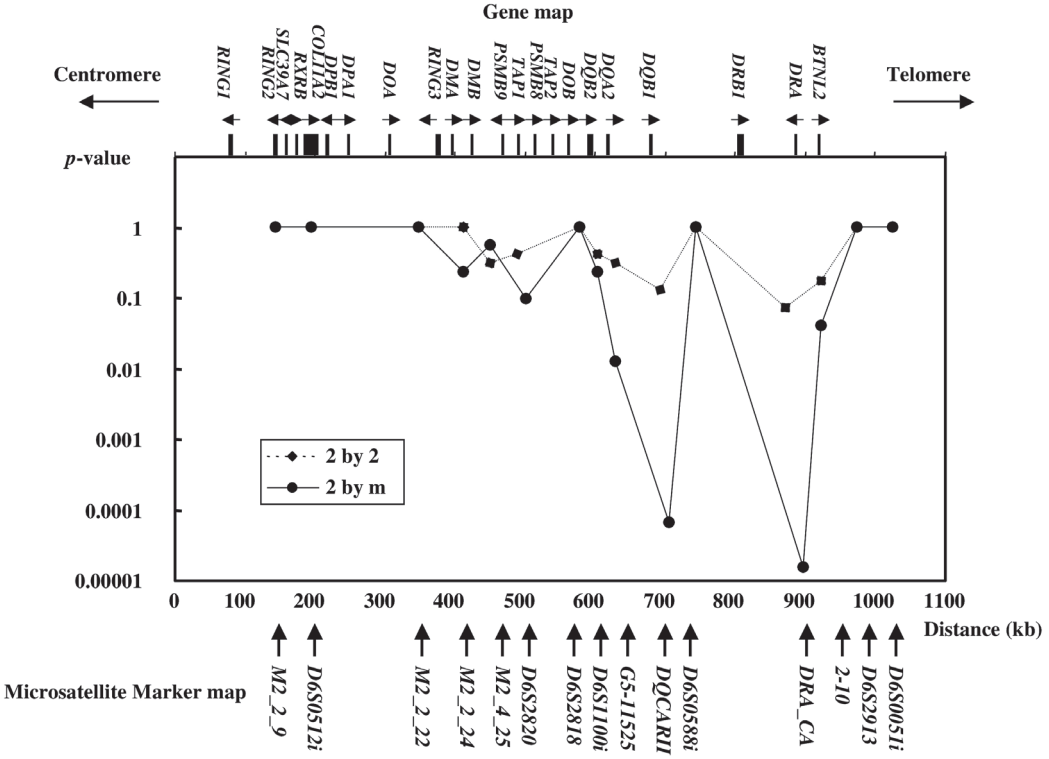

Fig. 1 Map of psoriasis susceptibility loci within the HLA class II region. The *p*-value plot shows the relative differences of probability of association (*pc*-values) obtained for 15 microsatellite frequencies by the Fisher's exact test. The vertical-axis is the log scale of *p*-values and horizontal-axis is the class II genomic region in kb. The rectangles above the *p*-value plot present the gene map of the known genes within the HLA class II region. The arrows below the *pc*-value plot present the location of 15 polymorphic microsatellite markers used in this study. Arrows show the transcriptional orientation of genes.

to estimate the probability of deviation from Hardy-Weinberg proportions [30]. All markers tested were in Hardy-Weinberg's equilibrium in the healthy controls ($p > 0.05$). In contrast, only *DQCARII* significantly deviated from the probability test of Hardy-Weinberg's equilibrium in the cases ($p = 0.03$).

The smallest *p*-values were corrected for multiple comparisons by the Bonferroni adjustment and presented in Table 3 as the corrected *p*-value, *pc*-value. Statistically significant differences with a *pc*-value (2 by m) of less than 0.05 were found at three microsatellite loci. The most significant association with psoriasis vulgaris was found for the *DRA_CA* allele 148 ($pc = 0.0000135$), the second was for the *DQCARII* allele 134 ($pc = 0.0000840$) and the third was for the *G5_11525* allele 215 ($pc = 0.0240$). Statistically significant differences with a *pc*-value (2 by 2) of less than 0.05 was not found.

Figure 1 shows the gene and microsatellite marker map of the HLA class II region (900 kb) and the *p*-value plot, which is the correlation between the *p*-values (exact 2 by 2 and exact 2 by m) and the 15 polymorphic microsatellite markers used for this study. There are at least 22 annotated genes in this genomic region, including the DP, DO, DM, DQ and DR family members of the HLA class II genes. The *p*-value plot in Fig. 1, has revealed two distinct regions of significant differences between the cases and the controls from the microsatellite markers *G5-11525* (36 kb centromeric of the *HLA-DQB1*) to the *2_10* (20 kb telomeric of the *BTNL2* gene) that represent two distinct psoriasis susceptibility loci near the *DQA2*, *BTNL2*, *DRA* and *DRB1* genes and the *DQB1* gene, respectively. For the m alleles at a given marker, 2 by m contingency table was formed, with columns corresponding to allele in case and control subjects. Rows were ordered by increasing allele size, and rows containing cells with expected values. This approach is expected to be powerful when several alleles occur in different frequencies in case and control subjects [31].

DISCUSSION

Recent genome-wide linkage studies and association studies have identified many psoriasis susceptibility loci and putative psoriasis candidate loci on several chromosomes [32]. However, the HLA region located on chromosome 6p21.3 is regarded as one of the major psoriasis susceptibility loci because of the expression of the HLA class I and class II genes whose products interact with $CD8^+$ and $CD4^+$ T cells that have an

Table 4 Published associations between psoriasis and HLA-DR and -DQ.

Year	Reference	Methods	Population	Allele or loci
1981	[13]	serological HLA typing	Japanese	A1, B13, B17, Cw6, DRw6, DR7
1981	[38]	serological HLA typing	Caucasian	B17, Cw6, DR7
1982	[39]	serological HLA typing	Caucasian	DR7
1985	[40]	serological HLA typing	-	B13, B17, B37, Cw6, DR7
1989	[41]	serological HLA typing	South Indian	Bw57, DR7
1991	[42]	serological HLA typing	Japanese	A2-Cw11-Bw46-C2C-BFS-C4A4-C4B2-DRw8 haplotype
1993	[15]	HLA allele typing (genotyping)	-	DRB1*0701/2-DQA1*0201-DQB1*0303 extended haplotype
1996	[43]	HLA allele typing (genotyping)	Caucasian	Cw6-B57-DRB1*0701-DQA1*0201-DQB1*0303 haplotype
1996	[44]	serological HLA typing HLA allele typing (genotyping)	Caucasian	Cw6-DR7-DQA1*0201 haplotype
1998	[45]	HLA allele typing (genotyping)	Japanese	DRB1*1502, DQB1*0601
1998	[46]	HLA allele typing (genotyping)	Taiwanese	DRB1*0701, DRB1*1401
1998	[47]	serological HLA typing HLA allele typing (genotyping)	Thai	A2, B46, B57, DQB1*0303 A1-B57-DRB1*0701-DQA1*0201-DQB1*0303 haplotype A2-B46-DRB1*0901-DQA1*0301- DQB1*0303 haplotype
2000	[48]	serological HLA typing HLA allele typing (genotyping)	Korean	A1, A30, B13, B37, Cw*0602, DRB1*07, DRB1*10, DQA1*02, DQB1*02, DPB1*1701 A30-B13-Cw*0602-DRB1*10-DQA1*01-DQB1*05 haplotype A1-B37-Cw*0602-DRB1*07-DQA1*02-DQB1*02-DPB1*1701 haplotype
2000	[49]	HLA allele typing (genotyping)	Croatian	B13, B17, Cw*0602, DR7
2002	[50]	serological HLA typing	Turkish	A30, Cw3, Cw6, DR7, DR14, DQ8, DQ9
2002	[51]	HLA allele typing (genotyping)	North-eastern	A*01, A*0207, A*30, B*08, B*13, B*4601, B*57, Cw*01, Cw*0602, DRB1*07
			Thai	A*0207-B*4601-Cw*01-DRB1*09-DQB1*0303 haplotype A*01-B*57-Cw*0602-DRB1*07-DQB1*0303 haplotype A*30-B*13-Cw*0602-DRB1*07-DQB1*02 haplotype (associated with type I psoriasis) A*0207, A*30, Cw*01, DRB1*1401 (associated with type II psoriasis)
2004	[14]	HLA allele typing (genotyping)	Chinese Han	DQA1*0104, DQA1*0201
2005	[52]	HLA allele typing (genotyping)	Brazilian	DRB3*02, DRB1*0102 DRB1*0102/DQB1*05, DRB1*0701/DQB1*03
2006	This study	Association study	Japanese	DRB1 subregion

integral role in psoriasis. Many association studies have supported a role for the classical HLA class I and class II genes in psoriasis (see the genes, populations and references in Table 4). However, a number of recent studies have narrowed the psoriasis susceptibility locus down to a region telomeric of the HLA-C gene, the so called PSORS1 loci within HLA class I region [16-19]. This region continues to be investigated vigorously with at least 3 genes suggested as possible candidate genes, *PSORS1C1* (*SEEK1*), *PSORS1C2* (*SPR1*), *PSORS1C3* [33, 34].

The $CD4^+$ T cells that interact with HLA class II molecules are believed to have a role in the onset of psoriasis [6-10]. Therefore, it is likely that the HLA class II gene products are also involved in psoriasis because antigen-presenting cells, such as dendritic cells, B cells and macrophages, use their HLA class II molecules to present foreign peptides to the $CD4^+$ T cells. Indeed, the association between psoriasis and the HLA class II antigens have been reported on numerous occasions (see references in Table 4). For example, HLA DR7 antigen was associated with psoriasis in Japanese-patients [13] and psoriasis in other ethnic groups was associated with some alleles of the HLA-DQA1 and -DQB1 [14], and with the HLA-DRB1*0701/2-DQA1*0201-DQB1*0303 extended haplotype [15]. The most major gene for psoriasis probably locates on so called PSORS1 loci within HLA class I region, but HLA class II gene also play an important role in inducing and maintaining psoriasis.

However, because some studies did not support an association between psoriasis and the HLA class II region [16-18], we decided to examine the HLA class II region by undertaking a case-control association study using only microsatellite markers. To our knowledge, this is the first study using high-density microsatellite markers to identify the psoriasis susceptibility loci within the HLA class II region in Japanese. The results of our study, as summarized by the p-value plot in Fig. 1, revealed two distinct regions of significant difference between the cases and the controls from the microsatellite markers *G5-11525* (36 kb centromeric of the *HLA-DQB1*) to the *2_10* (20 kb telomeric of the *BTNL2* gene), which in turn represent two distinct psoriasis susceptibility loci near the *BTNL2*, *DRA* and *DRB1* genes and the *DQA2* and *DQB1* genes, respectively. However the possibility of two susceptible regions in this region may be not so high, because recombination intensity between *DQB1* and *DRB1* loci was low. Further studies including different populations and more high density markers are being carried out to confirm the presence of two independent loci.

Although we have not genotyped the *HLA-DQB1* or *DRB1* genes in our patients and control groups, it is noteworthy that at least 18 studies have associated psoriasis with DR7 or DRB*0701 and at least 11 studies have associated psoriasis with DQB1*02 or DQB1*0303 (see Table 4 for references and a summary of the studies). This connection between previous studies and our present study highlights the usefulness of applying microsatellite markers to experimentally confirm and support the findings of previous studies on the association of candidate genes with disease.

The psoriasis susceptibility region encompassing the genes around the microsatellite marker *DRA_CA* in this study also highlights *BTNL2* as a possible non-HLA class II candidate disease gene. To our knowledge, *BTNL2* has not been previously considered as a candidate psoriasis gene. However, *BTNL2* has a polymorphic locus and it is a member of the immunoglobulin superfamily implicated as a costimulatory molecule involved in T cell activation [35]. *BTNL2* binds to a putative receptor on activated T cells and functions to inhibit the proliferation of T cells [36]. In addition, the polygenic immune disorder sarcoidosis has been associated with a truncated splice site mutation in *BTNL2* [37]. In this regard, *BTNL2* sequence variation between psoriasis patients and healthy controls warrants future investigation.

In conclusion, we have found a psoriasis susceptibility region using microsatellite markers within the HLA class II genomic region that encompasses 242 kb from the microsatellite *G5-11525* (36 kb centromeric of the *HLA-DQB1*) to the microsatellite *DRA_CA* (28 kb telomeric of the *BTNL2* gene). This association between the microsatellites and disease was determined by statistical analysis of allelic distribution (Fisher's exact test) and deviation from the Hardy-Weinberg's equilibrium. The results suggest that there are psoriasis susceptibility genes located within the HLA class II region apart from the HLA class I region as previously described.

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