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Letter to the Editor

Genetic polymorphisms in the *IL22* gene are associated with psoriasis vulgaris in a Japanese population

Keywords:
Genetic polymorphisms; *IL22* gene;
Psoriasis vulgaris; Japanese population

To the Editor,

Psoriasis vulgaris (PsV) is an inflammatory skin disease histologically characterized by epidermal hyperplasia, inflammatory cell infiltration and vascular changes in which T-lymphocytes and associated cytokines play a central role [1]. A dysregulated cutaneous immune response occurs in genetically susceptible individuals and the features of inflammation are characterized by tumor necrosis factor (TNF)-α dependence and exaggerated helper T cell 1 (Th1) and 17 (Th17) activation. Interleukin (IL)-22 is an IL-10 family cytokine member produced by Th17 cells and plays a role in the promotion of inflammation and tissue repair at barrier surfaces [2]. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation [3], and circulating IL-22 levels are significantly higher in psoriatic patients than in normal subjects [4,5]. Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that is basically considered to be a Th-2 type disease. However, a recent study suggests a possible role of Th17 cells in AD [6]. The study has shown that the number of Th17 cells is increased in the peripheral blood and acute lesional skin of AD and that IL-17 and IL-22 synergistically enhance the production of IL-8 from keratinocytes [6]. Since there are few genetic studies of the polymorphisms of *IL22* in populations of Asian and European ancestry, we conducted association studies to assess whether *IL22* gene variants contribute to the susceptibility to PsV or AD in a Japanese population.

We recruited a total of 236 patients with PsV (mean age 53, 11–85 years, male:female ratio = 1.0:2.8), and all subjects were diagnosed by clinical and histopathological findings. A total of 916 patients with AD (mean age 30, 3–77 years, male:female ratio = 1.0:2.2) and 844 controls (mean age 50, 20–75 years, male:female ratio = 1.0:1.3) were recruited as described [7]. Patients with AD were diagnosed according to the criteria of Hanifin and Rajka, and control subjects were never diagnosed with AD or PsV. All individuals were unrelated Japanese and gave written informed consent to participate in the study. The study was approved by the ethical committees at the Institute of Physical and Chemical Research (RIKEN), the University of Tokyo and the Jikei University School of Medicine. Genomic DNA was prepared in accordance with standard protocols.

We resequenced the *IL22* gene regions with genomic DNA from 36 individuals and identified a total of 32 polymorphisms (Table 1). We next examined the linkage disequilibrium (LD) between identified SNPs (Fig. S1). Pairwise LD coefficients D' and r^2 were calculated among the 24 SNPs with minor allele frequencies (MAF) of greater than 5% using Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/). We selected a total of 11 tag SNPs for association studies using tagger in Haploview 4.2, and the 11 tag

Table 1
Frequencies of polymorphisms of the *IL22* gene in a Japanese population.

	SNP[a]	Allele	Location	MAF[b]	NCBI[c]
1	−2479	T/C	5′-Flanking region	0.319	rs57947370
2	2378	C/T	5′-Flanking region	0.278	rs11177135
3	−2375	T/C	5′-Flanking region	0.014	rs77156535
4	−2161	G/A	5′-Flanking region	0.319	rs7139027
5	−1905	A/G	5′-Flanking region	0.278	rs2227472
6	1810	G/A	5′-Flanking region	0.319	rs2227473[d]
7	−1588	T/A	5′-Flanking region	0.292	rs2227476[d]
8	−1536	C/T	5′-Flanking region	0.028	rs2227477
9	−1394	T/C	5′-Flanking region	0.431	rs2227478[d]
10	−1114	C/T	5′-Flanking region	0.111	rs2227480
11	−1113	C/T	5′-Flanking region	0.278	rs2227481
12	−1089	AT/del	5′-Flanking region	0.000	rs35774195
13	1075	AT repeat	5′-Flanking region	0.000	rs10699698
14	−948	T/A	5′-Flanking region	0.292	rs2227483
15	−701	C/T	5′-Flanking region	0.111	rs2227484[d]
16	−485	C/T	5′-Flanking region	0.278	rs2227485[d]
17	201	A/G	5′-Flanking region	0.014	rs141972126
18	393	T/A	Intron 1	0.264	rs17224704[d]
19	708	A/G	Intron 2	0.278	rs2227491
20	1254	A/C	Intron 3	0.375	rs2046068[d]
21	1366	G/T	Intron 3	0.028	rs3782552
22	1945	G/C	Intron 4	0.014	
23	2178	G/C	Intron 4	0.361	rs1179251[d]
24	2385	T/C	Intron 4	0.097	rs1179250[d]
25	2449	C/A	Intron 4	0.097	rs1179249
26	2611	T/A	Intron 4	0.278	rs1012356
27	3270	C/A	Intron 4	0.278	rs2227501
28	3531	A/G	Intron 4	0.278	rs2227503
29	3635	T/C	Intron 4	0.014	rs976748
30	5301	A/T	3′-Flanking region	0.097	rs2227508[d]
31	5433	CT/del	3′-Flanking region	0.014	
32	5697	A/T	3′-Flanking region	0.444	rs1182844[d]

[a] Numbering according to the genomic sequence of IL-22 (NC_000012.11). Position 1 is the A of the initiation codon.
[b] MAF (minor allele frequencies) in the screening population (N = 36).
[c] NCBI, number from the dbSNP of NCBI (http://www.ncbi.nlm.nih.gov/SNP/).
[d] SNPs were genotyped in this study. Genotyping of the 11 SNPs in IL-22 were performed by the TaqMan™ allele-specific amplification (TaqMan-ASA) method (Applied Biosystems) and multiplex-PCR based Invader assay (Third Wave Technologies).

Table 2
Summary of association results for the *IL22* gene.

dbSNP allele 1/2 position	Subject	Genotype			Total	Frequency			MAF	1 vs. 2 alleles		
		11	12	22		11	12	22		*P* value	OR	95% CI
rs2227473	PsV	130	94	10	234	0.556	0.402	0.043	0.244	0.0095	1.38	1.08–1.76
G/A	AD	604	274	32	910	0.664	0.301	0.035	0.186	0.79	1.02	0.86–1.21
−1810	Control	550	262	28	840	0.655	0.312	0.033	0.189			
rs2227476	PsV	136	88	9	233	0.584	0.378	0.039	0.227	0.0051	1.43	1.11–1.84
T/A	AD	633	256	26	915	0.692	0.280	0.028	0.168	0.84	1.02	0.85–1.21
1588	Control	578	242	23	843	0.686	0.287	0.027	0.171			
rs2227478	PsV	98	101	37	236	0.415	0.428	0.157	0.371	0.00014	1.51	1.22–1.88
T/C	AD	470	363	82	915	0.514	0.397	0.090	0.288	0.60	1.04	0.90–1.20
−1394	Control	429	353	59	841	0.510	0.420	0.070	0.280			
rs2227484	PsV	187	41	7	235	0.796	0.174	0.030	0.117	0.30	1.18	0.86–1.64
C/T	AD	735	166	12	913	0.805	0.182	0.013	0.104	0.74	1.04	0.83–1.29
−701	Control	674	154	7	835	0.807	0.184	0.008	0.101			
rs2227485	PsV	92	105	38	235	0.391	0.447	0.162	0.385	0.024	1.27	1.03–1.57
C/T	AD	289	447	179	915	0.316	0.489	0.196	0.440	0.84	1.01	0.89–1.16
−485	Control	258	418	163	839	0.308	0.498	0.194	0.443			
rs17224704	PsV	146	82	7	235	0.621	0.349	0.030	0.204	0.023	1.35	1.04–1.75
T/A	AD	653	242	18	913	0.715	0.265	0.020	0.152	0.54	1.06	0.88–1.27
393	Control	595	225	22	842	0.707	0.267	0.026	0.160			
rs2046068	PsV	104	102	26	232	0.448	0.440	0.112	0.332	0.0018	1.42	1.14–1.78
A/C	AD	501	342	71	914	0.548	0.374	0.078	0.265	0.69	1.03	0.89–1.20
1254	Control	458	329	53	840	0.545	0.392	0.063	0.259			
rs1179251	PsV	124	85	26	235	0.528	0.362	0.111	0.291	0.54	1.07	0.86–1.34
G/C	AD	454	372	90	916	0.496	0.406	0.098	0.301	0.75	1.02	0.89–1.18
2178	Control	408	351	82	841	0.485	0.417	0.098	0.306			
rs1179250	PsV	134	84	17	235	0.570	0.357	0.072	0.251	0.23	1.15	0.91–1.46
T/C	AD	487	354	74	915	0.532	0.387	0.081	0.274	0.77	1.02	0.88–1.19
2385	Control	439	338	66	843	0.521	0.401	0.078	0.279			
rs2227508	PsV	187	41	7	235	0.796	0.174	0.030	0.117	0.11	1.31	0.94–1.81
T/A	AD	737	162	12	911	0.809	0.178	0.013	0.102	0.32	1.12	0.90–1.40
5301	Control	694	141	7	842	0.824	0.167	0.008	0.092			
rs1182844	PsV	83	108	43	234	0.355	0.462	0.184	0.415	0.54	1.07	0.87–1.31
A/T	AD	327	428	158	913	0.358	0.469	0.173	0.407	0.61	1.04	0.91–1.19
5697	Control	310	391	140	841	0.369	0.465	0.166	0.399			

SNPs captured 24 of the 24 alleles with $r^2 > 0.92$. We genotyped the 11 SNPs in *IL22* gene by the TaqMan® SNP Genotyping Assays (Life Technologies).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2013.04.002.

The results for genotype frequencies of the 11 tag SNPs in the case and control group are shown in Table 2. All 11 SNPs were in Hardy–Weinberg equilibrium, and we then compared differences in the allele frequencies by using a contingency χ^2 test. Odds ratios (ORs) with 95 percent confidence intervals (95% CI) were calculated. We applied Bonferroni corrections, the multiplication of *P* values by 11, the number of tag SNPs. In the association study, corrected *P* values of less than 0.05 were judged to be significant.

We identified significant associations between *IL22* gene variants and PsV under the allelic model (rs2227478; corrected $P = 0.0015$; OR = 1.51, rs2046068; corrected $P = 0.020$; OR = 1.42) (Table 2). Weger et al. evaluated a total of 10 common polymorphisms of the *IL22* gene in an Austrian population and reported no association between the variants and chronic plaque psoriasis [8]. Recent genome-wide association studies (GWASs) of PsV revealed a total of 36 psoriasis-associated regions in individuals of European ancestry, and the regions encode several proteins engaged in the TNF, IL-23 and IL-17 signaling pathways [9]. However, the *IL22* locus did not contain the susceptible regions identified by European GWASs. Since heterogeneous association signals are often seen among different ethnic populations, further genetic studies using Asian populations seem to be needed for further focusing attention of polymorphisms of the *IL22* gene in this disease. Although a validation study in an independent population is needed, our findings imply that *IL22* variants play a role in the pathogenesis of PsV in the Japanese population.

A number of features shared by AD and PsV including a Th17 cell pathway and common gene loci, were reported [10], but we did not find a significant association in this study between *IL22* SNPs and susceptibility to AD ($P = 0.32$–0.84) (Table 2). Since a recent study has shown a role of Th17 cells in exacerbation of AD [6], genetic variants of *IL22* might influence the exacerbation of the disease rather than susceptibility to it.

In summary, our data suggested important genetic influences of the polymorphisms in *IL22* on the susceptibility to PsV but not to AD in the Japanese population. Higher concentrations of IL-22 are observed in the peripheral blood and tissues of patients with PsV [2,4,5], and expression of IL-22 and IL-22-regulated genes in keratinocytes is reduced by antipsoriatic therapies [4]. Further evaluation of the clinical significance of the susceptible *IL22* gene variants would help better understand the etiology of PsV.

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Letter to the Editor

HLA-B*58:01 strongly associates with allopurinol-induced adverse drug reactions in a Japanese sample population

To the Editor,

Allopurinol, an inhibitor of xanthine oxidase, is widely used for the treatment of hyperuricemia associated with chronic gout, acute uric acid nephropathy, recurrent uric acid stone formation, certain enzyme/blood disorders, and cancer chemotherapy. It has been shown that severe cutaneous adverse drug reactions (ADRs) caused by allopurinol were strongly associated with HLA-B*58:01 in a Han Chinese sample population [1]. Odds ratio (OR) for the association of HLA-B*58:01 with allopurinol-induced severe cutaneous ADR in this population was 580.3 and 95% CI was 34.4–9780.9. Although the relationship between HLA-B*58:01 and allopurinol-induced Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) has subsequently been studied in European and Japanese patients, the association was much weaker than that reported in Han Chinese patients [2,3]. The association study in Japanese patients was examined in only a limited number of allopurinol-induced ADR cases. We therefore conducted a case-controlled study to determine HLA types associated with allopurinol-induced ADR in a Japanese sample population.

All patients were recruited from Shimane University Hospital between 2010 and 2012. These included 7 patients with allopurinol-induced ADR (3 patients with SJS and 4 patients with erythema exudativum multiforme (EEM)) and 25 patients who had been receiving allopurinol for more than 3 months without drug eruption. Diagnoses of SJS were made according to the diagnostic criteria established by Roujeau [4]. Allopurinol-induced ADR was diagnosed using medical histories, indicating that symptoms occurred within 3 months of starting allopurinol administration, and the symptoms resolved upon the withdrawal of allopurinol. If the patients were given other drugs, in addition to allopurinol, 3 months prior to the appearance of symptoms, a drug-induced lymphocyte stimulation test and a patch test were performed with allopurinol/oxypurinol. Allopurinol-induced ADRs were diagnosed by the single medication of allopurinol in 4 of the 7 patients (No. 1, 3, 4, 7), by the positive allopurinol-induced lymphocyte stimulation test in 2 of the 7 patients (No. 2, 6), and by the positive patch test with allopurinol in the patient No. 5. The indication for which drug had been prescribed was the level of hyperuricemia detected in all the patients. All patients were interviewed by investigators regarding the histories of their biological parents and grandparents, and were confirmed as being ethnically Japanese. This study was approved by the ethics committee of Shimane University Faculty of Medicine (approval no. 221).

Low-resolution HLA typing with DNA extracted from peripheral blood was performed using the reverse sequence-specific oligonucleotide with polymerase chain reaction (PCR-rSSO) method [5]. High-resolution HLA-B genotyping was determined using the polymerase chain reaction-sequence based typing (PCR-SBT) method [5]. Statistical analysis of the differences in each allele frequency among patients with ADR and control subjects was performed by Fisher's exact test. The strength of association was estimated by calculating the OR. The OR was determined using Haldane's modification, which adds 0.5 to all cells to accommodate