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A Possible Association of Single-Nucleotide Polymorphisms in the Alpha-Helix Coiled-Coil Rod Homologue Gene with Psoriasis in a Japanese Population

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Abstract The α-helix coiled-coil rod homologue (*HCR*) gene is known to be associated with the susceptibility of various races to psoriasis vulgaris (PV). This study aimed to analyze single-nucleotide polymorphisms (SNPs) in *HCR* in Japanese PV patients and investigate the association between these SNPs and PV in the Japanese population. The PV group comprised 75 PV patients from our hospital; the control group, 75 healthy individuals. By using the direct sequencing method, we analyzed SNPs in *HCR* at exons 4, 13/14, and 18, genetic polymorphisms in which are associated with susceptibility to PV in Caucasian and Taiwanese populations. We then investigated the association between these SNPs and PV. The association between PV and *HCR* -404*T, *HCR*-1802*T, and *HCR*-2406*T was statistically significant. Other SNPs were not statistically significant at 5% level. Compared with the previous study in Finnish and Taiwanese populations, in our study, the frequencies of SNPs at the risk alleles were lower in both PV patients and healthy controls. *HCR*-2406*G showed the most frequent SNPs (17.3% vs 4.0% in controls; odds ratio = 5.03, 95% confidence interval = 1.37-18.5; p = 0.015). These observations suggest that *HCR* may be a candidate gene for susceptibility to PV in the Japanese population.

Key words: psoriasis, *HCR*, SNPs

Introduction

Psoriasis vulgaris (PV) is a common chronic inflammatory skin disorder. The prevalence rate of PV is less than 0.1% in the Japanese population,[1] compared to approximately 2% in Caucasian populations.[2] This inflammatory disease is considered to be multifactorial, caused by the concerted action of multiple genetic and environmental factors. The pathogenesis of this disease involves both genetic predisposition and immune response disorder.[3] PV is clinically characterized by pink-to-reddish papules and plaques with thick silver-white scales; the lesions particularly occur in the intertriginous regions such as the elbow and knee.[4] Histopathological examination of the affected areas in PV has shown keratinocyte hyperproliferation and incomplete differentiation, angiogenesis, inflammatory leukocyte infiltration into the epidermis, and papillary dermis.[5]

The fundamental causes of PV have not been completely elucidated thus far. Previous studies have shown a strong association between HLA-Cw*0602 and PV among various races, but the degree of association differed among the populations.[6-9] It has been reported that HLA-Cw6 and HLA-Cw7 are

strongly associated with PV in the Japanese population.[10]

PSORS1 is the locus of the candidate genes within the class 1 region of the major histocompatibility locus antigen (HLA) cluster. It contains *HCR* (Online Mendelian Inheritance in Man [OMIM] 605310), *OTF3* (OMIM 164177), *TCF19* (OMIM 600912), and *CDSN* (OMIM 602593) in addition to HLA, particularly HLA-Cw*0602 (Fig. 1). Of the above candidate genes, *HCR* is located at 110 kb telomeric to the HLA cluster and consists of 18 exons. It has been shown that there are 27 SNPs in *HCR* (Table 1), and it is considered that those at positions 386 (exon 4), 404 (exon 4), 1802 (exon 14), and 2406 (exon 18) are the most important SNPs for the development of psoriasis. These SNPs are more frequent in PV patients than in healthy controls.[13–17] SNPs in *HCR* in the Japanese population have been analyzed in a previous study, but the report did not provide any detailed data such as those regarding the frequency of each SNP.[12] Thus, we analyzed the association between PV and genetic polymorphisms at the risk alleles of *HCR* in the Japanese population to elucidate the genetic factors involved in susceptibility to PV.

Patients and methods

DNA samples

We consecutively recruited 75 unrelated Japanese patients with PV (53 males and 22 females; mean age, 55.7 ± 20.3 years) from the outpatients and inpatients at the Department of Dermatology, Yamaguchi University Hospital. All the patients provided their written informed consent prior to participation according to the protocols approved by the research ethics board of the hospital. The healthy controls comprised 75 unrelated disease-free individuals (50 males and 25 females; mean age, 35.5 ± 15.5 years); they also provided their written informed consent prior to participation. Venous blood from the PV patients and healthy controls was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes. Genomic DNA was isolated from 10 mL of whole blood by using QIAamp DNA Blood Maxi Kit (QIAGEN K.K., Tokyo, Japan).

Genotyping

HCR (accession no. XM_041760) consists of 18 exons, and its mRNA contains 2580 bp. We amplified exons 4, 13/14, and 18 by using the following primer sets: 5′-CTATGTTTATGC CCTCAACTA-3′ (forward) and 5′-ACTGCCC TCCACAATAC-3′ (reverse) for exon 4; 5′-GC TGGGTGATTTCTCCTGACT-3′ (forward) and 5′-ACCGGCCATATGCTGTTTC-3′ (reverse) for exon 13/14; and 5′-AGCCCTGTTTCCTC TGTAACC-3′ (forward) and 5′-CCCAAACA TTTCCAAAGCTG-3′ (reverse) for exon 18.[18] Each polymerase chain reaction (PCR) was performed in a total volume of 50 μL, including 20 μL of 10× buffer (containing 100 mM Tris-HCl [pH 8.3], 500 mM KCl, and 15 mM $MgCl_2$; Takara Inc., Shiga, Japan), 16 μL of 200 μM deoxynucleotide triphosphates (an equimolar mixture of dATP, dCTP, dGTP, and dTTP; Takara Inc.), 10 pmol of each primer, 500-ng template DNA, and 0.5 U of Ex Taq DNA polymerase (Takara Inc.). The PCR conditions were as follows: denaturation at 94 ℃ for 30 s, annealing at 52 ℃-58 ℃ for 30 s, and extension at 72 ℃ for 60 s through 30 cycles. The PCR products were electrophoresed in 1.2% agarose gel containing 0.5 mg/mL ethidium bromide. The gels were run in 1× Tris-acetate-EDTA (TAE) buffer for 60 min at 8 V/cm. Subsequently, the PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN K.K.). The purified products were directly sequenced using fluorescent-dye-terminator cycle chemistry (ABI PRISM; PE Biosystems, Foster City, CA, USA). Sequences were obtained from both ends by using the same primers as used in PCR and read on a 3730 DNA sequencer (ABI PRISM; PE Biosystems).

Statistics

Differences in allele frequencies between the PV patients and healthy controls were assessed by performing Fisher's exact test on 2 × 2 contingency tables. Odds ratios (ORs), 95% confidence intervals (CIs), and significance values were calculated by SPSS. We could not calculate the OR and 95% CI for an empty cell in the table.

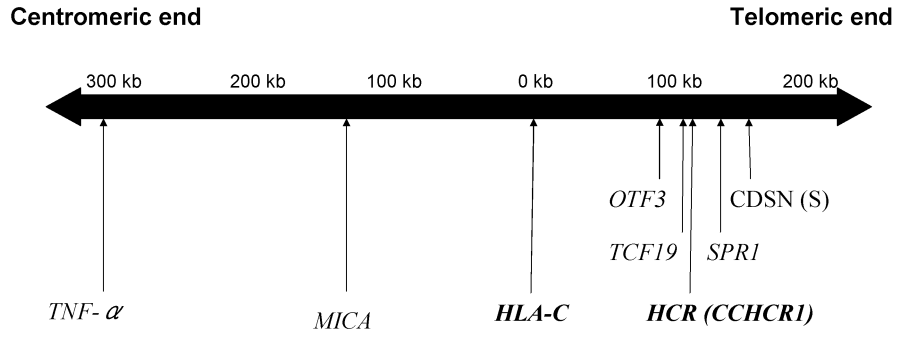

Fig. 1 Map of *PSORS1*.

Table 1 Single-nucleotide polymorphisms in *HCR* and the corresponding amino acid changes

Exon	Base[a]	Polymorphism	Amino acid change
4	384	G→A	Arg→Gln
	386	C→T	Arg→Trp
	404	C→T	Arg→Trp
	556	C→T	No change
	571	C→G	Ser→Arg
5	614	T→A	Leu→Glu
6	850	C→G	No change
	904	A→C	Glu→Asp
9	1178	G→A	Ala→Thr
10	1328	C→T	Arg→Trp
	1329	G→A	Arg→Gln
	1354	C→T	No change
	1364	C→T	No change
11	1426	G→A	No change
	1471	C→A	No change
13	1579	T→C	No change
	1601	C→T	No change
14	1716	A→G	Lys→Arg
	1802	G→T	Gly→Cys
15	1959	G→A	Arg→Gln
16	1990	G→A	No change
	1996	G→T	Gln→His
17	2254	A→G	No change
	2257	A→T	No change
	2277	C→T	Ala→Val
	2278	A→G	No change
18	2406	C→G	Ser→Cys

[a]According to GenBank accession no. AB_029343.

Results

Table 2 shows the results of each analysis of SNPs in *HCR*. In the PV group, we found SNPs at *HCR*-404*T (9.3% [=7/75] vs 0% [=0/75], p = 0.013, OR: not applicable); *HCR* -1802*T (9.3% [=7/75] vs 0% [=0/75], p = 0.013, OR: not applicable); and *HCR*-2406*G (17.3% [=13/75] vs 4.0% [=3/75], p = 0.015, OR = 5.03 [1.37-18.5]), compared with the healthy controls. However, no statistically significant difference was found between *HCR*-384*A (32.0% [=24/75] vs 20.0% [=15/75], p = 0.136 [not significant], OR: 1.88, 95% CI [0.98-3.97]); *HCR*-386*T (6.7% [=5/75] vs 0% [=0/75], p = 0.058 [not significant], OR: not applicable); *HCR*-556*T (0% [=0/75] vs 4.0% [=3/75], p = 0.244 [not significant], OR: not applicable); *HCR*-571*G (50.6% [=38/75] vs 64.0% [=48/75], p = 0.137 [not significant], OR: 0.57, 95% CI [0.30-1.11]); *HCR*-1579*T (65.3% [=49/75] vs 66.7% [=50/75], p = 1.00 [not significant], OR: 0.94, 95% CI [0.48-1.35]; *HCR*-1601*T (26.7% [=20/75] vs 33.3% [=25/75], p = 0.476 [not significant], OR: 0.72, 95% CI [0.36-1.47]); or *HCR* -1716*G (16.0% [=12/75] vs 20.0% [=15/75], p = 0.671 [not significant], OR: 0.76, 95% CI [0.33-1.76]), compared with the healthy controls. The results showed that the frequencies of the 3 SNPs at *HCR*-404*T (exon 4), *HCR*-1802*T (exon 14), and *HCR*-2406*G (exon 18) were significantly high ($p < 0.05$), whereas the frequencies of other SNPs at exons 4, 13/14, and 18 were not significant at 5% level.

Discussion

The present study showed a strong association between genetic polymorphisms in *HCR* and susceptibility to psoriasis in the Japanese population. The association between *HCR* and PV was first reported in 2000.[11] In 2002, 4 risk alleles of *HCR* for psoriasis were identified, which were *HCR*-386*T (exon 4), *HCR*-404*T (exon 4), *HCR*-1802*T (exon 14), and *HCR*-2406*G (exon 18).[13] Therefore, we focused on exons 4, 14, and 18 of *HCR* in Japanese PV patients.

As shown in Table 1, SNPs at *HCR*-386*T and *HCR*-404*T cause a functional arginine (R)-to-tryptophan (W) residue change; one at *HCR*-1802*T causes a functional glycine (G)-to-cysteine (C) residue change; and an SNP at *HCR*-2406*G causes a functional serine (S)-to-cysteine (C) residue change. C has a characteristic feature, owing to which disulfide bonds (i.e., S-S bonds) can be formed between 2 C side chains in proteins.[19] A study on

Table 2 Distribution of SNP frequencies at exons 4, 13, 14, and 18 of *HCR* in Japanese PV patients and healthy controls

Exon	Allele[a]	Patients	Controls	OR	95% CI	p value[b]
4	*HCR*-384*A	32.0% (24/75)	20.0% (15/75)	1.88	0.89—3.97	0.136 (NS[d])
	HCR-386*T	6.7% (5/75)	0.0% (0/75)	NA[c]	-	0.058 (NS)
	HCR-404*T	9.3% (7/75)	0.0% (0/75)	NA	-	0.013
	HCR-556*T	0.0% (0/75)	4.0% (3/75)	NA	-	0.244 (NS)
	HCR-571*G	50.6% (38/75)	64.0% (48/75)	0.57	0.30—1.11	0.137 (NS)
13	*HCR*-1579*T	65.3% (49/75)	66.7% (50/75)	0.94	0.48—1.35	1.00 (NS)
14	*HCR*-1601*T	26.7% (20/75)	33.3% (25/75)	0.72	0.36—1.47	0.476 (NS)
	HCR-1716*G	16.0% (12/75)	20.0% (15/75)	0.76	0.33—1.76	0.671 (NS)
	HCR-1802*T	9.3% (7/75)	0.0% (0/75)	NA	-	0.013
18	*HCR*-2406*G	17.3% (13/75)	4.0% (3/75)	5.03	1.37—18.5	0.015

[a]According to GenBank accession no. AB_029343.

[b]determined by Fisher's exact test.

[c]NA = not applicable (we could not calculate the values for empty cells in the 2 × 2 table).

[d]NS = not significant.

Table 3 Comparison of high-risk alleles of psoriasis-susceptible *HCR* used in the present study with those used in previous studies

Allele	Japanese (our data)		Finnish[a]		Taiwanese[b]	
	patients	controls	patients	controls	patients	controls
HCR-404*T	9.3% (7/75)	0.0% (0/75)	42.0% (42/100)	19.4% (18/93)	18.7% (43/230)	6.3% (13/206)
HCR-1802*T	9.3% (7/75)	0.0% (0/75)	4.0% (4/100)	3.2% (3/93)	17.8% (41/230)	6.8% (14/206)
HCR-2406*G	17.3% (13/75)	4.0% (3/75)	12.0% (12/100)	6.5% (6/93)	20.4% (47/230)	9.7% (20/206)

[a]Asumalahti, K.: *Hum Mol Genet* 9:1533-1542, 2000.
[b]Chang, Y.T.: *Br J Dermatol* 150:1104-1111, 2004.

another gene has indicated that aberrant disulfide bonds cause structural alteration and consequent instability of the gene.[20] Therefore, it is conceivable that formation of a disulfide bond(s) induced the structural alteration of *HCR* and functional disturbance of the protein.

In the present study, we found the highest frequency of SNPs at *HCR*-2406*G; this result was consistent with that of a previous study, wherein high frequencies of SNPs were found at *HCR*-2406*G in Finnish (12.0% vs 6.5%) and Taiwanese (20.4% vs 9.7%) populations.[12] The frequency of SNPs at *HCR*-1802*T in Japanese and Taiwanese PV patients was significantly high, whereas no statistically significant difference was observed in the frequency of SNPs between Finnish PV patients and healthy controls (4.0% vs 3.2%). No SNPs were found at *HCR*-386*T, *HCR*-404*T, and *HCR*-1802*T in Japanese healthy controls. To confirm the differences in the frequencies of SNPs at the alleles, it is necessary to collect a larger sample than the one used in our study. Furthermore, Asumalahti *et al.* studied *HCR* in the members of 419 families from 6 populations and suggested a 4-SNP haplotype, *HCR**WWCC* (*HCR*-386*T, *HCR*-404*T, *HCR*-1802*T, and *HCR*-2406*G), located at *PSORS1* as the putative susceptibility gene for psoriasis.[13] We could not compare our data to theirs, because we did not have any data of families for haplotype analysis.

The association between the HLA region and PV can be explained by 2 mechanisms. First, there is a possibility that the association of *HCR* variants with PV might be due to their strong linkage disequilibrium with *HLA-C*, particularly HLA-Cw*0602, which might be the main candidate gene of *PSORS1*. Second, it has been suggested that *HCR* is one of the regulators of steroidogenesis and vitamin D_3 metabolism via vitamin D receptor.[21] Since active forms of vitamin D_3, such as 1α, 25 dihydroxyvitamin D_3, have been used in psoriasis therapy, it is possible that *HCR* can control keratinocte differentiation by exerting an indirect effect on vitamin D_3-vitamin D receptor complex. Thus, there is a possibility that *HLA-C* and *HCR* are independently associated with susceptibility to PV.

Currently, we are investigating the relationship between *HLA-C* and SNPs in *HCR*, using a larger number of Japanese PV patients.

In conclusion, the 2 genes *HCR* and *HLA-C* may play important roles-either interdependent or independent-in the development of PV.

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