# Specific Restriction Fragment Length Polymorphism on the HLA-C Region and Susceptibility to Psoriasis Vulgaris

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In psoriasis vulgaris, the HLA class I Cw6 specificity has previously been recognized as the most commonly associated antigen serologically. This study was carried out to investigate whether or not the gene controlling the susceptibility to psoriasis vulgaris existed on the HLA, especially the HLA-C region. At first, we analyzed the restriction fragment length polymorphism (RFLP) of 13 patients with psoriasis vulgaris and 6 healthy controls who were all positive for at least one allele of HLA-Cw6. To characterize RFLP in psoriasis patients who did not have HLA-Cw6, 12 patients and 10 healthy controls who had HLA-Cw7 were also examined. Southern hybridization of genomic DNA demonstrated that DNA polymorphisms of the HLA-C antigen gene could not be found in any psoriasis vulgaris patient whether HLA-Cw6 or Cw7. However, a 4.5 kb BamHI fragment and a 3.1 kb PstI fragment were lacking in some healthy controls who had either HLA-Cw6 or Cw7. This study suggests that the presence of RFLP in the HLA-C gene is associated with psoriasis vulgaris. These specific fragments may help predispose individuals to psoriasis vulgaris, or may be essential for the development of the disease. *J Invest Dermatol 90:402–405, 1988*

The etiology of psoriasis vulgaris is still unknown. In 1972, the first articles reporting an association between HLA antigens and susceptibility to psoriasis were published by Russell et al [1] and White et al [2]. During the past 10 yr, many studies have been carried out to clarify the etiology of psoriasis vulgaris, and a relationship between HLA and this disease has been confirmed [3]. The association of HLA antigens HLA-A1, B13, Bw16, B17, B37, Cw6, Cw7, and DR7 with psoriasis vulgaris were recognized [3]. Table I summarizes the data from our laboratory and demonstrates the association between these HLA antigens and psoriasis vulgaris.

However, the findings that not only single HLA alloantigen types but also other HLA isotypes from different genes were recognized in one disease, may be questionable. Therefore, we again statistically analyzed the association between HLA-Cw6, which was the most associated antigen with relative risk of 24.0 (Table I), and the other associated HLA types. From the result, the possibility of linkage disequilibrium between HLA-Cw6 and the other associated HLA alloantigen type was recognized statistically [5]. In our family study, all patients had the same haplotype: namely, HLA-A3, B13, Cw6, and DR7 [6]. The associated HLA-A, B, and DR antigens in psoriasis vulgaris may be secondary to a primary increase of HLA-Cw6, judging from both population and family studies [7].

If HLA-Cw6 is really associated with psoriasis vulgaris, a high frequency of Cw6 in the population may lead to a high incidence of the disease clinically. From data of both the frequency of HLA-Cw6 in the population and the clinical incidence of psoriasis vulgaris in 12 countries or races, a significant relationship ($r = 0.69$; $t = 3.02$; $p < 0.02$) was clearly recognized [8].

Although HLA-Cw6 is the most associated HLA antigen in psoriasis vulgaris, an increased frequency of HLA-Bw16(Bw39) associated with HLA-CW7, which was the secondary associated HLA-C antigen, was also observed [7]. Although many mechanisms of HLA disease associations have been postulated [3], in psoriasis the possibility of linkage between locus C and the gene controlling the susceptibility to this disease or a receptor for a virus or other organisms, rather than locus B or D/DR, was suggested [9].

Recently, Southern hybridization analysis was developed to define polymorphisms at the genomic levels. In any population, a given stretch of DNA may have variations in nucleotide sequences that affect restriction sites for certain endonucleases [10]. They are known as restriction fragment length polymorphisms (RFLPs). For example, a case of digestion with BamHI was shown in Fig 1. The DNA molecule is digested with BamHI when the nucleotide sequence of G-G-A-T-C-C is the restriction site. In the case of A, there are three restriction sites. Therefore, two fragments will be recognized after BamHI digestion. However, if there is any variation in nucleotide sequence at the restriction site, the DNA molecule is not cut after BamHI digestion. Thus, in the case of B, a nucleotide sequence in one of three sites is different from the case of A. Namely, next to G is G in the case of A, but in the case of B, next to G is C. Therefore, at the site, the DNA molecule is not digested with BamHI. Only one fragment is gained in the case of B. The polymorphisms at the genomic level in a disease can be defined using the technique to investigate such a difference of restriction fragments with a certain endonuclease. Essential and important

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Abbreviations:
HLA: Human leukocyte antigen
kb: kilobase
RFLP: restriction fragment length polymorphism
SDS: sodium dodecyl sulfate
SSC: sodium chloride-sodium citrate

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**Table I.** HLA Frequencies and Psoriasis Vulgaris

| HLA | Psoriasis Vulgaris | Controls | Relative Risk | $\chi^2$ |
|---|---|---|---|---|
| A1[a] | 7.84% | 1.05% | 8.6 | 38.72 |
| B13[a] | 8.58% | 4.00% | 2.2 | 9.20 |
| Bw16[a] | 16.04% | 6.11% | 2.6 | 27.09 |
| B17[a] | 0.37% | 1.69% | 0.2 | 2.69 |
| B37[a] | 10.45% | 1.05% | 15.2 | 60.97 |
| Cw6[b] | 25.96% | 1.37% | 24.0 | 155.28 |
| Cw7[b] | 17.71% | 2.11% | 10.0 | 83.19 |
| DR7[c] | 11.54% | 1.02% | 12.7 | 49.51 |

Relative risk is defined as how much more likely a person with a specific HLA antigen is to get a disease than a person who does not have that HLA antigen [3]. These HLA typing data of all unrelated Japanese patients with psoriasis vulgaris were examined at our laboratory from 1975 to 1985. HLA frequencies of Japanese controls were referred to the data of the Eighth International Histocompatibility Workshop [4].

[a] Two hundred sixty-eight patients and 949 controls.

[b] One hundred four patients and 950 controls.

[c] One hundred four patients and 884 controls.

questions raised by this method are whether there is any specific enzyme or protein which is associated with the disease, and whether there are DNA probes for detecting the genes that encode the specific enzyme or protein.

In this study, restriction fragment length polymorphisms at the genomic level were analyzed between psoriatic patients and healthy individuals who had the HLA-C alloantigen types associated with this disorder by the Southern hybridization technique to investigate whether or not possibilities of the gene controlling the susceptibility to psoriasis vulgaris existed on the HLA, especially the HLA-C region.

## MATERIALS AND METHODS

**Subjects** Twenty-one unrelated Japanese patients with psoriasis (nine patients with HLA-Cw6, eight with HLA-Cw7, and four with both) and sixteen healthy control subjects (six with HLA-Cw6 and ten with HLA-Cw7) were examined in this study.

**Probes** The cDNA clone (pDP001 encoding the HLA-B7 mRNA [11]) was used as the probe for detecting the HLA class I antigen genes, locus A, B, and C. Four types of PvuII fragments of the HLA-C genomic clone P42 [12] were used as the probe for detecting the region around the HLA-C gene: The Pvu II 1.7-kilobase (kb) fragment obtained from the HLA-C genomic clone P42 encodes the leader peptide, the $\alpha_1$ domain, and the $\alpha_2$ domain region (Fig 2). The Pvu II 1.2-kb fragment (the 3′-flanking DNA of the HLA-C gene), the Pvu II 1.0-kb fragment (the 3′-untranslated region of the HLA-C gene), and the Pvu II 0.6-kb fragment (part of the $\alpha_3$ domain and the transmembrane region of the HLA-C gene) were used in this study.

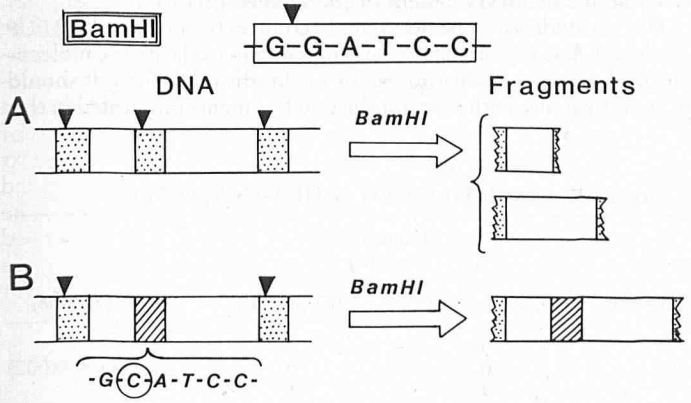


**Figure 1.** Restriction fragment length polymorphisms (RFLPs). *Arrows* indicate the restriction site with BamHI. In *B*, *circle* indicates a different nucleotide sequence.

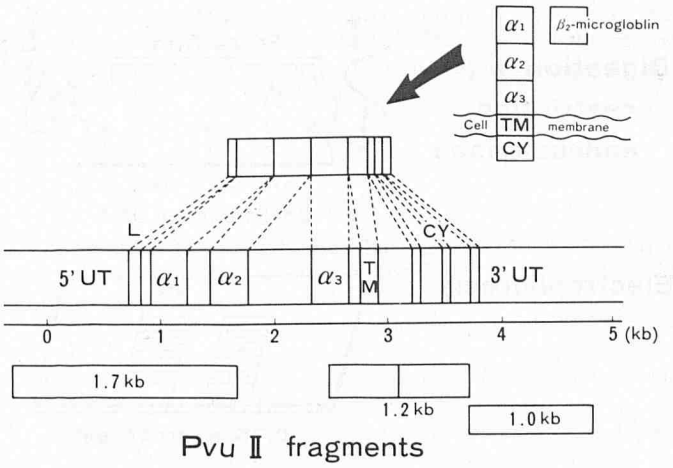


**Figure 2.** The map of the HLA-C antigen gene and the PvuII fragments. *L:* the leader peptide; *5′UT:* the 5′-untranslated region; *TM:* the transmembrane; *CY:* the cytoplasmic region; *3′UT:* the 3′-untranslated region.

**Methods** DNAs were isolated from peripheral lymphocyte ($2 \times 10^7$ cells) from patients and controls using the proteinase K-SDS phenol method [13]. Each isolated DNA sample was digested with the restriction endonucleases EcoRI, PstI, or BamHI (20–30 U/7.5–10 $\mu$g of DNA) (Fig 3). Digested genomic DNA (7.5 $\mu$g) were subjected to electrophoresis on 0.7% agarose gel and transferred to nitrocellulose paper [14,15]. After transfer, the filters were prehybridized in the hybridization mixture (0.9 M NaCl, 90 mM Tris HCl [pH7.4], 6 mM EDTA, 0.5% SDS, 5× Denhardt's solution, and heat-denatured 100 $\mu$g/ml salmon sperm DNA) at 65°C for 4 h. Radioactively labeled cDNA inserts from cDNA clone and DNA were added to a concentration of $1 \times 10^6$ cpm/ml and $1 \times 10^4$ cpm/ml in the hybridization mixture, respectively, and incubated with the filters at 65°C for 16 h. The filters were washed in 3× SSC, (0.1% SDS for 5 min at room temperature), 2×SSC (0.1% SDS for 5 min at room temperature), 1×SSC, (0.1% SDS for 20 min at 68°C), and 0.2× SSC (0.1% SDS for 20 min at 68°C). The restriction fragments were probed with $^{32}$P-labeled cDNA probes, and the filters were exposed to XRP-5 Kodak film with an intensifying screen at −70°C.

## RESULTS

**Southern Hybridization Analysis with the PvuII 1.7-kb Fragment from the 5′-Half Region of the HLA-C Gene and the cDNA Clone pDP001 after BamHI Digestion** One of the autoradiograms is shown in the Fig 4 in this series. Four patients with psoriasis and nine control subjects with HLA-Cw7 were examined in the autoradiogram. The BamHI 4.5-kb fragment was found in all four patients, but was absent in four of nine healthy controls. As shown in Table II the BamHI 4.5-kb fragment was found in all 21 patients, both those with HLA-Cw6 and those with HLA-Cw7. However, this fragment was absent in 5 of 16 control subjects; these five all had HLA-Cw7. Similar results were obtained when the cDNA clone pDP001 for HLA-B7 was used as a probe. That is, the BamHI 4.5-kb fragment was absent in the same five healthy individuals.

**Southern Hybridization Analysis with the PvuII 1.7-kb Fragment of the HLA-C Gene after PstI Digestion** When the PstI restriction enzyme was used for digestion, significant differences between patients and control subjects were recognized in hybridization band patterns by using the PvuII 1.7-kb fragment of the HLA-C gene as a probe. The results of autoradiogram of blot hybridization analysis of seven psoriatic patients and nine controls is shown in Fig 5. The PstI 3.1-kb fragment was recognized in all seven patients; however, this fragment was absent in two controls with HLA-Cw6 and was recognized in the others with either HLA-Cw6 or -Cw7. Finally, in this series, the band patterns in all patients

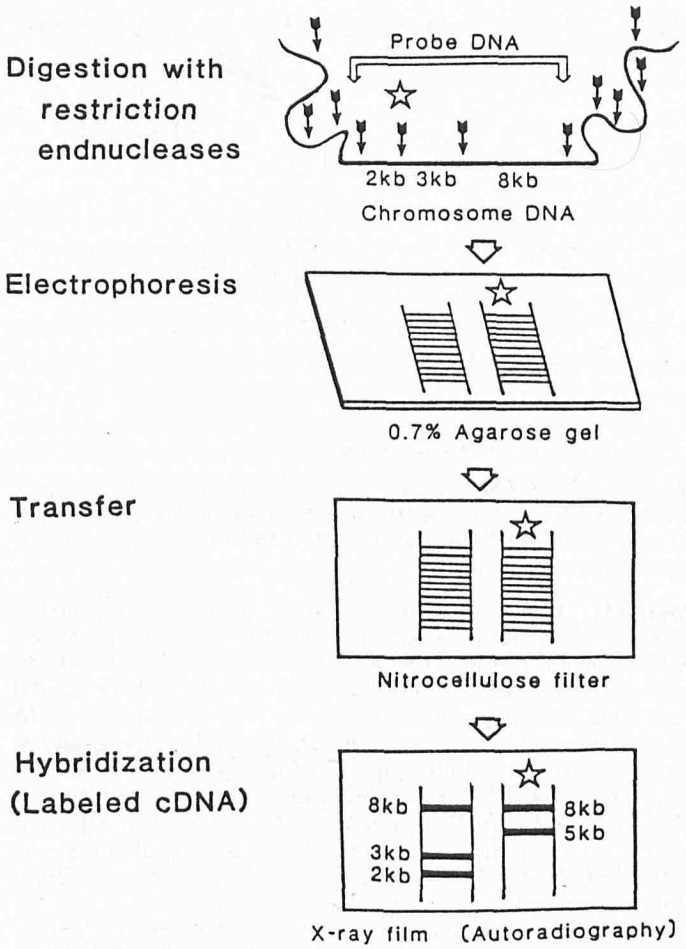


**Figure 3.** The Southern hybridization technique. The *star* shows the difference of a restriction site. If the DNA molecule is not cut at the site of the star, only one fragment of 5 kb is recognized in autoradiogram. Two fragments (2 and 3 kb) are recognized when the DNA molecule is cut at the site of the star.

with HLA-Cw6 and HLA-Cw7 were identical. The 3.1-kb fragment was absent in 4 of 16 controls (two controls had HLA-Cw6 and others had HLA- Cw7) (Table II). However, this fragment was found in all 21 patients with either HLA-Cw6 or HLA- Cw7. The HLA-B7 cDNA clone (pDP001) could not distinguish between hybridization band patterns in patients and controls after PstI digestion.

**Southern Hybridization Analysis with the PvuII 1.7-kb Fragment of the HLA-C Gene after EcoRI Digestion** Hybridization band patterns in all cases (seven patients and nine controls), after treatment with EcoRI, were completely similar.

**Southern Hybridization Analysis Using Other Probes** Hybridization using other gene probes, PvuII 1.2 kb, PvuII 1.0 kb, and

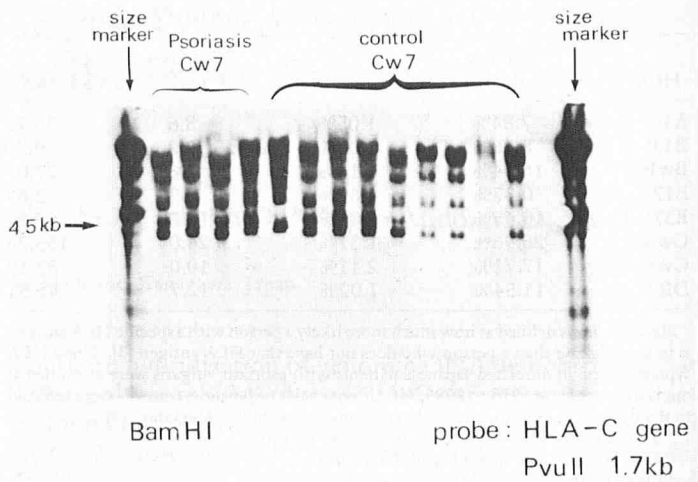


**Figure 4.** Autoradiogram of blot hybridization analysis using the PvuII 1.7-kb fragment of the HLA-C gene as a probe of psoriasis patients and controls after BamHI digestion. The BamHI 4.5-kb fragment is absent in four healthy controls who had HLA-Cw7. *Arrow* indicates the BamHI 4.5-kb fragment. Size marker was a mixture of HindIII- and (EcoRI + HindIII)-digested λDNA.

PvuII 0.6 kb, did not reveal different hybridization patterns between patients and controls after EcoRI, PstI, and BamHI digestion.

## DISCUSSION

It is well known that nucleotide sequences of the HLA class I alloantigen genes are very similar to each other. Therefore, significant differences in DNA band patterns cannot be easily found by genomic Southern hybridization analysis for the HLA class I antigen genes. In this study, significant differences between patients with psoriasis and controls were not found around the HLA-C antigen gene and the HLA class I antigen genes at the genomic level. However, detailed and extensive analysis of RFLP clearly demonstrated significant differences in hybridization patterns between psoriasis patients and healthy controls.

Recently, three restriction fragments, specific for narcoleptic patients but not healthy controls, were reported by Southern hybridization analysis using a DQβ cDNA clone [16]. The association of these specific fragments with the susceptibility to narcolepsy and its molecular mechanism were speculated.

In our data, all 21 HLA-Cw6 and/or HLA-Cw7 patients with psoriasis vulgaris had the BamHI 4.5-kb ($\chi^2 = 10.5$; $p < 0.002$) and the PstI 3.1-kb ($\chi^2 = 8.8$; $p < 0.005$) fragments when the 5′-half region DNA from the HLA-C gene was used as a probe, whereas in some healthy controls these fragments were lacking (Table II). These results imply that these specific HLA gene fragments are essential for the development of psoriasis vulgaris.

Further studies may be necessary to confirm the presence of RFLP on the HLA-C region in psoriasis vulgaris and to clarify the molecular mechanism in the pathogenesis of this disorder. First, it should be investigated whether or not the two fragments implicated in this

**Table II.** Restriction Fragment Frequencies in Psoriasis Vulgaris Patients with HLA-Cw6 or HLA-Cw7, or Both

| Probe: PvuII 1.7-kb Fragment | | Psoriasis Vulgaris (n = 21) Cw6 (n = 9) | Cw6/7 (n = 4) | Cw7 (n = 8) | Controls (n = 16) Cw6 (n = 6) | Cw7 (n = 10) | $\chi^2$ ($p$ value) |
|---|---|---|---|---|---|---|---|
| BamHI 4.5 kb fragment[a] | (+) | 9 | 4 | 8 | 6 | 5 | |
| | (−) | 0 | 0 | 0 | 0 | 5 | 10.5 ($p < 0.002$) |
| PstI 3.1 kb fragment[b] | (+) | 9 | 4 | 8 | 4 | 8 | |
| | (−) | 0 | 0 | 0 | 2 | 2 | 8.8 ($p < 0.005$) |

[a] The Southern hybridization analysis, using pDP001 as the probe, was the same.
[b] The Southern hybridization analysis, using pDP001 as the probe, was no different between psoriatic patients and healthy controls.

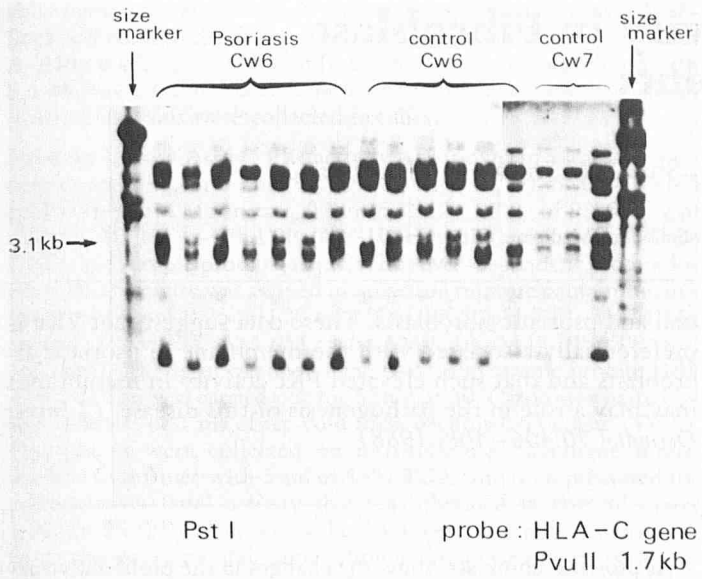


**Figure 5.** Autoradiogram of blot hybridization analysis using the PvuII fragment (1.7 kb) after PstI digestion. The 3.1-kb fragment was absent in two controls who had HLA-Cw6. *Arrow* indicates the PstI 3.1-kb fragment. Size marker was a mixture of HindIII-and (EcoRI + HindIII)-digested λDNA. The bands in the fourth psoriasis patient are a little bit faint, but the original autoradiogram, where in a higher resolution is obtained, indicates the presence of 3.1-kb band.

study are in fact the same, inasmuch as they were obtained using two different endonucleases; however, it would not be unexpected to find multiple different fragments necessary for development of psoriasis, given the heterogenity of this disorder. Next, it should be clarified whether or not these specific fragments are recognized in all psoriasis patients with any serologic HLA-C specificities except HLA-Cw6. In this study, the same two specific fragments were also recognized in all psoriasis patients with HLA-Cw7. This finding may suggest that the same hybridization band patterns will be recognized in patients, even those who have different serologic specificities from HLA-Cw6.

It will also be necessary to perform family studies — not to find the linkage between the HLA specificity and the disease, such as in the previous HLA studies, but to investigate which RFLPs on the HLA-C region in psoriasis vulgaris are caused from hereditary or acquired factors. These studies should include both some relatives with the same disorder and some patients not in the family at all.

Finally, there is a possibility that these two fragments, BamHI 4.5 kb and PstI 3.1 kb, do not come from the HLA-C gene region, but from another (e.g., HLA-A or B gene region) cross-hybridized region with the PvuII 1.7-kb fragment under stringent conditions because the HLA-B7 cDNA clone can also detect the BamHI 4.5-kb fragment.

These studies present only the first step in characterizing the molecular mechanism of the genetic basis for psoriasis vulgaris, and the genetic basis of the linkage of certain class I HLA specificities with this disease.

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