

## Evidence that HLA-Cw6 Determines Early Onset of Psoriasis, Obtained Using Sequence-specific Primers (PCR-SSP)

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Psoriasis vulgaris has previously been shown to associate with certain HLA alleles. HLA-Cw6 is considered to be the primary association, based on calculations of relative risk after serological typing. This association is reportedly more pronounced in early- than in late-onset psoriasis. We performed a PCR-based typing with sequence-specific primers, which has been shown to give a more complete result than serology. Two hundred and one unrelated patients with psoriasis, with a mean age of 40 years, and 77 healthy controls were typed. Two thirds (67%) of the patients were positive for one or two copies of the allele, while the corresponding figure for the control group was 12%. A significant peak for age at onset of 21 or younger was seen for the Cw6 carriers. For patients older than 21 at onset, the frequency of Cw6 was significantly lower; e.g. for patients with an age at onset between 30 and 35 the frequency was comparable to the level of the control group. The high frequency of Cw6 among patients with an age at onset of 21 or younger is in agreement with data of other groups. In comparison with this age-at-onset group the frequency of Cw6 is sharply reduced among patients with an age at onset of 22 years or older, which contrasts with earlier studies. This may reflect differences between population groups but may also be due to the higher sensitivity of the PCR-based HLA-Cw6 typing method. In view of these findings, we suggest that psoriasis is a genetically determined disease, in which the additional presence of HLA-Cw6 is associated with the characteristic of early onset.

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Psoriasis is one of several immunologically mediated diseases that show significant HLA-association. Many serologically defined class I and class II antigens or alleles have been reported to associate with the disease. The most consistently reported relative risk has been with HLA-Cw6 in many populations (1). It has therefore been suggested that the primary association is with HLA-Cw6 and that other associations are due to linkage disequilibrium, i.e. a tendency of specific combinations of alleles at two linked loci to occur together due to a low recombination fraction between them. The fact that the association is by no means perfect may, on the other hand, indicate that the gene for HLA-C itself is in linkage disequilibrium with a susceptibility gene. This feature of an incomplete association with a specific HLA-antigen is shared with other HLA-associated diseases and, until now, no significant linkage for psoriasis to the HLA locus has been reported (2).

The relative risk with Cw6 for psoriasis vulgaris differs in various populations: Caucasian: 9.1, (3) 11.0 (the latter only for patients with age at onset before 40 years of age) (4);

Finnish: 10.7 (5); and Japanese: 8.22 (6). All these studies have been carried out using serological techniques with typing antisera. However, these methods have some disadvantages. Especially the HLA-C locus has been persistently difficult to type; more than 20% type blank in most populations (7). This is partly due to a lack of proper typing reagents but may also depend on the fact that the HLA-C antigens are expressed to a lower extent (10%) on the cell surfaces than HLA-A and B (7).

More accurate PCR-based typing methods have therefore been developed. One of these was used in a linkage study of eight psoriasis families (8). These new methods are suitable for larger population studies. In the "phototyping" method described by Bunce et al. (9), sequence-specific primers (SSP) are used. The principle of the PCR-SSP is that the primer pair amplifies only a specific allele, due to exact matching to the nucleotide sequence specific for the serological specificity. The presence or absence of an amplified product is determined after agarose-gel electrophoresis. Apparently, this method did not fail to identify the alleles in any sample, either the serologically defined or the undefined ones (9).

We performed a typing with regard to HLA-Cw6 on a Swedish psoriasis population and related the results to the age at onset of the patients.

### MATERIAL AND METHODS

#### *Assumptions, definitions and statistical analysis*

Two hundred and one unrelated psoriasis patients were selected from our large Swedish material presented earlier (10). From a total of 22,000 questionnaires sent out to the members of the Swedish Psoriasis Association, data was collected from 14,008 families. To check the diagnosis given by the proband, all persons typed in this study were also examined by the same physician (AI).

DNA was obtained from 201 randomly selected probands. Only one member of each family was selected, as one expects siblings to share haplotypes to some extent, thus leading to a certain bias. The median age of these patients was 40 years, with a lower quartile of 34.0 and a higher quartile of 45.5, and with a maximum of 56 years and a minimum of 23 years. This is well above the expected peak of age at onset reported previously by several groups (4, 11), including ours (12). This fact decreases the overrepresentation of patients with early onset, although, to a certain degree, this will always be the case.

The typing of healthy family members included 58 siblings and 146 parents from 73 families with two unaffected parents.

Age at onset, as defined by us earlier (12), is regarded as the age at which the patient, or his or her relatives, becomes aware that the patient has a skin disease, which at that time or later is diagnosed as psoriasis.

When age-at-onset distribution curves are analysed, it is important to compare groups of patients with equal mean age at the time of examination, since psoriasis may start at any age. We therefore compared the fraction of age at onset for the Cw6 positives and negatives with the information about age at onset given by 2,547

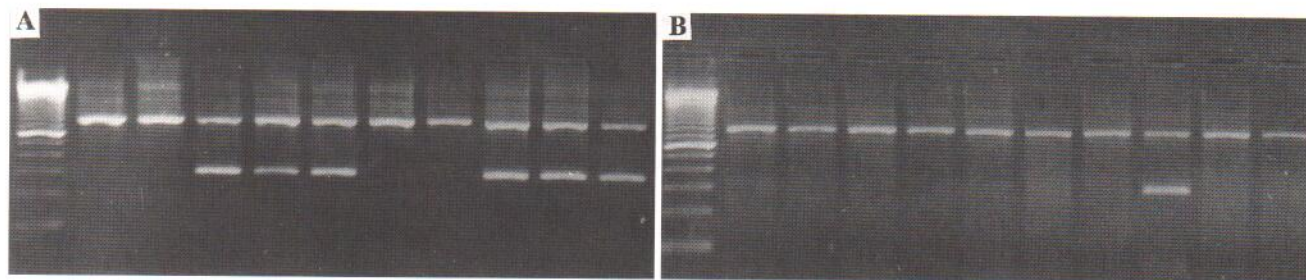


Fig. 1. Significantly higher frequency of Cw6 among psoriatics than among healthy controls. PCR-SSP analysis of presence of Cw6 among (A) 10 psoriatics and (B) 10 healthy controls. The Cw6 allele is seen as a 300 base pair fragment in addition to the 700 base pair control fragment.

psoriatic patients with an age between 35 and 45, thus a mean age as in the typed group.

A 95% confidence interval has been calculated assuming a binominal distribution.

#### DNA analysis and PCR-conditions

Venous blood samples anticoagulated with EDTA were obtained from 201 unrelated patients with psoriasis, 204 unaffected relatives and 77 healthy controls of Swedish origin. Genomic DNA was extracted from blood using standard procedures.

The sequence-specific primer PCR was carried out according to the procedures described by Bunce et al. (9). The primers identifying HLA-Cw6 were used together with amplification control primers, which gave rise to a 796 base pair fragment from the third intron of HLA-DRB1 (13). Both primer pairs were designed based on published nucleotide sequences (14). To obtain optimal results, the PCR conditions were modified according to two serologically determined positive HLA-Cw6 controls, kindly donated by Dr Lena Sandberg, Sahlgrenska Hospital. The PCR-products were separated by electrophoresis in 2.0% agarose (Nusieve) gels, containing 2.5 µl ethidium bromide in 75 ml gel. Buffer consisted of 1xTBE. The fragments were separated at 85 V for 30 min and visualised using UV illumination (Fig. 1).

## RESULTS

Two hundred and one unrelated patients with psoriasis vulgaris were examined with regard to HLA-Cw6, using a PCR-SSP method. Among these, 66.7% showed the presence of at least one Cw6 allele (the method does not discriminate between homo- and heterozygosity). In the control group, consisting of 77 healthy blood-donors, the frequency of HLA-Cw6 was 11.7%. On the other hand, among healthy family members the frequency of Cw6 was elevated. Thirty-eight per cent of healthy siblings expressed Cw6 and 44.5% of healthy parents.

We related the age at onset of the probands to the presence of HLA-Cw6. Fig. 2 shows the age-at-onset curves for the Cw6 carriers and for the non Cw6 carriers. The relative fraction of patients in each year at onset is presented. Between 7.5 and 22.5 years of age at onset there is a significant peak for the patients carrying Cw6. This peak is not seen in patients lacking the Cw6 allele.

We then compared the age-at-onset curve for the HLA-Cw6 positives and negatives with the curve based on information given by 2,547 psoriatics with a mean age of 40 (also Fig. 2). The curve for the typed psoriatics has a similar shape to the curve for all probands.

Among the 201 typed probands 74% had an age at onset of less than 22 years of age, and in this group 77% were shown to be Cw6-positive. On the other hand, 26% had an age at

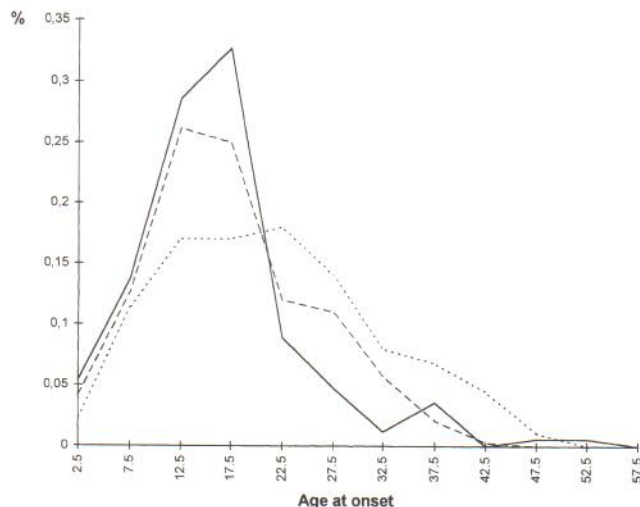


Fig. 2. The relative distribution of age at onset for Cw6 positive (—) and Cw6 negative (...) psoriasis patients is shown. For comparison, the age at onset distribution for an age-matched group of 2,547 psoriatics (---) is given. Compared to a larger material, the Cw6 carriers represent the majority of the peak at young ages, whereas the Cw6 negatives contribute to the peak seen for later age at onset.

onset of 22 years or older. In this group only 38% showed Cw6 positivity (Table I).

We then divided the patients into groups according to their age at onset (Fig. 3). The fraction of Cw6 for each age group and a 95% confidence interval are shown. From 0–20 years there is a high and almost stable proportion of patients carrying Cw6: 72–80%. After that its frequency decreases rapidly, and at an age of onset between 30 and 35 it has fallen to a frequency comparable with that of our control group (12.5 vs 10.7).

Table I. Presence of Cw6 alleles in unrelated psoriasis patients (n=201)

Age at onset	<22	>22
Total no. of patients	148	53
%	74	26
Fraction of patients with Cw6	114	20
%	77	38

$p < 0.00001$ .

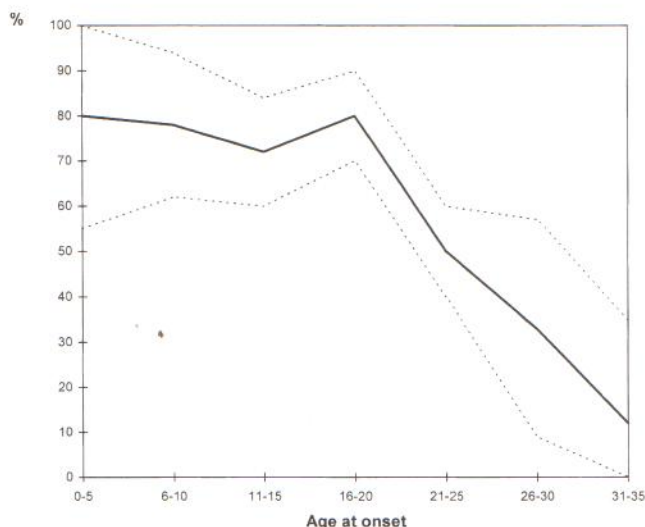


Fig. 3. Rapid diminution of the association with Cw6 for psoriatics with an age at onset older than 21. Presence of Cw6 among patients with different ages at onset (—) with a 95% confidence interval (...).

## DISCUSSION

The HLA antigens are genetic markers for biologic individuality and are encoded by highly linked genes on chromosome 6p. They are considered to act as receptors for foreign antigens and present them to the proper subpopulation of T-cells. Like many other HLA-associated diseases, psoriasis has an unknown aetiology. The disease also has a hereditary pattern and immunological abnormalities are present. Linkage to the HLA locus has therefore been sought, so far without success (2).

The significance of the HLA association still remains an enigma. It is obvious that a certain HLA type is not an absolute requirement for the development of psoriasis. As mentioned in our prior publication on age at onset in psoriasis (12), we find it possible that the HLA alleles influence or define some characteristics in the disease, such as age at onset, not necessarily defining a specific type of psoriasis.

The most persistently reported relative risk has been with Cw6, and a weaker association has been reported with B13, B17, Cw7, DR4 and DR7, among others. The frequency of these antigens is lower compared to Cw6 among the psoriatics and they are also more frequent in the general population, resulting in a lower relative risk. As previously mentioned, the other associations are considered to be due to linkage disequilibrium (15). The strength of linkage disequilibrium in HLA indicates that Cw6 may not be the primary association. The high frequency of Cw6 among psoriatics may also reflect population stratification, illustrated by populations in which Cw6 accounts for a much smaller proportion of psoriasis patients (e.g. Japan) (6).

The presence of HLA Cw6 has previously been related to the age at onset of patients with psoriasis (16, 4), giving evidence for a higher frequency among those with early onset. Henseler & Christophers (17) characterized two types of psoriasis, one with early onset (<40 years) with higher frequency of Cw6 (73.8%), and one with late onset (>40 years), expressing Cw6 in 31.8%. It is probable that there is a heterogeneity in psoriasis (18). Whether this means that there

is one type that is determined by a gene in the vicinity of the HLA locus or that the different types are due to primary genes on other locations and that HLA antigens only influence the age at onset is not yet known.

Our material presented a significant peak for the Cw6 carriers at an age at onset of 21 years or younger. In comparison with the larger material, it seemed obvious that the Cw6 carriers represent the majority of the peak at young ages. The Cw6 negatives contribute to the peak seen for later age at onset. Comparing the shape of the curves for the typed psoriatics with that of the larger material, we find our typed samples representative of this age-group.

Seventy-four per cent of the patients had an age at onset lower than 22 years, indicating an overrepresentation of young ages. Of these, 77% expressed the Cw6 antigen, in agreement with previous findings. The overrepresentation at young ages could not influence the finding that the group with age at onset over 22 years only presented Cw6 in 38%. As mentioned above, a similar low frequency after 40 years at onset has been reported (17). It is striking that even at such an early onset as 22 years, the frequency of Cw6 decreases rapidly towards levels shown in the control population.

When the relative risk is estimated, it is of great importance to choose a proper control group. The difference in frequency of HLA-Cw6 among blood-donors and healthy siblings illustrates this fact. The higher frequency of Cw6 among healthy siblings may be due to ascertainment bias and mendelian inheritance. However, a way of understanding and estimating a patient's individual risk of developing the disease obviously requires further investigation.

We conclude that psoriasis is a genetically determined disease, most probably heterogenous, and suggest that the presence of Cw6 associates strongly with early onset.

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