Linkage Analysis of Human Leukocyte Antigen (HLA) Markers in Familial Psoriasis: Strong Disequilibrium Effects Provide Evidence for a Major Determinant in the HLA-B/-C Region

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Summary

Although psoriasis is strongly associated with certain human leukocyte antigens (HLAs), evidence for linkage to HLA markers has been limited. The objectives of this study were (1) to provide more definitive evidence for linkage of psoriasis to HLA markers in multiplex families; (2) to compare the major HLA risk alleles in these families with those determined by previous case-control studies; and (3) to localize the gene more precisely. By applying the transmission/disequilibrium test (TDT) and parametric linkage analysis, we found evidence for linkage of psoriasis to HLA-C, -B, -DR, and -DQ, with HLA-B and -C yielding the most-significant results. Linkage was detectable by parametric methods only when marker-trait disequilibrium was considered. Case-control association tests and the TDT identified alleles belonging to the EH57.1 ancestral haplotype as the major risk alleles in our sample. Among individuals carrying recombinant ancestral haplotypes involving EH57.1, the class I markers were retained selectively among affecteds four times more often than among unaffecteds; among the few affected individuals carrying only the class II alleles from the ancestral haplotype, all but one also carried Cw6. These data show that familial and "sporadic" psoriasis share the same risk alleles. They also illustrate that substantial parametric linkage information can be extracted by accounting for linkage disequilibrium. Finally, they strongly suggest that a major susceptibility gene resides near HLA-C.

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Introduction

Psoriasis (MIM 177900) is a common, human leukocyte antigen (HLA)–associated skin disease affecting 1%–2% of the Caucasian population (Christophers and Sterry 1993). There is increasing evidence that immune-system activation plays a central role in the keratinocyte hyperproliferation that is an obvious feature of psoriasis. The marked efficacy of cyclosporine A treatment (Ellis et al. 1991) has focused attention on actively dividing T cells that accumulate in psoriatic lesions (Morganroth et al. 1991). In addition, a variety of other antigen-presenting, effector, and inflammatory cells are also present in psoriatic skin (de Boer et al. 1994), and keratinocytes themselves may modulate immune functions via a variety of mechanisms (Barker et al. 1991).

Candidate psoriasis-susceptibility loci recently have been identified in the HLA region, as well as on chromosomes 2p, 4q, 8q, 16q, 17q, and 20p (Tomfohrde et al. 1994; Matthews et al. 1996; Nair et al. 1997; Trembath et al. 1997). Independent confirmation has been reported for the HLA region (Nair et al. 1997; Trembath et al. 1997), 17q (Tomfohrde et al. 1994; Nair et al. 1997), and 20p, for which two groups (Nair et al. 1997; Trembath et al. 1997) reported evidence for linkage to markers within a 10-cM region. All disease genes remain to be identified. Consistent with expectations for a multifactorial disorder (Lander and Schork 1994), both HLA association and familial aggregation increase markedly with decreasing age at onset (Henseler and Christophers 1985).

Numerous HLA phenotypes, corresponding to alleles at the HLA-A, -B, -C, -DRB1, -DQA1, and -DQB1 loci, have been associated with psoriasis. Among these, the class I phenotypes Cw6 and B57 have been most consistently reported worldwide (see Elder et al. 1994). The alleles that correspond to these Cw6 and B57 phenotypes are in linkage disequilibrium in normal individuals and compose the proximal class I end of an ancestral haplotype, termed "EH57.1" (Degli-Esposti et al. 1992).

The class II side of the EH57.1 ancestral haplotype contains the DRB1*0701, DQA1*0201, and DQB1*03032 alleles, which also display strong associations with psoriasis (Schmitt-Egenolf et al. 1993). A recent analysis of ancestral haplotypes has implicated the class I end of this haplotype to be the region that contains the major HLA-linked determinant of psoriasis susceptibility (Schmitt-Egenolf et al. 1996). Two additional HLA-B alleles (B13 and B37) known to be in linkage disequilibrium with Cw6 have been associated with psoriasis (see Elder et al. 1994). Nonetheless, many affected individuals do not carry Cw6 or any detectable portion of the EH57.1 ancestral haplotype.

These studies suggest at least three possible genetic etiologies for psoriasis. First, more than one allele at a single locus could confer susceptibility. Second, alleles at more than one locus within the HLA region could confer susceptibility. Third, the "true" disease locus could be linked to, but separate from, one or more known HLA genes (Thomson and Bodmer 1977). The available HLA-association data favor the third possibility, but they do not rule out the other two possibilities.

In a recent genome scan performed in our laboratory (Nair et al. 1997), evidence of linkage to HLA was present over a rather broad interval and was not as robust as might be expected, given the strong HLA associations characteristic of psoriasis. Thus, the objectives of the current study were threefold: (1) to use linkage-disequilibrium studies to provide stronger evidence for linkage to HLA; (2) to determine whether the same HLA risk alleles are involved in familial and in sporadic psoriasis; and (3) to localize the disease allele more precisely.

Subjects and Methods

Juvenile-onset probands (<40 years of age) were identified from billing records, referrals, or clinic encounters and were used to identify sibships with at least two affected members. Most individuals were residents of southeast Michigan or northern Germany. The age-at-onset criterion was based on the observed bimodal age-at-onset distribution characteristic of psoriasis (Henseler and Christophers 1985). No age-at-onset criterion was applied to other affected family members. All individuals were examined by a dermatologist and were considered to be affected if they displayed two or more characteristic skin, scalp, nail, or joint lesions (Christophers and Sterry 1993) or if a single lesion occupied >1% of the total body surface area. Parents and siblings of affected sib pairs were examined, and blood was sampled whenever possible. Pedigrees that extended beyond the nuclear family were analyzed when at least four affected first- or second-degree relatives were available for study. Each individual ascertained under these criteria was included in the analysis (Cannings and Thompson 1977), and no individuals were excluded on the basis of marker data. Informed consent was obtained from all subjects, under protocols approved by the institutional review boards of the University of Michigan and the University of Kiel. These families were essentially the same as those used for our genome scan (Nair et al. 1997).

HLA-A and -B were typed serologically, and HLA-C, -DRB1, and -DQB1 were typed by use of DNA-based techniques (Kimura and Sasazuki 1993; Bunce et al. 1994). Typing was performed on suspensions of fresh leukocytes or immortalized lymphoblasts, prepared as described elsewhere (Nair et al. 1995). The nomenclature used for serologically defined HLA specificities is the name of the locus followed by the number of the specificity (e.g., B57) and, for genotyped alleles, the name of the locus followed by an asterisk and the number of the allele (e.g., Cw*0602 or DQB1*03032). For class II phenotypes, the nomenclature is complicated by the fact that antigens are coded by two polymorphic loci. For example, the phenotype DQ9 is coded by the DQA1*0201 and DQB1*03032 alleles (Bodmer et al. 1995). For consistency, we used serological designations for class I alleles and genotypic designations for class II alleles, even though HLA-C was typed by use of DNA-based techniques.

Parametric linkage was assessed by use of the LINKAGE package (Lathrop et al. 1984). The prevalence of psoriasis (ϕ) varies within the range of .01–.028 in Caucasian populations (Johnson 1978; Christophers and Sterry 1993). We assumed $\phi = .01$. The disease-allele frequency (p) was assumed to be .05 under dominance and .25 under recessivity, with 10% of psoriasis cases attributable to nongenetic causes. These assumptions are very similar to those recommended for linkage analysis of complex-trait data (Risch et al. 1989). Under Hardy-Weinberg equilibrium, these assumptions yielded the penetrances $f_{DD} = f_{Dd} = .092$ and $f_{dd} = .0011$, for the dominant model, and $f_{DD} = .144$ and $f_{Dd} = f_{dd} = .0011$, for the recessive model. Increasing ϕ to .02 (under the assumption of Hardy-Weinberg equilibrium) had little or no effect on the results (for supplemental data, see the Psoriasis Genetics Research web site).

The LINKAGE package can be programmed to "recognize" the existence of linkage disequilibrium between the marker and the trait (Terwilliger and Ott 1994). Otherwise, the program assumes that either phase between the marker and the disease alleles is equally likely, in phase-unknown matings. We estimated disease-marker haplotype frequencies by use of the EH program (Terwilliger and Ott 1994) and then used these frequencies to incorporate marker-trait disequilibrium into our parametric linkage calculations, following the same procedure for both the null and the alternative hypotheses. Haplotype frequencies were estimated by comparison of an unrelated group of local controls (189 U.S. and 124

German) with either case sample 1 (54 U.S. and 107 German unrelated affected pedigree members typed at all five HLA loci) or case sample 2 (237 German unrelated psoriatics typed for HLA-A and HLA-B). An individual other than the proband was chosen for inclusion in case sample 1 only when he or she was typed more completely than the proband. HLA allele sharing in affected sib pairs was analyzed by use of SIBPAIR (Satsangi et al. 1996).

Transmission of HLA alleles from heterozygous parents to affected offspring was assessed by use of the transmission/disequilibrium test (TDT). The biallelic TDT (Spielman et al. 1993) was performed for all alleles at loci for which the number of scoreable transmission plus nontransmission events was >10. A multiallelic extension of the TDT also was employed (Spielman and Ewens 1996). The TDT was used both as a test of linkage (by scoring all informative parent-offspring trios) and as a test of linkage disequilibrium (by scoring only one randomly chosen trio per pedigree). The recommendations of Curtis and Sham (Curtis and Sham 1995) were followed when a parental genotype for a trio was missing.

Tests for HLA associations with the psoriasis phenotype were performed by comparison of the HLA phenotypes of the individuals in case sample 1 with those of the unrelated local controls described above, by use of 2 × 2 contingency tables. Significance tests were performed only for HLA phenotypes reported in the literature to be associated with psoriasis (Svejgaard and Ryder 1994).

HLA haplotypes were estimated by use of the HAPCHILD program of the Genetic Analysis System package, version 2.0 (Young 1993), and were validated by visual inspection of pedigrees. Individuals were considered to carry the class I end of the EH57.1 haplotype if at least one of their HLA haplotypes was positive for Cw6 and B57 but negative for both DRB1*0701 and DQB1*03032 and to carry the class II end of this haplotype if the converse was true.

Results

The U.S. and German cohorts were comparable in terms of percentages of males and females affected (table 1). The percentage of affected parents (37%–44%) was higher than that previously reported for juvenile-onset psoriasis overall (13.5%) (Christophers and Henseler 1990). Although the age-at-onset criterion was applied only to probands, >91% of the affected relatives of the probands also were <40 years of age at onset, compared with 73% of unrelated psoriatic individuals (Henseler and Christophers 1985).

Parametric two-point linkage calculations performed under the assumption of marker-trait equilibrium revealed only weak evidence for linkage under dominant or recessive models (LOD $\leqslant 0.7$), and there also was no evidence for sharing of HLA alleles, among affected siblings, at any HLA locus (table 2*A*). However, maximum LOD scores (Z_{max}) increased markedly after we accounted for linkage disequilibrium between the marker and the trait, particularly for HLA-C and -B (table 2). The largest increases in Z_{max}—from 0.06 at recombination fraction $\theta = .41$ to 8.43 at $\theta = .16$—were observed under the dominant model, for HLA-C. These results were obtained by use of a sample of unrelated affected individuals from our pedigrees to define disease-marker haplotype frequencies, which is a potential source of bias (Chakravarti et al. 1984). Therefore, we repeated these calculations for HLA-A and -B, using an independent sample of unrelated German psoriatics, to estimate disease-marker haplotype frequencies. LOD scores obtained for HLA-B again increased markedly under the assumptions of dominance and marker-trait linkage disequilibrium, whereas those obtained for HLA-A did not (table 2*B*).

When applied to all informative parent-offspring trios, the TDT is also a test for linkage in the presence of linkage disequilibrium. When used in this fashion, the TDT confirmed the parametric linkage results (table 3): *P* values obtained for HLA-C and -B were much more significant than those obtained for HLA-DRB1 and -DQB1, and the results obtained for HLA-A were not significant at the $P = .05$ level.

When all trios studied are independent (i.e., derived from different families), the TDT is a test of linkage disequilibrium (Spielman and Ewens 1996). We identified risk alleles at each locus, using two overlapping but

Table 1

Selected Characteristics of the Study Cohorts

Characteristic	U.S. Cohort	German Cohort
General:		
No. of extended pedigrees	16	22
No. of nuclear sib-pair families	33	16
Affected/total males (%)	79/182 (43.4)	66/159 (41.5)
Affected/total females (%)	97/223 (43.5)	77/184 (41.8)
Affected/total individuals (%)	176/405 (43.5)	143/343 (41.7)
Mean age at onset (years)	19.2	21.4
No. of affected children, by parental affected status:[a]		
One parent affected (%)	42 (75)	23 (65.7)
Neither parent affected (%)	14 (25)	8 (22.9)
Both parents affected (%)	0 (0)	4 (11.4)
Total	56	35
No. of affected parents/total no. of parents (%)	42/112 (37.5)	31/70 (44.2)

[a] Includes only cases for which both parents were evaluated. For cases of sibships with multiple affected individuals, parental status was counted only once per sibship.

Table 2

Allele Sharing and Parametric Linkage Results (ILINK) for HLA Markers

HLA Marker	Distance from HLA-A[a] (Mb)	Hetero-zygosity	Affected-Sib-Pair LOD Score[b]	Dominant Model				Recessive Model			
				Marker-Trait Equilib-rium		Marker-Trait Disequilib-rium		Marker-Trait Equilib-rium		Marker-Trait Disequilib-rium	
				Z_{max}	θ_{max}	Z_{max}	θ_{max}	Z_{max}	θ_{max}	Z_{max}	θ_{max}
	A. Pooled U.S. and German Cohorts[c]										
A	.0	.84	.138	.474	.29	2.106	.23	.000	.50	.858	.26
C	1.3	.84	.000	.064	.41	8.435	.16	.159	.30	1.983	.30
B	1.4	.92	.011	.705	.32	6.228	.23	.000	.50	1.259	.32
DRB1	2.2	.87	.157	.605	.33	2.761	.28	.323	.26	2.333	.22
DQB1	2.3	.82	.150	.198	.38	2.998	.25	.706	.19	1.747	.19
	B. German Cohort Only[d]										
A	.0	.84	...	.075	.35	.890	.20	.000	.50	.035	.45
B	1.4	.92	...	.586	.29	5.259	.14	.004	.44	.770	.36

[a] Map distances are from the physical map of the HLA region (Campbell and Trowsdale 1993) and are expressed to the nearest 0.1 Mb.

[b] Calculated from pooled U.S. and German cohorts, by use of the SIBPAIR program from the ANALYZE package (Satsangi et al. 1996). Data shown include downweighting for multiple sib pairs in a sibship.

[c] Marker-trait–equilibrium data were calculated by use of marker allele frequencies generated from pooled U.S. and German founder individuals, under the assumption of linkage equilibrium between marker and trait loci. Marker-trait–disequilibrium data were calculated by use of case sample 1 and pooled local controls to generate marker-disease haplotype frequencies, as described in Subjects and Methods.

[d] Marker-trait–equilibrium data were calculated by use of marker allele frequencies generated from German founder individuals, under the assumption of linkage equilibrium between marker and trait loci. Marker-trait–disequilibrium data were calculated by use of case sample 2 and German local controls to generate marker-disease haplotype frequencies, as described in Subjects and Methods.

complementary methods: the TDT, performed using only one randomly selected trio per pedigree, and case-control association analysis. Both studies revealed that the most strongly associated alleles/phenotypes were Cw6, B57, DRB1*0701 (DR7), and DQB1*03032 (DQ9) (tables 3 and 4). Case-control testing revealed very similar results for the U.S. and German cohorts (table 4). In both cohorts, the most significant results were obtained for HLA-Cw6.

Although the foregoing results generally favored the class I loci HLA-B and -C over the class II loci HLA-DRB1 and -DQB1, none of these tests could exclude involvement of a gene in the class II region. In an effort to address this question, we compared disease prevalence among individuals carrying fragments of the EH57.1 ancestral haplotype (Cw6–B57–DRB1*0701–DQB1*03032). It was notable that 35 (11.8%) of 296 affected individuals selectively retained only the class I end of this haplotype, compared with only 12 (3.4%) of 347 unaffected individuals. In contrast, only 9 (3.0%) affected and 6 (1.7%) unaffected individuals selectively retained the class II end of this haplotype. Of the 9 affected individuals selectively retaining the class II end, 5 unrelated persons were extracted. Of these, 4 carried Cw6 (3 in *trans* and 1 in *cis*). This trend was reversed among the unrelated unaffected individuals, since 3/4 were Cw6 negative.

Discussion

The presence of a disease gene in the HLA region has long been suspected, on the basis of HLA-association studies (reviewed in Elder et al. 1994). However, the precise genetic basis of HLA association in psoriasis has remained elusive, as it has in other autoimmune disorders. Previous studies based on limited numbers of families found only weak evidence (Russell et al. 1972; Karvonen et al. 1976; Civatte et al. 1977; Suarez-Almazor and Russell 1990; Lin et al. 1991; Cao et al. 1993; Tomfohrde et al. 1994) or no evidence (Lin et al. 1991) for linkage to the HLA region. Recently, stronger evidence for linkage to the HLA region has been found in the context of two genome scans, including a scan performed in our laboratory (Nair et al. 1997; Trembath et al. 1997).

Given that HLA associations are well established for psoriasis and that linkage disequilibrium is the most common explanation for allelic association, it was curious that our genome scan yielded only suggestive evidence for linkage to the HLA region, under two-point

Table 3

TDT Results

HLA Marker (tel→cen)	Distance from HLA-A[a] (Mb)	Hetero-zygosity	Risk Allele[b]	All Trios			One Trio per Family		
				T:NT[c]	Best Allele *P* Value[d]	Multiallelic *P* Value[e]	T:NT[c]	Best Allele *P* Value[d]	Multiallelic *P* Value[e]
A	.0	.84	…	…	1.00	.54	…	1.00	.62
C	1.3	.84	Cw6	85:32	.0000086	.000012	38:13	.0023	.000040
B	1.4	.92	B57	61:14	.00000097	.0000028	27:3	.000082	.012
DRB1	2.2	.87	DR7	86:39	.00024	.0013	44:10	.000029	.000086
DQB1	2.3	.82	DQ9	40:13	.0015	.0053	22:6	.015	.00678

[a] Map distances are from the physical map of the HLA region (Campbell and Trowsdale 1993) and are expressed to the nearest 0.1 Mb.

[b] Most significant risk allele identified by the biallelic TDT.

[c] Ratio of transmitted (T) to nontransmitted (NT) alleles from heterozygous parents.

[d] Corrected for number of valid biallelic TDT tests performed at the locus (Spielman et al. 1993).

[e] Calculated over all alleles at the indicated locus, by use of a multiallelic extension of the TDT (Spielman and Ewens 1996).

parametric linkage analysis ($Z_{max} = 2.59$ at $\theta = .14$, for TNFB, a marker mapping just 5′ to the tumor necrosis factor-β gene), and that little, if any, evidence for linkage emerged from two-point or multipoint analysis of allele sharing (SIBPAIR $P = .026$ and GENEHUNTER $P = .022$, for D6S291; SIBPAIR $P = .011$ and GENEHUNTER $P = .059$, for TNFB) (Nair et al. 1997). Moreover, since most individuals carrying HLA risk alleles are heterozygous for these alleles (Russell et al. 1972; Karvonen et al. 1976; Civatte et al. 1977), it also was curious that the mode of inheritance under which we found evidence for linkage to HLA in the genome scan was recessive rather than dominant. Inspection of our pedigrees revealed many asymptomatic carriers of Cw6 among the founders and unaffected spouses, with numerous examples of transmission of this allele to affected individuals. A typical example is shown in figure 1. Previously published family studies had not reported this phenomenon (Russell et al. 1972; Karvonen et al. 1976; Civatte et al. 1977), perhaps because such families were considered to be bilineal for the disease chromosome and therefore may have been excluded. However, it is not surprising that this situation arose, given that the allele frequency of Cw6 was substantial in both sets of local controls (9.3% in the German cohort and 9.9% in the U.S. cohort) and that our ascertainment criteria were intentionally biased toward multiplex involvement. Given that Cw6 is associated with disease when found on at least three HLA-B-Cw6 haplotypes (Cw6-B13, Cw6-B37, and Cw6-B57), it seems reasonable to assume that many, if not all, Cw6-positive individuals are carriers of the disease allele (see below). However, only ~10% of Cw6-positive individuals are affected, presumably owing to lack of other genetic and/or environmental factors (reviewed in Elder et al. 1994). This assumption is incorporated into our parametric model by the low penetrance (9%) and high frequency (5%) specified for the disease allele. However, unless the phase of the disease allele is specified correctly, the power of the linkage analysis is greatly reduced, even for a fully penetrant disorder (Clerget-Darpoux 1982). By combination of low penetrance with appropriate specification of phase, the linkage parameters much more closely approach the actual situation encountered in our pedigrees, as exemplified in figure 1. Consistent with the fact that most Cw6-positive psoriatics carry only one copy of this allele, incorporation of marker-trait disequilibrium not only markedly increased the LOD score but also changed the apparent mode of inheritance, from recessive (as was suggested by our genome scan) to dominant.

Case-control association tests and the TDT both yielded risk alleles very similar to those identified by previous case-control association studies of Caucasian populations. Since only one-third of psoriatics are aware of an affected relative (Farber and Nall 1974), most prior association studies can be assumed to be comprised of mainly "sporadic" cases. Therefore, our results argue against any meaningful distinction between "familial" and "sporadic" psoriasis, at least in terms of the HLA-linked component.

Our study yielded relatively strong associations with HLA-DR7, as have numerous prior case-control studies. This result may be explained by the fact that the ancestral haplotype EH13.1, carrying Cw6 and B13, also encodes DR7 (DRB1*0701) (Degli-Esposti et al. 1992). The existence of these two psoriasis-associated ancestral haplotypes probably explains why the number of TDT transmission events involving Cw6 and DR7 greatly outnumber those involving B57 and DQ9 (table 3). The association of psoriasis with at least two ancestral haplotypes carrying the same alleles at HLA-C and -DRB1 would appear to be inconsistent with linkage outside the C–DR interval, even after allowing for recent population expansion and endogamy (Thompson and Neel 1997).

Table 4

2 × 2 Contingency Tests for Association of Selected HLA Phenotypes with Psoriasis

Phenotype	Relative Risk	Pearson χ^2	*P* Value[a]	Corrected *P* Value[b]
	A. U.S. Cohort			
A1	1.70	2.73	.13	1.00
A2	.93	.06	.87	1.00
B13	2.62	4.62	.06	1.00
B37	3.79	2.93	.12	1.00
B39	.35	1.07	.46	1.00
B57	7.91	28.68	1.6×10^{-6}	3.8×10^{-5}
Cw6	6.55	34.72	1.9×10^{-8}	4.6×10^{-7}
Cw7	.54	3.93	.06	1.00
DR7[c]	2.80	9.54	3.8×10^{-3}	.09
DR10	2.07	.36	.48	1.00
DQ9[d]	5.82	16.65	1.6×10^{-4}	3.8×10^{-3}
73Ala[e]	2.81	2.89	.10	1.00
	B. German Cohort			
A1	1.23	.49	.55	1.00
A2	2.05	6.24	.02	.37
B13	4.69	12.91	5.5×10^{-4}	.013
B37	2.39	1.54	.31	1.00
B39	2.39	1.54	.31	1.00
B57	9.02	30.50	2.7×10^{-8}	6.5×10^{-7}
Cw6	15.11	53.08	$< 5 \times 10^{-10}$	$< 1.2 \times 10^{-8}$
Cw7	1.00	.00	1.00	1.00
DR7[c]	4.28	22.91	2.0×10^{-6}	4.8×10^{-5}
DR10	1.33	.08	1.00	1.00
DQ9[d]	3.92	13.57	3.1×10^{-4}	7.4×10^{-3}
73Ala[e]	4.68	11.87	9.1×10^{-4}	2.2×10^{-2}

[a] As determined by use of Fisher's exact test.

[b] Bonferroni correction applied for 24 tests (12 on the U.S. cohort and 12 on the German cohort).

[c] DR7 phenotype denotes one or two copies of the DRB1*0701 genotype.

[d] DQ9 phenotype denotes one or two copies of the DQB1*03032 genotype.

[e] Defined as phenotypically positive for Cw4, Cw6, Cw7, Cw12, Cw13, or Cw15.

However, the two haplotypes differ in their intervening class III and HLA-B alleles (Degli-Esposti et al. 1992), suggesting that further localization should be possible.

Because we have followed transmission of HLA haplotypes through families, we have been able to identify recombinant ancestral haplotypes with confidence. We have used these "broken" haplotypes to confirm prior case-control studies (Schmitt-Egenolf et al. 1996), in which the recombinant haplotypes could only be inferred. By studying individuals carrying recombinants of EH57.1, we found that the class I end of this haplotype was selectively retained among affected individuals nearly four times more often than among unaffected individuals and that nearly all of the few affected individuals who carry only the class II end of this haplotype also carried Cw6 (usually in *trans*). Statistical analysis of these results was not possible, because of the limited number of genetically independent individuals carrying suitable fragments of the EH57.1 haplotype. Nevertheless, our results are very consistent with those of Schmitt-Egenolf et al. (1996), who found a highly significant result by use of a case-control design. However, at present, we cannot rule out the possibility that a second determinant may reside in the class II region.

In agreement with prior association studies, the family data presented here all point to HLA-Cw6 as the strongest predictor of psoriasis risk, among the HLA alleles tested. However, we believe that Cw6 is unlikely to be the direct determinant of susceptibility, for three reasons: First, only one Cw6 coding sequence has been identified, and, yet, three psoriasis-associated B-Cw6 haplotypes do not carry equal risk in any given population. Second, the risk of psoriasis as a function of the B-Cw6 haplotype varies from population to population. Thus, B13-Cw6 and B37-Cw6 are the strongest B-Cw6 risk haplotypes inferred from Japanese association studies (Tsuji et al. 1979; Nakagawa et al. 1991), whereas Cw6-B57 is predominant in Caucasian (table 4) (Tiwari and Terasaki 1985) and Chinese (Cao et al. 1993) populations. Third, if HLA-C is the direct determinant of susceptibility, this would imply that more than one allele at HLA-C must predispose to disease (under the assumption of locus homogeneity). However, our study alone has identified >100 examples of Cw6-negative psoriatics. Prompted by the high prevalence of HLA-Cw7 in Japanese psoriatics (Nakagawa et al. 1991; Muto et al. 1995), Asahina et al. (1991) suggested that the presence of alanine (rather than threonine) at amino acid residue 73 of HLA-C (73Ala) confers risk for psoriasis. 73Ala is present in alleles Cw4, Cw6, Cw7, Cw12, Cw13, and Cw15 and

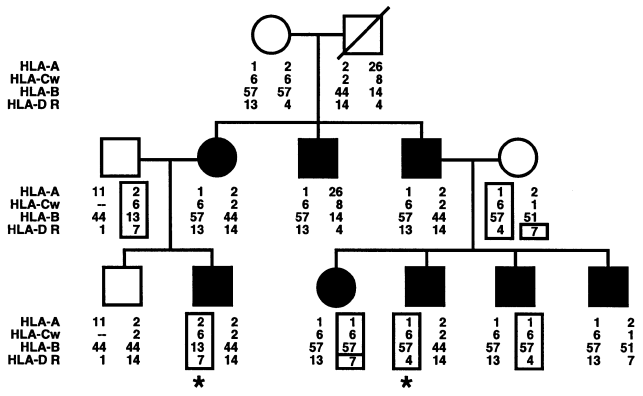

Figure 1 Psoriasis pedigree, demonstrating segregation of HLA haplotypes, determined as described in Subjects and Methods. Note four transmissions of Cw6 from married-ins in the second generation (boxed haplotypes), generating two apparent recombination events (marked by asterisks [*]).

therefore is found in at least two-thirds of Caucasian HLA-C molecules (Bunce et al. 1996). Although a weak association with 73Ala was identified in the German cohort, there was no association with this collection of alleles in the U.S. cohort (table 4). Moreover, neither association tests nor the TDT supported involvement of Cw7 in our pedigrees. Overall, the most parsimonious explanation for our results is that the disease determinant is neither 73Ala nor any feature of HLA-C itself but that it is an allele at a locus in very tight disequilibrium with HLA-Cw6. If so, the variability in identity of the accompanying HLA-B alleles could be explained by ancestral recombination or gene-conversion events that are more recent than the emergence of the disease allele, and the variation in relative risk associated with each B-Cw6 haplotype could reflect haplotype-specific variants of the disease allele, variations in population prevalence of the susceptibility haplotypes, or both.

Recombination in the vicinity of the B and C alleles would have to be very rare in order to preserve the observed associations between these loci and the disease allele in diverse world populations. This would be consistent with the emerging concept that the HLA region is composed of five "polymorphic frozen blocks," within which recombination appears to be strongly suppressed (Marshall et al. 1993). Our data suggest that a major psoriasis-susceptibility locus resides within the β block, which extends from just telomeric to TNFB to ≈100 kb telomeric to HLA-C (Marshall et al. 1993). This region contains a high density of genes (Leelayuwat et al. 1995), several of which are polymorphic and in strong linkage disequilibrium with known B-C haplotypes (Foissac et al. 1997). Recently, one of the genes within the β block, known as "MICA" or "PERB11," has been shown to display a near-perfect association with Behçet disease (Mizuki et al. 1997). We predict that a similar scenario will emerge for psoriasis, when the correct locus within the β block is identified.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for psoriasis susceptibility [177900])

Psoriasis Genetics Research, Department of Dermatology, University of Michigan, http://www.psoriasis.umich.edu/ (for supplemental linkage data)

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