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Comparison of MHC class I risk haplotypes in Thai and Caucasian psoriatics shows locus heterogeneity at *PSORS1*

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Abstract

Earlier studies have shown that psoriasis in Japan and Thailand is associated with two different major histocompatibility complex (MHC) haplotypes – those bearing *HLA-Cw6* and those bearing *HLA-Cw1* and *HLA-B46*. In an independent case-control sample from Thailand, we confirmed the association of psoriasis with both haplotypes. No association was seen in Thai *HLA-Cw1* haplotypes lacking *HLA-B46*, nor was *HLA-Cw1* associated with psoriasis in a large Caucasian sample. To assess whether these risk haplotypes share a common origin, we sequenced genomic DNA from a Thai *HLA-Cw1-B46* homozygote across the ~300 kb MHC risk interval, and compared it with sequence of a *HLA-Cw6-B57* risk haplotype. Three small regions of homology were found, but these regions share equivalent sequence similarity with one or more clearly non-risk haplotypes, and they contain no polymorphism alleles unique to all risk haplotypes. Differences in psoriasis phenotype were also observed, including lower risk of disease, greater nail involvement, and later age at onset in *HLA-Cw1-B46* carriers compared with *HLA-Cw6* carriers. These findings suggest locus heterogeneity at *PSORS1* (psoriasis susceptibility 1), the major psoriasis susceptibility locus in the MHC, with *HLA-Cw6* imparting risk in both Caucasians and Asians, and an allele other than *HLA-Cw1* on the *HLA-Cw1-B46* haplotype acting as an additional risk variant in East Asians.

Introduction

Human leukocyte antigen (HLA) associations with psoriasis have been known for nearly 40 years (1). Earlier studies localized the disease determinant to the class I end of the MHC (2, 3) and assigned the name PSORS1 [OMIM (Online Mendelian Inheritance in Man) # 177900] to this locus (4). Association with *HLA-Cw6* is particularly strong in many different world populations (5), and recent sequencing and haplotype analyses of Caucasian and Chinese Han psoriatics have indicated that *HLA-Cw6* itself is likely to be the susceptibility determinant on these chromosomes, rather than any of 10 nearby genes in the 300-kb *PSORS1* candidate interval (6, 7). The prevalence of psoriasis differs markedly throughout the world (8). While it is unclear whether genetic or environmental factors are primarily responsible for this variation, it has been suggested that the rarity of psoriasis in Australian aborigines and several Amerindian populations is correlated with the absence of HLA haplotypes carrying *HLA-Cw6* (8).

Several studies have documented a strong association between psoriasis and another *HLA* haplotype that is common in Japan and Thailand, but extremely rare in Caucasians (*HLA-A*0207, -B*4601, -Cw*01*) (9–13). This haplotype was associated with psoriasis when found in *cis* to any of three HLA class II haplotypes (10), suggesting that the disease determinant resides on the class I end of these haplotypes. Interestingly, all studies but one (9) found that the *HLA-Cw1-B46* haplotype imparts considerably lower risk for psoriasis than does *HLA-Cw6*. There is also some evidence that this determinant produces a different clinical form of psoriasis as *HLA-Cw1-B46* is equally associated with early or late-onset disease (9, 10), whereas *HLA-Cw6* is much more strongly associated with early-onset psoriasis in both Thais (10) and Caucasians (14).

A major goal of this study was to determine whether the *HLA-Cw1-B46* psoriasis risk haplotype found in Japanese (12, 13, 15) and Thai (9, 10) populations and the *HLA-Cw6*-bearing psoriasis risk haplotypes found in both Caucasians and Asians represent allelic or locus heterogeneity at the *PSORS1* locus, or if they share a disease locus inherited identical by descent (IBD) from a common ancestor. To this end, we cloned and sequenced the *PSORS1* candidate interval of the *HLA-Cw1-B46* haplotype for comparison with sequences for the *HLA-Cw6-B57* and *HLA-Cw6-B50* risk haplotypes and nine non-risk MHC haplotypes that were derived by us (6) and the Sanger Centre (16). In addition, we looked for differences in associated relative risk and phenotype of the *HLA-Cw1-B46* and *HLA-Cw6*-bearing haplotypes in a previously unreported sample of 206 Thai cases and 114 Thai controls. Finally, we compared the haplotype compositions and odds ratios (ORs) for association of *HLA-Cw1* in our Caucasian and Thai samples. Together, these analyses confirm that *HLA-Cw1-B46* is a psoriasis risk haplotype in the Thai population, demonstrate that *HLA-Cw1* is unlikely to be a direct determinant of risk for psoriasis in the Caucasian or Thai populations, and strongly suggest that the disease determinants carried on these two ancestral haplotypes are not derived from a common ancestor.

Materials and methods

Subjects

Informed consent was obtained from all subjects under protocols adherent to the Declaration of Helsinki principles and approved by the Institutional Review Boards of the participating institutions. In the Caucasian sample, which consisted of 2438 cases and 2311 controls, most affected individuals were identified through the dermatology services of the University of Michigan Medical Center, the Ann Arbor Veterans Affairs Medical Center, and Henry Ford Hospital of Detroit. A few psoriatics were also provided by the National Psoriasis Foundation Tissue Bank. Individuals were defined as affected if chronic plaque or guttate psoriasis lesions covered more than 1% of the total body surface area (TBSA) or if at least two skin, scalp, nail or joint lesions were clinically diagnostic of psoriasis (17). Controls were recruited from the southeast Michigan area, and were required to be unrelated to each other or to any case, and to be free of a family history of psoriasis. For this study, only cases and controls of self-reported European Caucasian origin were analyzed. The Thai sample consisted of 206 psoriasis cases and 114 normal controls, all collected at the Institute of Dermatology in Bangkok, Thailand, using the same inclusion and exclusion criteria used for the Caucasian sample.

DNA preparation

Genomic DNA was prepared from heparinized whole blood using previously established methods (18). Blood samples collected in Bangkok were transported to Ann Arbor for DNA preparation within 4 days.

Genotyping

Eight single-nucleotide polymorphisms (SNPs) in exons 2 and 3 of the *HLA-C* gene were genotyped – *rs1131151*, *rs28732105*, *rs1050409*, *rs1131123*, *rs1131118*, *rs1050384*, *rs17839985*, and *rs41547419* at positions 89, 213, 218, 341, 361, 387, 459, and 540 of the coding sequence. These SNPs allow absolute discrimination of *HLA-C* to a triallelic level (*Cw1*/*Cw6*/neither), even in the absence of external phasing information, for all known alleles in release 2.10.00 of the IMGT-HLA Sequence Database (19); URL: http://www.ebi.ac.uk/imgt/hla). Six SNPs in exons 2 and 3 of the *HLA-B* gene were also genotyped – *rs713031*, *rs41562914*, *rs1131204*, *rs41553715*, *rs1071652*, and *rs2308466* at positions 142, 206, 277, 299, 362, and 560 of the coding sequence. These SNPs provide typing of *HLA-B* to a biallelic level (*B46*/other) in the absence of phasing information for all known alleles in release 2.16.00 of the IMGT-HLA database. All SNPs were typed by single-base primer extension, as implemented in the SnapShot assay protocol (Applied Biosystems, Foster City, CA), per the manufacturer's instructions. Polymerase chain reaction (PCR) amplification and SnapShot extension primer sequences are provided in Table S1 (*Supporting Information*). Microsatellites were genotyped by PCR amplification using fluorescently labeled forward and unlabeled reverse primers followed by size determination by capillary electrophoresis on an Applied Biosystems 3100 Genetic Analyzer.

Cloning and sequencing

A Thai psoriatic who was homozygous for the *HLA-Cw1-B46* haplotype throughout the 300-kb *PSORS1* candidate region (31.129–31.429 Mb on build 36.3 of the human reference sequence for chromosome 6) was identified by genotyping *HLA-C*, *HLA-B*, and 10 microsatellite markers extending from *MICA* to telomeric of *CDSN*. A fosmid library was prepared from the genomic DNA of this individual and screened for the region of interest, as previously described (6). Thirteen overlapping fosmid clones that provided complete coverage of the risk interval were selected, with no attempt made to distinguish clones from the maternal or paternal chromosome. Inserts of each fosmid clone were subjected to shotgun sequencing, as described previously (6). High quality sequence coverage from at least two different plasmid subclones – and from both strands, whenever possible – was required for the entire fosmid insert, which resulted in a 22-fold average depth of coverage. The published sequence of the *HLA-Cw7-B8* haplotype of the Cox homozygous cell line (20) was used as both a reference for sequence alignment and for the numbering of the coordinate system used in the

tables and figures of the present study, which starts at the first base of the 5′ primer (GCAACTTTTCTGTCAATCCA) used to amplify microsatellite marker *D6S273* and extends in the telomeric direction. Overlapping fosmid clone sequences were assembled into a single contig; the resulting 337.1 kb of *HLA-Cw1-B46* haplotype sequence (spanning 31.105–31.446 Mb on human reference) has been deposited in Genbank (accession number GQ472773).

Association analysis

Single marker association was evaluated using a chi-squared contingency test of allelic counts; asymptotic *P*-values are reported. Haplotype inference and haplotype-based association tests were carried out with v1.07 of PLINK (21); http://pngu.mgh.harvard.edu/purcell/plink. For standard haplotype association, a logistic regression model with an allele dosage term was used, and *P*-values were determined with 1 million permutations of case-control status. For conditional haplotype-based association, a test of whether *HLA-Cw1* has effects independent of *HLA-B46* was constructed as a likelihood ratio test comparing an alternative model with separate effects for each of the three *HLA-Cw1-B46* haplotypes to a null model which groups the *Cw1+/B46−* and *Cw1−/B46−* haplotypes together. An analagous conditional test for independent effect of *HLA-B46* compares association of the *Cw1+/B46+* and *Cw1+/B46−* haplotypes. Meta-analysis of disease associations in the two Thai studies used Cochran-Mantel-Haenszel test procedures. Power calculations were carried out with version 3.1 of G*POWER (22); http://www.psycho.uni-duesseldorf.de/aap/projects/gpower.

Sequence analysis

The *HLA-Cw1-B46* sequence was aligned with the other 18 MHC haplotype sequences using SeqMan (DNASTAR, version 8.0.2); whenever necessary, sequence alignments were manually adjusted to yield the minimum possible number of polymorphisms. For each polymorphism, its location in the Cox *HLA-Cw7-B8* reference sequence and its alleles for all 19 haplotypes were recorded. MHC haplotype sequences were then compared with that of the *HLA-Cw6-B57* haplotype by determining the percentage difference of polymorphic alleles over 2.5-kb intervals. Regions of similarity between pairs of MHC haplotype sequences were delineated by a two-step approach (23). Rough bounds were first obtained using a moving window of 2.5 kb with a lag of 100 bp and a criterion of at least 80% identity of alleles for all included polymorphisms. Recursive entropic segmentation (24) with a stopping criterion based on the Bayesian information criterion (25) was then applied as a second-stage refinement. Version 0.97-600-1000 of the MINCOV program (26); (http://www.stanford.edu/group/molepi/free_software.html) was used to search for minimal combinations of polymorphism alleles unique to risk haplotypes. MHC haplotype sequences within regions of homology were clustered using an average-distance agglomerative hierarchical method with a metric of percentage difference of polymorphism alleles. Multiple instances of a single MHC haplotype (viz., two *HLA-Cw6-B57*, two *Cw7-B7*, two *Cw8-B65*, three *Cw7-B8*, two *Cw3-B62,* and two *Cw12-B38* haplotypes) were consolidated into a single representative consensus sequence before comparison of sequence similarity and combinatorial analysis. All 19 available MHC sequences were used for clustering.

Phenotype analysis

Five phenotypic aspects of psoriasis were measured at entry into the study: age at onset of disease, TBSA involvement of lesions, toenail involvement, fingernail involvement, and arthritis. All traits were compared for four different *HLA-CB* phenotypes (carriage of *HLA-Cw1-B46* but no *HLA-Cw6*, carriage of *HLA-Cw6* but no *HLA-Cw1-B46*, carriage of both *HLA-Cw6* and *HLA-Cw1-B46*, carriage of neither *HLA-Cw1-B46* nor *HLA-Cw6*), as well as all six possible *HLA-CB* genotypes involving these two alleles. Age at onset and TBSA were analyzed by one-way analysis of variance (ANOVA) after transforming the variables to approximate normality with the optimal Box–Cox power transformation (power of 0.8 for age at onset and 0.3 for TBSA); *P*-values were determined using 10,000 randomizations of the response variable observations. There were no significant departures from the assumption of homogeneity of variances for either of the transformed variables, as assessed by a randomization version of Levine's test. Sheffe's modified S-method (27, 28) was used for an unplanned comparison of mean age at onset of *HLA-Cw6* carriers *vs* noncarriers; it controls the experimentwise error rate at the nominal level for all possible linear contrasts of the group means. Nail involvement and arthritis were analyzed by unordered two-way contingency tables, using Fisher's exact test to determine the significance of association between phenotype variables and *HLA-C* status. Standardized Pearson residuals were examined to determine the relative contributions of different cells of the contingency table to the overall test result, and a 2×2 contingency table was used to analyze nail involvement for *HLA-Cw1-B46* carriers *vs* noncarriers.

Results

Association analysis

As shown in Table 1, in our Thai sample, *HLA-Cw6*, *HLA-Cw1* and *HLA-B46* all are significantly associated with psoriasis ($P = 3.2 \times 10^{-6}$, 0.0011 and 0.0017, respectively). The associations with *HLA-Cw1* and *HLA-B46* are driven entirely by association with an underlying *HLA-Cw1-B46* haplotype ($P = 0.0016$, Table 2). In this sample *HLA-B46* is invariably linked with *HLA-Cw1* (i.e. no *HLA-Cw1*$^{-}$*/B46*$^{+}$

Table 1 Single marker analysis of *HLA-Cw1*, *HLA-Cw6*, and *HLA-B46* associations with psoriasis in Thais and Caucasians

	Thais				Caucasians			
	Frequency (proportion) in				Frequency (proportion) in			
Allele	Cases	Controls	OR (95% CI)[a]	P[b]	Cases	Controls	OR (95% CI)[a]	P[b]
HLA-Cw1	94 (0.2338)	26 (0.1238)	2.16 (1.35, 3.46)	0.0011	161 (0.0330)	182 (0.0394)	0.83 (0.67, 1.03)	0.097
HLA-Cw6	71 (0.1766)	9 (0.0429)	4.79 (2.34, 9.80)	3.2×10^{-6}	1139 (0.2336)	420 (.0909)	3.05 (2.70, 3.44)	1.3×10^{-78}
HLA-B46	79 (0.1955)	22 (0.0991)	2.21 (1.33, 3.66)	0.0017	0[c] (0.0000)	0[c] (0.0000)	—	—

[a]Odds ratio and its 95% confidence interval for the allelic association test.
[b]*P*-value for allelic association test; multiallelic *P*-value for *HLA-C* is 3.4×10^{-8} in Thais and 3.2×10^{-77} in Caucasians.
[c]Frequency of *HLA-B46* in Caucasians is based on typing all *HLA-Cw1*-positive individuals (160 cases and 174 controls successfully typed) and a large subsample of *HLA-Cw1*-negative individuals (505 cases and 667 controls successfully typed).

Table 2 Association of *HLA-Cw1-B46* haplotypes with psoriasis in Thais

HLA haplotype		Frequency	Frequency		
Cw1	B46	cases	controls	OR (95% CI)[a]	P[b]
+	+	0.1965	0.0991	2.25 (1.34, 3.80)	0.0016
+	−	0.0373	0.0283	1.34 (0.51, 3.57)	0.63
−	+	0.0000	0.0000	—	—
−	−	0.7662	0.8726	0.46 (0.28, 0.75)	0.0012

[a]Odds ratio and its 95% confidence interval in logistic regression dosage model for association.
[b]Global *P*-value = 0.0036; all the *P*-values based on 1 million permutations.

haplotypes); however, *HLA-Cw1* haplotypes lacking *HLA-B46* do occur at a low frequency of ~3%, but they show a much smaller effect size (OR = 1.34) and are not significantly associated with psoriasis ($P = 0.63$). Similarly, conditional haplotype-based association testing found no evidence that *HLA-Cw1* is associated independently of *HLA-B46* ($P = 0.33$), or vice versa ($P = 0.52$).

Although *HLA-Cw6* appears to be more strongly associated with psoriasis than *HLA-Cw1-B46* (OR = 4.79 *vs* 2.16), the difference in ORs is not significant as their 95% confidence intervals (CI) overlap. Because of increased power, a meta-analysis that combines the allelic counts in our Thai sample with those of a previous Thai study (10) is able to show a significantly greater association of psoriasis with *HLA-Cw6* (OR = 5.10, 95% CI = 3.23–8.06, $P = 2.1 \times 10^{-14}$) than with *HLA-Cw1-B46* (OR = 2.16, 95% CI = 1.61–2.88, $P = 1.4 \times 10^{-7}$). The other Asian studies (9, 11–13) used serological typing, so their results could not be easily combined with the more recent allele-based studies, but it is noteworthy that three of four of these older studies also show a greater strength of association for *HLA-Cw6*.

We also looked for *HLA-C* associations in a Caucasian sample of 2438 cases and 2311 controls (Table 1). While *HLA-Cw6* was very strongly associated with psoriasis in this sample (OR = 3.05, $P = 1.3 \times 10^{-78}$), *HLA-Cw1* was clearly unassociated (OR = 0.83, $P = 0.097$). Despite the lower allele frequency of *HLA-Cw1* in the Caucasian cohort (3.9% in Caucasian controls versus 12.4% in the Thai controls), our large sample had essentially 100% power to detect association if it indeed exists, assuming a multiplicative model, a significance level $\alpha = 0.05$, and an OR of 2.16 (similar to that in Thais); our sample had 80% power to detect an association with an OR as low as 1.32. *HLA-B46* did not occur in our Caucasian sample, neither among all 334 *HLA-Cw1* -positive individuals nor among a large typed subset of 1172 *HLA-Cw1* -negative individuals (95% CI for *HLA-B46* frequency = 0.0000–0.0026 in 730 randomly selected controls and 0.0000–0.0035 in 545 randomly selected cases).

Sequence comparisons

In order to determine the DNA sequence of the *PSORS1* risk region on the *HLA-Cw1-B46* haplotype, an affected Thai individual homozygous for this haplotype was identified by HLA typing and microsatellite genotyping, and a fosmid library was prepared and screened as described in Methods. A total of 13 overlapping fosmid clones were isolated that provided complete coverage of a 337 kb region extending from 15 kb telomeric of *HLA-B* to 90 kb centromeric of *CDSN*. The sequenced interval fully includes a 298-kb candidate region for *PSORS1* shared by all known *HLA-Cw6* risk haplotypes (6). We then compared this sequence to a collection of genomic DNA sequences generated by ourselves (6) and by the MHC Haplotype Project (16). Besides providing new examples of haplotypes we previously sequenced (*HLA-Cw7-B8, Cw7-B7, Cw3-B62,* and *Cw6-B57*), inclusion of the MHC Haplotype Project sequences contributed sequences for four new haplotypes (*HLA-Cw5-B18, Cw5-B44, Cw16-B44,* and *Cw3-B60*) that all appear to be non-risk from our previous analysis (6). All together, 19 sequences were available, including those for 11 distinct MHC haplotypes that were complete enough for sequence comparison in the candidate interval.

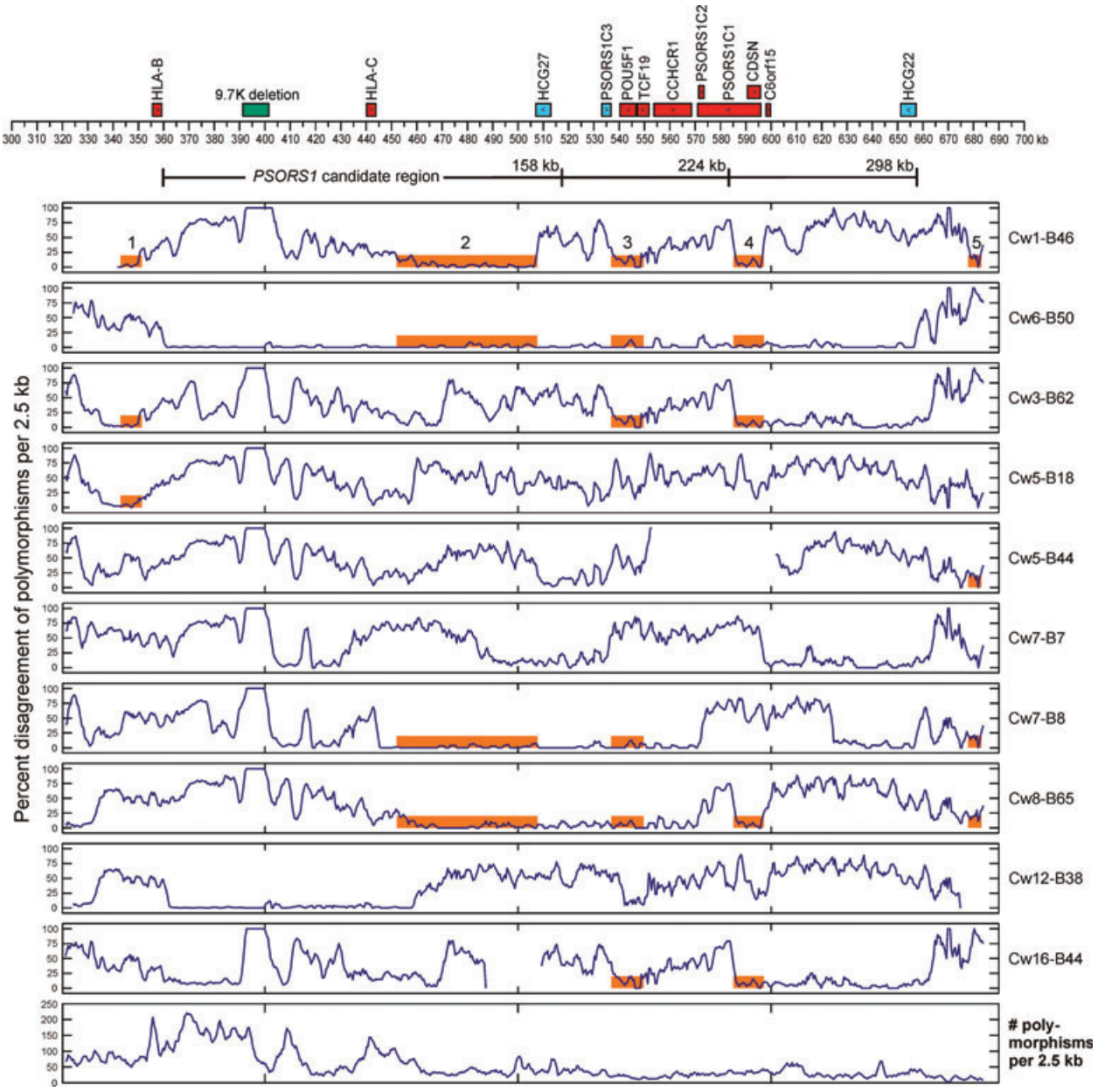

Figure 1 Sequence comparison of 10 MHC class I haplotypes with the *HLA-Cw6-B57* risk haplotype. Known genes and their direction of transcription, as well as a 9.7-kb indel are shown above the coordinate axis. Genes expressing non-coding RNA are colored cyan, and those expressing protein are colored red. Three delineations of the *PSORS1* candidate region (6) are shown below the coordinate axis. The per cent disagreement of polymorphism alleles, when compared with the *HLA-Cw6-B57* haplotype, is plotted for each haplotype using a moving 2.5-kb window with a 500-bp lag. The bottom panel plots the number of polymorphisms that are variable among all sequenced haplotypes; only these polymorphisms were considered when computing per cent disagreement. Regions of sequence homology at least 5 kb in length between the *HLA-Cw1-B46* and *HLA-Cw6-B57* haplotypes are mapped as five-numbered orange bars in the top panel; these bars are also shown on all other haplotypes sharing the same regions of homology.

As shown in Figure 1, the *HLA-Cw1-B46* and *HLA-Cw6-B57* haplotypes exhibit substantial allelic divergence for more than three-quarters of the sequenced interval. Nevertheless, within the 331 kb region encompassing most sequences, four of 7364 qualifying polymorphisms carry an allele common to all three psoriasis risk haplotypes (*HLA-Cw6-B57*, *Cw6-B50*, and *Cw1-B46*) that is not found on any of the eight non-risk haplotypes (Table 3). Furthermore, there are more than 59,000 two-way combinations and 2 billion three-way combinations of polymorphism alleles fulfilling this same criterion for a potential IBD disease locus. Progressively narrowing the region of comparison to each of three different *PSORS1*

Table 3 Polymorphism analysis of *PSORS1* candidate regions and regions of sequence homology between *HLA-Cw1-B46* and *HLA-Cw6-B57*

				No. of polymorphisms[c]		Per cent disagreement *Cw1-B46 vs Cw6-B57*[d]		No. of combinations of polymorphisms common and unique to risk haplotypes[e]		
Region[a]	Bounds (kb)	Length (kb)	No. of haplotypes[b]	all	SNPs and indels	All	SNPs and indels	1-way	2-way	3-way
1	343.0–351.3	8.3	11	218	214	4.1	3.7	0	0	0
2	452.1–507.6	55.5	10	915	881	4.5	4.0	0	31	13,272
3	536.8–597.1	12.8	11	92	91	12.0	11.0	0	0	0
4	585.1–597.1	12.0	10	159	158	5.0	5.1	0	0	0
5	677.9–683.1	5.2	11	34	33	11.8	12.1	0	0	0
158 kb	359.9–517.1	157.6	10	4983	4912	46.9	46.7	4	13,817	3.8×10^7
224 kb	359.9–583.4	223.6	9	5673	5571	46.1	45.7	4	17,421	5.4×10^7
298 kb	359.9–657.6	297.7	9	6608	6470	46.5	46.0	4	38,109	1.8×10^8
Entire	343.0–673.8	330.8	9	7364	7211	44.7	44.1	4	59,555	2.5×10^9

[a]Region of MHC sequence as shown in Figure 1; 'entire' refers to the full interval for which most MHC haplotypes were sequenced.
[b]Number of different haplotype sequences being compared that are fully sequenced for the region; this number varies among regions because three of the Sanger Centre sequences have gaps in coverage.
[c]Number of polymorphisms that are variable for sequences being compared; separate tallies are given for all types of polymorphisms and for SNPs and indels only (i.e. excluding STRs and poly-A/T variations, which tend to have higher mutation rates).
[d]Per cent disagreement of polymorphism alleles between the *HLA-Cw1-B46* and *HLA-Cw6-B57* risk haplotypes.
[e]Number of one-way, two-way, and three-way allelic combinations of polymorphisms that are both common and unique to the three psoriasis risk haplotypes (*Cw1-B46*, *Cw6-B57*, and *Cw6-B50*) when compared with all non-risk haplotypes.

candidate regions (298, 224, and 158 kb), which were delineated by previous work (6), only modestly reduces the number of potential disease loci (Table 3). However, if *HLA-Cw6* and *HLA-Cw1-B46* risk haplotypes are indeed descended from a common *PSORS1*-bearing ancestor, then the disease locus should occur within a region of sequence homology. Five such regions at least 5 kb in length, marked in orange in Figure 1, could be delineated. Two of these (regions 1 and 5) occur outside the 298-kb candidate interval that is the shortest region common to all known *HLA-Cw6* risk haplotypes, and two of the remaining three (regions 3 and 4) are unpromising candidates for an IBD disease region as these short (12.8 and 12.0 kb, respectively) intervals bear no polymorphism alleles or combinations of alleles unique to the risk haplotypes. Furthermore, region 4 falls ouside of a 224-kb *PSORS1* candidate interval firmly established by ancestral recombinant haplotype analysis, and region 3 falls outside of a probable although not definitively established 158-kb candidate interval.

The final and largest region of homology, region 2, is a 55.5-kb interval between *HLA-C* and *HCG27* with 95.5% allelic identity at all 915 variable polymorphisms and a 96.0% allelic identity among the 881 more stable SNPs and indels [i.e. excluding highly mutable poly-A/T and short tandem repeat (STR) variations]. Region 2 bears no single polymorphism with an allele restricted to risk haplotypes, but it does have 31 two-way and 13,272 three-way combinations of polymorphism alleles unique to risk. However, as can be seen in Figure 1, the three risk haplotypes share roughly equivalent levels of sequence similarity in region 2 with two clearly non-risk haplotypes (*HLA-Cw7-B8* and *Cw8-B65*). This visual comparison is confirmed more rigorously by the clustering dendograms of Figure 2. For region 2, the clustering distance separating the non-risk *HLA-Cw7-B8* haplotypes from any of the *HLA-Cw6* risk haplotypes is substantially less than the distance between the *HLA-Cw6* and *HLA-Cw1-B46* risk haplotypes, and the former distance is actually slightly less than that between the two different *HLA-Cw6* haplotypes, which are almost certainly IBD in this region. Furthermore, the non-risk *HLA-Cw8-B65* haplotype is only slightly more different from *HLA-Cw1-B46* in region 2 than is the latter haplotype from the *HLA-Cw6* haplotypes. Figure 2 shows a similar situation for regions 3 and 4. Extended regions of sequence similarity where only a few common haplotypes are observed (haplotype blocks) are commonplace within the MHC (29) and elsewhere in the human genome (30), which makes it difficult to test for identity by descent among these risk haplotypes. The lack of any known expressed genes or single polymorphism alleles unique to risk in region 2 argues strongly against an IBD disease locus in this interval, but a risk-specific haplotype of an unknown gene or of an intergenic regulatory element within the 55 kb defined by region 2 could conceivably be common to all three risk haplotypes, as long as it spans at least 2.3 kb (the minimum interval encompassed by any of the two-way or three-way allelic combinations unique to risk haplotypes).

Phenotype comparisons

We next undertook a comparison of psoriasis phenotype of Thais carrying *HLA-Cw1-B46 vs HLA-Cw6,* under the hypothesis that if the two HLA risk haplotypes share a common causative variant, then the resulting disease phenotype should

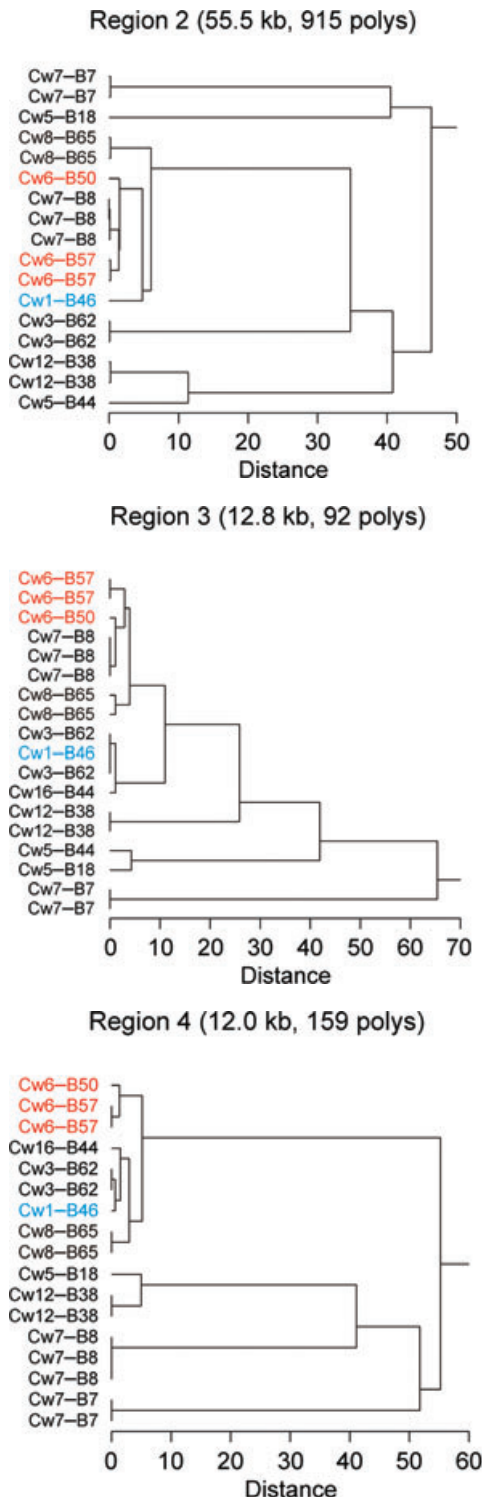

Figure 2 Sequence variation among MHC class I haplotypes within three regions of homology between *HLA-Cw1-B46* and *HLA-Cw6-B57*. Hierarchical clustering dendrograms are shown. *HLA-Cw6* haplotypes are shown in red, and the *HLA-Cw1-B46* haplotype in cyan. The clustering distance metric is per cent disagreement of polymorphism alleles within the region.

be similar in the same genetic population. As shown in Table 4, among the four *HLA-CB* phenotypes significant differences were observed for mean age at onset, toenail involvement, and fingernail involvement ($P = 0.043$, 0.0048, and 0.0070, respectively). No significant differences in TBSA involvement or arthritis were observed ($P = 0.098$ and 0.84, respectively).

Inspection of group means in Table 4 shows that age at onset is about 6 years earlier in the two groups of *HLA-Cw6* carriers (30.0 and 30.2 years) than for either the *HLA-Cw1-B46* only carriers (36.9 years) or carriers of neither risk haplotype (35.3 years). The contrast of the average of the mean transformed age at onset for the two groups of *HLA-Cw6* carriers compared with the average of the mean transformed onset for the two groups of *HLA-Cw6* noncarriers is significant ($P = 0.037$). Conversely, inspection of standardized Pearson residuals for the four *HLA-CB* phenotype groups indicates that greater nail involvement for the two groups of *HLA-Cw1-B46* carriers (36.4% and 26.3% for toenail, 36.4% and 31.6% for fingernail) *vs* lesser involvement for either *HLA-Cw6* only carriers (10.2% and 14.3% for toenail and fingernail) or carriers of neither risk haplotype (15.4% and 14.1% for toenail and fingernail) is largely responsible for the significant test findings of the 4×2 contingency table. Collapsing the contingency table to a 2×2 format based on *HLA-Cw1-B46* carriage yields a strong positive association for both toenail involvement (OR = 3.30, $P = 0.0010$) and fingernail involvement (OR = 3.28, $P = 0.00074$). TBSA trends higher in *HLA-Cw6* carriers (34.2% *vs* 25.5%), and the marginal lack of significance for variation among groups may be because of inadequate power of our Thai sample, as increased TBSA has been shown to be associated with *HLA-Cw6* in Caucasians (31). The findings for arthritis have little meaning given the low incidence of this trait (1.5%) among Thai affecteds in the sample.

Differences of disease phenotype among *HLA-CB* genotypes were similar to those seen among *HLA-CB* phenotypes, with mean age at onset lower in all groups carrying one or more copies of *HLA-Cw6*, and toenail and fingernail involvement higher in all groups carrying one or more copies of *HLA-Cw1-B46* (data not shown). However, only the differences in toenail and fingernail involvement were significant ($P = 0.018$ and 0.024, respectively). The weaker significances for *HLA-CB* genotype compared with *HLA-CB* phenotype may be a simple outcome of subdividing a relatively small sample into six *vs* four categories with a concomitant reduction in power.

Discussion

Genome-wide linkage scans (17, 32) as well as more recent genome-wide association studies (33–36) have made it clear that the major genetic determinant of psoriasis resides within the MHC. We have identified *HLA-Cw6* as the predominant *PSORS1* disease allele in the Caucasian population (6), and

Table 4 Variation of disease phenotype in Thai psoriatics as a function of *HLA-CB* phenotype

HLA-CB phenotype[a]	n[b]	Age at onset[c] (mean ± SE)	TBSA[c] (mean ± SE)	Per cent toenail involvement (mean ± SE)	Per cent fingernail involvement (mean ± SE)	Per cent arthritis (mean ± SE)
Cw1-B46 only	55	36.9 ± 1.6	26.2 ± 2.4	36.4 ± 6.5	36.4 ± 6.5	1.8 ± 1.8
Cw6 only	49	30.0 ± 2.3	34.2 ± 3.6	10.2 ± 4.3	14.3 ± 5.0	0.0 ± 0.0
Cw6 + *Cw1-B46*	19	30.2 ± 3.2	34.2 ± 6.3	26.3 ± 10.1	31.6 ± 10.7	0.0 ± 0.0
Neither	78	35.3 ± 1.8	24.7 ± 2.1	15.4 ± 4.1	14.1 ± 3.9	2.6 ± 1.8
Missing	5	34.8 ± 8.3	28.2 ± 8.9	40.0 ± 21.9	40.0 ± 21.9	0.0 ± 0.0
Total	206	34.0 ± 1.1	28.3 ± 1.5	21.4 ± 2.9	22.3 ± 2.9	1.5 ± 0.8
P[d]		0.043	0.098	0.0048	0.0070	0.84

[a] *HLA-CB* phenotype is based on the carriage of *HLA-CB* haplotypes by the individual, where *HLA-C* is typed to a *Cw1*/*Cw6*/neither level and *HLA-B* to a *B46*/other level; i.e. '*Cw1-B46* only' designates individuals with one or two copies of a *HLA-Cw1-B46* haplotype but no copies of a *HLA-Cw6* haplotype, 'Cw6 only' means carriage of one or two copies of *HLA-Cw6* but no copies of *HLA-Cw1-B46*, '*Cw6* + *Cw1-B46*' means carriage of one *HLA-Cw6* and one *HLA-Cw1-B46* haplotype, 'neither' means carriage of neither a *HLA-Cw6* nor a *HLA-Cw1-B46* haplotype, and 'missing' means the *HLA-CB* haplotypes are unknown because of typing failures. In this sample, *HLA-B46* haplotypes always carry *HLA-Cw1* (see Table 3).

[b] Number of individuals

[c] Mean and standard error for raw variable values are shown, but before analysis data were transformed to approximate normality using the optimal Box–Cox power transformation (power of 0.8 for age at onset and 0.3 for TBSA).

[d] *P*-values for age at onset and TBSA are for one-way ANOVA, based on 10,000 random permutations of the response variable observations; *P*-values for toenail and fingernail involvement and arthritis are for Fisher's exact test on an unordered two-way contingency table. All tests excluded individuals with a missing *HLA-CB* phenotype.

this has been confirmed in the Han Chinese (7). However, considerable evidence indicates that *HLA-Cw6* is not the only psoriasis susceptibility allele in the MHC. Psoriatic arthritis has also repeatedly been associated with *HLA-B38* and *HLA-B39* (splits of *HLA-B16*) (37–43) and with *HLA-B27*, especially when axial involvement is present (39, 40, 42, 44). Moreover, we have recently shown that additional, albeit less genetically robust, association signals are present in the MHC class III region (35, 45). Together with the *HLA-Cw1-B46* association, which is the focus of this study, these findings suggest that genetic heterogeneity is likely to be present at *PSORS1*, with various effects on the phenotype.

Our interest in the *HLA-Cw1-B46* haplotype stemmed from several prior demonstrations of disease association in Asian populations (9, 10, 12, 13, 15, 46). Taking advantage of a collection of Thai psoriasis patients and normal controls independent of those collected previously, we were able to robustly confirm the association of psoriasis with *HLA-Cw6*, *HLA-Cw1*, *HLA-B46,* and the *HLA-Cw1-B46* haplotype in our Thai sample (Table 1). In order to assess whether these associations might be a result of allelic heterogeneity at *HLA-C*, we tested for *HLA-Cw1*-specific associations with psoriasis in the Thai and Caucasian populations. In our Thai sample, haplotypes carrying *HLA-Cw1* but lacking *HLA-B46* showed no significant association with psoriasis (Table 2), but our sample lacks adequate power given the relatively low frequency of this haplotype. However, similar findings, in a Japanese study (11) where *HLA-Cw1* not on *HLA-B46* haplotypes trended toward negative association with psoriasis (OR = 0.45, $P = 0.068$) and in a Thai study (9) where the strength of association of *HLA-B46* with psoriasis (OR = 4.23, $P = 1.4 \times 10^{-6}$) was much greater than that for *HLA-Cw1* (OR = 1.70, $P = 0.083$), increase the likelihood that *HLA-Cw1* is not a direct determinant of psoriasis in East Asians.

In our Caucasian sample, which showed highly significant evidence for association with *HLA-Cw6*, we found no evidence for association with *HLA-Cw1* despite >99% power to detect an association of the strength observed in the Thai population. No occurrences of the *HLA-B46* allele were seen for a large genotyped subset of our Caucasian sample, which includes all *HLA-Cw1* carriers, confirming the specificity of *HLA-Cw1-B46* for Asian populations. While two small studies have reported association of *HLA-Cw1* with psoriatic arthritis (47, 48), this may reflect the fact that *HLA-Cw1* is in linkage disequilibrium with *HLA-B27* in Caucasian populations. However, *HLA-Cw1* was clearly unassociated with 493 psoriatic arthritis cases in our own much larger Caucasian sample ($P = 0.38$), with an effect size nearly identical to that seen for 1,549 purely cutaneous psoriasis cases in the same sample (OR = 0.84 *vs* 0.85, respectively). One other study of 50 pediatric Kuwaiti psoriatics and 120 controls yielded a positive association with *HLA-Cw1*, but no association with *HLA-Cw6* (49). Whether this divergent finding is the result of small sample size, different ethnicity (predominantly Arab), very early onset (<12 years), or the presence of arthritis (which was not reported) remains to be determined. Overall, it appears highly unlikely that *HLA-Cw1* itself is a psoriasis risk determinant in either Thais or Caucasians. Hence another MHC locus, perhaps *HLA-B46* itself, is driving the observed associations with the *HLA-Cw1-B46* haplotype.

Consistent with data presented by others, we noted that *HLA-Cw6* appears to be more strongly associated with psoriasis than is *HLA-Cw1-B46* in the Thai population. While the 95% CI for the two ORs estimated from our sample

overlapped (Table 1), the greater strength of the *HLA-Cw6* association could be statistically established after combining our study with the only other relevant Thai study with allele-based HLA genotyping (10). In addition, three out of four of the older serological studies (11–13) corroborate the greater risk of disease imparted by *HLA-Cw6* compared with *HLA-Cw1-B46* in Asian populations.

To our knowledge there is no evidence for an association between guttate psoriasis and the *HLA-Cw1-B46* haplotype, in contrast to its strong association with *HLA-Cw6* (50). It is also notable that the *HLA-Cw1-B46* haplotype has been associated with other autoimmune diseases, including myasthenia gravis and Graves disease (51), whereas *HLA-Cw6* has not. Moreover, Romphruk et al. reported that *HLA-Cw1-B46* is equally associated with early and late-onset disease in Thai psoriatics, whereas *HLA-Cw6* is more strongly associated with early-onset disease (46). Taken together with the aforementioned difference in strength of association, these findings suggested that the psoriasis susceptibility determinants carried on these two haplotypes are different. We tested this hypothesis in two ways: by performing a sequence analysis of the two haplotypes, and by comparing the phenotypes of known carriers of each haplotype.

Detailed sequence comparison with eight non-risk haplotypes (Figure 1 and Table 3) found no single variants unique to the *HLA-Cw1-B46* and *HLA-Cw6* risk haplotypes within potential IBD regions of homology in the *PSORS1* candidate interval. Although two-way and three-way combinations of variants unique to these two risk haplotypes do exist, they are confined to a 55 kb region that contains no known genes and that has equivalent similarity with two non-risk haplotypes (Figure 2). Nevertheless, based on sequence analysis alone, we cannot completely exclude the possibility that this region contains a variant that is IBD within a regulatory element or a novel expressed gene.

Phenotypic analysis provided additional support for the hypothesis of genetic heterogeneity, although its conclusions must be tempered by sample size considerations. We found that Thai psoriatics carrying *HLA-Cw1-B46* have a later age at onset and greater nail involvement than do carriers of the *HLA-Cw6* risk haplotype (Table 4). However, it is important to note that age at onset correlates with the presence or absence of *HLA-Cw6* but not of *HLA-Cw1-B46*, and likelihood of nail involvement with the presence or absence of *HLA-Cw1-B46* but not of *HLA-Cw6*. Together, the weight of evidence from these sequencing and phenotype comparisons strongly favors the hypothesis that the *HLA-Cw1-B46* and *HLA-Cw6* risk haplotypes do not derive from a common ancestral risk chromosome.

Given these findings in support of genetic heterogeneity, the evolutionary history of *HLA-B46* is of interest. In 1992, Parham and colleagues dissected a complicated serological determinant known as Cw1x3 antigen (also called Cw11, CwB, Cx46, Cw1+3, C-Bangkok, and CSH1). In doing so, they showed that the *HLA-B46* allele is the result of an unusual gene conversion event in which a 31-bp segment of *HLA-Cw1* encoding residues 66–76 of the α1 helix replaced the corresponding sequences of the *HLA-B62* allele (52). Haplotypes containing *HLA-B62* and *HLA-Cw1* are not uncommon in Asian populations (53), supporting the notion of a gene conversion event rather than recombination. *HLA-B62* has a worldwide distribution, whereas *HLA-B46* is specific for Asian populations, showing that *HLA-B62* is the ancestral allele and that the gene conversion occurred in an individual of Asian descent. *HLA-B46* is a common allele in Asian populations, suggesting that this event was followed by marked expansion in the population. Whether this expansion reflects positive selection for pathogen resistance, analogous to the postulated selection for *HLA-Cw6* in resistance to Streptococcal pneumonia (54), is unknown.

The 11 amino acids transferred from *HLA-Cw1* to *HLA-B62* by gene conversion differ from the corresponding residues of *HLA-Cw6* only at amino acid residue 73 (threonine in *HLA-Cw1 vs* alanine in *HLA-Cw6*). Because we have shown here that *HLA-Cw1* is not disease-associated, on the (unproven) hypothesis that this specific segment of *HLA-C* confers disease susceptibility, we could infer that alanine residue 73 is unlikely to be of critical importance, as previously suggested (55). However, there are many other possible explanations for the observed *HLA-B46* association. *HLA-B* and *HLA-C* are very similar to each other, reflecting a relatively recent gene duplication (56). As MHC class I genes, both *HLA-B* and *HLA-C* are involved in the presentation of peptides to CD8+ T cells, whose emigration into the epidermis appears to be necessary for the development of epidermal hyperplastic response (57). One possibility would be that the two alleles could be presenting different antigens. Alternatively, another nearby gene in the MHC class III region could be the causative agent on the *HLA-Cw1-B46* haplotype. Several of these genes are strong functional candidates. For instance, *MICA* and *MICB* are nonclassical MHC genes that participate in the regulation of CD8+ T cells and natural killer (NK) cells (58), and the tumor necrosis factor and lymphotoxin genes encode proteins whose blockade is highly therapeutically effective (59). While our earlier studies of recombinant ancestral haplotypes argue strongly against a primary role for MHC class III genes as the drivers of the *HLA-Cw6* association signal (6, 60), no comparable mapping studies exist as yet for the *HLA-Cw1-B46* disease association in Asians. Thus, at this stage it is premature to speculate that the genetic heterogeneity suggested by our data must involve *HLA-B46* itself, although it is certainly possible.

In conclusion, we have presented several lines of evidence for a distinct *PSORS1* locus in the Thai population. Future genetic studies of this allele in Asian populations should focus on increasing sample size and high-density genotyping of *HLA-B*, its flanking sequences, and the MHC class III region.

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Supporting Information

The following supporting information is available for this article:

Table S1. Amplification and single base extension primers for HLA-C and HLA-B SNPs.

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