Fine Mapping of the Psoriasis Susceptibility Locus

**PSORS1** Supports HLA-C as the Susceptibility Gene in the Han Chinese Population

Xing Fan1,2, Sen Yang1,2, Wei Huang3, Zhi-Min Wang3, Liang-Dan Sun1,2, Yan-Hua Liang1,2, Min Gao1,2, Yue-Qing Ren1,2, Kai-Yue Zhang3, Wen-Hui Du1,2, Yu-Jun Shen1,2, Jian-Jun Liu1,2,4, Xue-Jun Zhang1,2

1 Institute of Dermatology and Department of Dermatology at No. 1 Hospital, Anhui Medical University, Hefei, Anhui, China, 2 The Key Laboratory of Gene Resource Utilization for Genetic Diseases, Ministry of Education, Hefei, Anhui, China, 3 Chinese National Human Genome Center at Shanghai, Shanghai, China, 4 Genome Institute of Singapore, Singapore

Abstract

**PSORS1** (psoriasis susceptibility gene 1) is a major susceptibility locus for psoriasis. Several fine-mapping studies have highlighted a 300-kb candidate region of **PSORS1** where multiple biologically plausible candidate genes were suggested. The most recent study has indicated HLA-Cw6 as the primary **PSORS1** risk allele within the candidate region in a Caucasian population. In this study, a family-based association analysis of the **PSORS1** locus was performed by analyzing 10 polymorphic microsatellite markers from the **PSORS1** region as well as HLA-B, HLA-C and **CDSN** loci in 163 Chinese families of psoriasis. Five marker loci show strong evidence (P < 10-3), and one marker locus shows weak evidence (P = 0.04) for association. The haplotype cluster analysis showed that all the risk haplotypes are Cw6 positive and share a 369-kb region of homologous marker alleles which carries all the risk alleles, including HLA-Cw6 and **CDSN**TTTC, identified in this study. The recombinant haplotype analysis of the HLA-Cw6 and **CDSN**TTTC alleles in 228 Chinese families showed that the HLA-Cw6/**CDSN**TTTC recombinant haplotype is clearly not associated with risk for psoriasis (TNT = 29.57, p = 0.0025) in a Chinese population, suggesting that the **CDSN**TTTC allele itself does not confer risk without the presence of the HLA-Cw6 allele. The further exclusion analysis of the non-risk HLA-Cw6/**CDSN**TTTC recombinant haplotypes with common recombination breakpoints has allowed us to refine the location of **PSORS1** to a small candidate region. Finally, we performed a conditional linkage analysis and showed that the HLA-Cw6 is a major risk allele but does not explain the full linkage evidence of the **PSORS1** locus in a Chinese population. By performing a series of family-based association analyses of haplotypes as well as an exclusion analysis of recombinant haplotypes, we were able to refine the **PSORS1** gene to a small critical region where HLA-C is a strong candidate to be the **PSORS1** susceptibility gene.

Introduction

Psoriasis (OMIM*177900) is a common chronic inflammatory skin disorder affecting approximately 2–5% of Caucasian population [1] and 0.125% of the Chinese population [2]. It has long been widely accepted that psoriasis is a complex disease involving multiple genetic and environmental factors. Past genome-wide linkage analyses have identified nine susceptibility loci (designated **PSORS1** –9) and an additional 13 suggestive linkage loci for psoriasis. Among them, the **PSORS1** locus on 6p21.3 is a well-confirmed major susceptibility locus for psoriasis, which accounts for about 30% to 50% of the genetic contribution to the disease [3,4,5].

Many fine-mapping studies have been performed to refine the localization of the **PSORS1** gene. By using **HLA** types as markers, Schnitt-Egenolf et al [6,7] shown that familial psoriasis is associated with the class I end of the EHS7.1 haplotype. By performing a linkage disequilibrium (LD) mapping in the Caucasian population, Balendran et al [8] suggested a 285-kb critical region for **PSORS1** between the markers tn821 and **HLA-C**. Oka et al [9] further mapped the **PSORS1** gene to a 111 kb interval telomeric to **HLA-C** through an association analysis in the Japanese population. Similarly, Orru et al’s study also highlighted a 70-kb critical region around the **CDSN** gene that is not recombinant with **PSORS1** by identical-by-descent (IBD) haplotyping analysis in a Sardinian population [10]. A 130-kb region telomeric to **HLA-C** was also shown to be associated with psoriasis in a Jewish population [11]. More recently, Lench et al [12] performed a SNP-based association analysis and found strong association to a 46-kb interval telomeric to **HLA-C** in both Caucasian and Japanese populations. Helm et al [13] also performed a comprehensive case/control and family-based association study using SNPs and located the **PSORS1** to a haplotype block harboring **HLA-C**and distinct from **CDSN** and **HCR**. Nair et al [14] carried out a comprehensive analysis of the **MHC** region and narrowed the candidate interval for **PSORS1** to a 224-kb region in an American
Psoriasis is a common skin disease with strong genetic risk. The analysis of psoriatic families with multiple affected individuals has identified several genomic regions that are linked (showing linkage evidence) to the development of psoriasis. Of them, the region on 6p21.3 (PSORS1) is a well-confirmed major risk locus. The identification of the disease risk gene within the PSORS1 locus, however, has been difficult, largely due to the fact that several genes show evidence for association with the disease development and the evidences are highly correlated and hard to be separated from each other. In this study, we performed a fine mapping study of the PSORS1 locus in Chinese families with psoriasis. By analyzing recombinant haplotypes that carry different risk-associated genetic variants within different genes, we were able to separate the genetic effects observed within multiple genes and identify strong supporting evidence for HLA-C to be the primary risk gene of the PSORS1 locus. We have further demonstrated that the genetic variation within the HLA-C gene does not explain the full linkage evidence at the PSORS1 locus, suggesting that there might be other risk genes and/or alleles within the region. Our findings have improved our understanding about the genetic complexity of the PSORS1 locus.

Caucasian population. Taken together, although the results are not totally consistent with each other, these fine-mapping studies generally suggested a critical region of about 300 kb for PSORS1, containing HLA-C and at least 10 other genes.

As the PSORS1 locus contains the major histocompatibility complex (MHC) that is known to be involved in many autoimmune disorders, MHC genes have been intensively evaluated as the candidates for the PSORS1 susceptibility gene. HLA-Cw6 has long been known to be associated with susceptibility to psoriasis [15] and was suggested to be a marker allele in LD with the PSORS1 susceptibility allele because of the lack of its functional role in psoriasis [16,17,18]. However, a recent study indicated that there are distinct differential expression patterns of HLA-C in psoriasis and eczema, suggesting a functional role of HLA-C in psoriasis-related immune response rather than general inflammation [19]. In addition, several other genes within the PSORS1 locus, including MICA [20], CD58 [21,22,23], HCR [24,25,26,27,28], and PSORS1C3 [29,30], were shown to be expressed in skin cells and are, therefore, plausible candidates for the PSORS1 gene. Other genes like SEEK1 and SPR1 genes were also suggested as the candidates for PSORS1 [31]. Through a systematic screening of densely distributed SNPs within a 150-kb region around HLA-C by family-based analysis, Veal et al. [32] identified the strongest association evidence at two SNPs (n. 7 and n. 9) that are located 4 and 7 kb centromeric to HLA-C, respectively. Using the haplotype sharing statistic HSS, Foerster et al. [33] suggested the localization of PSORS1 to a small region telomeric of HLA-C and indicated that an endogenous retroviral dUTPase should be considered a candidate for the PSORS1 gene.

Identification of the PSORS1 susceptibility gene has been difficult due to the existence of extensive LD and multiple biologically plausible candidate genes within the PSORS1 region [34]. Most of the genes within the 300-kb critical region of PSORS1 have been shown to be strongly associated with psoriasis [24,29,31,35,36]. However, the risk alleles of these genes are inseparable from each other due to strong linkage disequilibrium among them. Single marker and haplotype-based association analyses have therefore not been able to determine the primary susceptibility allele. Functional analysis of the known genes within this critical region has also failed to shed light on this problem, because many of these genes have been shown or suggested to be involved in the development of skin tissue and/or the pathogenesis of psoriasis. To overcome this difficulty, Elder and his colleagues genetically dissected the PSORS1 locus by directly sequencing risk-associated haplotypes and subsequently analyzing recombinant haplotypes that can separate the risk-associated alleles of the several genes [14]. They demonstrated that of the eleven genes within the 300-kb critical region of PSORS1, only HLA-C and CD58 carry nonsynonymous alleles (protein alleles) that are unique to the risk haplotypes of the PSORS1 locus, making them the more likely candidates to be the PSORS1 gene. By analyzing the recombinant haplotypes that carry only either the HLA-C or CD58 risk allele, they further demonstrated that HLA-C is more likely to be the PSORS1 susceptibility gene than the CD58 gene.

In this study, we performed the first fine mapping analysis of the PSORS1 locus in Chinese population. The PSORS1 locus was confirmed in Chinese population by our previous genome-wide scan analysis [37]. Our previous case-control study had also shown association of certain class I and II MHC alleles with psoriasis [38]. However, no fine mapping analyses of PSORS1 have been done in Chinese population. By performing both population- and family-based association analysis of haplotypes as well as an exclusion analysis of recombinant haplotypes, we were able to refine the PSORS1 gene to a small critical region where HLA-C is a strong candidate of the PSORS1 susceptibility gene.

Materials and Methods

Ascertainment

A total of 228 Han Chinese psoriasis families, including 61 families used in our previous genome-wide linkage study, as well as additional 192 sporadic cases and 192 healthy controls were recruited in this study. All samples were recruited from the Dermatology Department at No. 1 Hospital of the Anhui Medical University using an ascertainment procedure described previously [37]. The structure and clinical characteristics of all the 228 pedigrees are summarized in Table 1 and Supplementary Table 1. All the samples were recruited with informed consent. The study was approved by the Ethics Committee of Anhui Medical University and conducted according to the Declaration of Helsinki Principles.

Genotyping Analysis

10 microsatellite markers (D6S1660, D6S1691, M6S187, C2_4_5, C2_4_4, C1_3_2, C1_2_6, M6S172, D6S273 and D6S1645) were genotyped in 163 families as well as the 192 sporadic cases and 192 healthy controls. The primer information of the 10 microsatellite markers was obtained from the UniSTS Database (http://www.ncbi.nlm.nih.gov/). Marker order and distances were obtained from the National Center for Biotechnology Information (NCBI) database (the build 36.2). HLA-B antigen alleles were also genotyped in the same 163 pedigrees by using DNA-based methods (PCR-SSP) described elsewhere [39].

HLA-C and CD58 were genotyped in the 163 families and additional 63 affected sib pairs. HLA-C alleles were determined by genotyping seven SNPs within HLA-C using the method developed by Nair et al. [14]. The seven SNPs are located in exons 2 and 3 of the HLA-C gene at positions 213, 218, 341, 361, 387, 459, and 540 (NM_000217.4). HLA-Cw6 genotype can be definitely distinguished from all other alleles by testing the observed genotypes for the seven HLA-C SNPs against those produced by all possible combinations of alleles in the IMGT/HLA database [14].
constructed HLA-C haplotypes were retained if they were the only possible outcome given the genotypes of that person and other family members or if the only other choices involved HLA-C haplotypes known to be very rare in the study population. The CDSN*TTC allele (defined as CDSN*632T-1249T-1256C) was determined by genotyping three SNPs within CDSN. The three polymorphisms within CDSN gene are located at positions 632, 1249, and 1256 of the coding sequence (NM_001264).

Genomic DNA was extracted from peripheral blood leukocytes by using standard procedures [40]. Microsatellite markers were genotyped by using a procedure described previously [41]. Briefly, PCR amplifications were performed in a thermocycler 9700TM (Perkin Elmer, Foster City, CA) and PCR products were pooled together and analyzed on an automated ABI Prism 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). GeneMapper 4.0 software (Applied Biosystems) was used for determining the fragment sizes of alleles that were further reviewed by two persons independently. All SNPs were genotyped by using the Snap-Shot method of the Applied Biosystems. Pedstats [42] was used to check for non-Mendelian inheritance of alleles and Hardy–Weinberg equilibrium in controls. Unlike genotypes were further identified by using Merlin (version 1.0.1) [43] and corrected by reviewing the raw genotyping results or re-genotyping if necessary.

Table 1. Details of Structures of all Pedigrees.

| No. of affected members | No. of nuclear families (2 gen) | Generation Total |
|-------------------------|---------------------------------|----------------|
|                         | triads dyads Sibships 3 gen 4 gen |                |
| 1                       | 2 2 1 0 0 0 0 3                  |                |
| 2                       | 17 14 17 15 13 1 15 60          |                |
| ≥4                      | 2 0 0 11 1 14                   |                |
| total                   | 73 48 80 25 2 228               |                |

Nuclear (2 generation) families are classified as either triads (affected child and one or more family sibs), dyads (affected child and one collected parent), or Sibships (affected child and one or more collected sibs, with or without parents). doi:10.1371/journal.pgen.1000038.t001

Association Test

Single-marker case-control association analysis was performed using the two-tailed chi-squared statistic to compare allele frequency differences. Correction for multiple testing was done by calculating the false discovery rate (FDR) using Benjamini and Hochberg’s method [44]. The odds ratio (OR) was calculated by 2×2 contingency tables.

A family-based transmission/disequilibrium test (TDT) was performed by using either single locus genotypes or the haplotype clusters. For all the pedigrees with at least one heterozygous parent where allele or haplotype transmission could be determined, a single affected child was chosen randomly for performing the TDT test. Inferred genotypes and haplotypes of founder individuals without DNA samples were not used in the TDT test due to the potential bias [45].

Family-based conditional association analysis was performed by using the WHAP program version 2.09 [46]. Single trio was randomly selected from each of the 163 families. The 7 microsatellites were treated as multi-allelic locus by using the –usat command. We first evaluated the association of every marker with disease phenotype after controlling for the genotypes of the surrounding loci and then the omnibus association evidence within the region after dropping the HLA-Cw6 or CDSN*TTC risk allele from the null model.

Recombination Breakpoints Mapping and Exclusion Analysis

The haplotypes of the HLA-Cw6 and CDSN*TTC alleles were constructed in the 228 families by using Merlin and the biallelic genotypes (+/+ , +/− and −/−) of the two loci. The recombinant haplotypes that carry either the HLA-Cw6 or CDSN*TTC risk allele were identified and tested for association with psoriasis in the same 228 families. Subsequently, the recombination breakpoints of the recombinant haplotypes were mapped in the 163 families where 10 microsatellite markers (in addition to the Cw6 and CDSN*TTC alleles) were genotyped. The recombination breakpoints were mapped to the last marker on both sides of the CDSN locus that bears a risk allele, to ensure that these recombinant haplotypes fully retained the portion of the candidate interval being tested for exclusion. TDT test was subsequently performed on the recombinant haplotypes sharing the same breakpoints to assess association between the different portions of the PSORSI candidate region and psoriasis. Due to the low frequency of the HLA-Cw6/CDSN*TTC recombinant haplotypes, only the HLA-Cw6*/CDSN*TTC* recombinant haplotypes were used in the analysis of identifying the maximum portion of the HLA-Cw6*/CDSN*TTC* risk haplotype that is retained by the non-risk HLA-Cw6*/CDSN*TTC* recombinant haplotypes.

Combined Linkage and Association Analysis

To evaluate the contribution of the HLA-Cw6 allele to the linkage evidence observed at the PSORSI locus, we performed the Linkage and Association Modeling in Pedigrees (LAMP) analysis [47] in the 163 families using 7 microsatellite markers surrounding the maximum LOD score peak identified in our previous genome-wide analysis as well as the HLA-C and CDSN loci. LAMP linkage test, direct association test and indirect association test have been performed to get the linkage signal and to assess whether there are other variants that can explain the linkage signal.

Results

Single-Locus Association Analysis

To follow up the linkage evidence at the HLA region (PSORSI) identified by our previous genome-wide analysis [37], we
performed a family-based association analysis by genotyping 10 microsatellite markers from the PSORS1 locus in 163 Chinese pedigrees (including the 61 families used in our genome-wide linkage analysis). As expected, the family-based TDT test revealed strong supporting evidence for our initial linkage finding (Fig 1). Of the 10 microsatellite markers analyzed, 5 markers (M6S107, C2_4_5, C2_4_4, C1_3_2 and M6S172) show strong evidence for association with psoriasis (TDT p<10^-3), and one marker (C1_2_6) yields weak evidence for association (p = 0.0437). The results from the family-based TDT test are consistent with the results from the association analysis of the same 10 microsatellite markers in 192 unrelated cases and 192 controls (Supplementary results from the association analysis of the same 10 microsatellite markers from the PSORS1 locus in 163 Chinese pedigrees). The four risk haplotypes share a 369-kb region of homologous alleles as well as the risk-associated alleles of the 5 microsatellite markers are all present on these four risk haplotypes. The four risk haplotypes seem to be derived from two known ancient risk haplotypes (HLA-Cw6-B57 and HLA-Cw6-B13) [36], and the 369-kb interval is the minimum fragment of the ancestral haplotypes retained by all four risk haplotypes. The clusters 24 and 27 also show moderate evidence for protective effect. However, because the moderate evidence becomes insignificant after correction for multiple testing and the two clusters do not show any allele sharing, the evidence for the cluster 24 and 27 is likely to be false positive.

Haplotype Association Analysis
We then performed a haplotype-based TDT analysis in the 163 families. The haplotypes were constructed using the genotypes of the 7 microsatellite markers showing single-locus association evidence as well as the HLA-B, HLA-Cw6, CDSN*TTC loci. After removing 31 inferred haplotypes, only 699 founder haplotypes from the 163 families were used in the following haplotype clustering and TDT test.

As described in Materials and Methods, the 699 founder haplotypes were grouped into 29 haplotype clusters where the last cluster (cluster 29) holds all the haplotypes that can not be clustered otherwise. All the haplotype clusters, except the cluster 29, were tested for association with psoriasis by using family-based TDT test, and the results are summarized in Table 3. The family-based association analysis of the haplotype clusters was also performed by using FBAT test, and the results are very consistent to the TDT results (data not shown). Of the 28 clusters tested, 4 haplotype clusters (cluster 4, 5, 6, and 7) are associated with risk.

Recombinant Haplotype Analysis
To evaluate which of the risk alleles within the 369-kb critical region is likely to be the primary cause of association, we employed the strategy of recombinant haplotype analysis where different risk alleles as well as the risk-associated alleles of the 5 microsatellite markers are all present on these four risk haplotypes. The four risk haplotypes seem to be derived from two known ancient risk haplotypes (HLA-Cw6-B57 and HLA-Cw6-B13) [36], and the 369-kb interval is the minimum fragment of the ancestral haplotypes retained by all four risk haplotypes. The clusters 24 and 27 also show moderate evidence for protective effect. However, because the moderate evidence becomes insignificant after correction for multiple testing and the two clusters do not show any allele sharing, the evidence for the cluster 24 and 27 is likely to be false positive.

Hence, the haplotype analysis determined a 369-kb critical region for the PSORS1 locus. However, due to the strong linkage disequilibrium within this region, association evidences observed at the HLA-Cw6 and CDSN*TTC alleles as well as other 5 marker alleles are not independent from each other, and the haplotype-based analysis can not discriminate which alleles is the primary cause of association with psoriasis observed within the PSORS1 locus.

Recombinant Haplotype Analysis
To evaluate which of the risk alleles within the 369-kb critical region is likely to be the primary cause of association, we employed the strategy of recombinant haplotype analysis where different risk allele or alleles are evaluated for association separately. First, we
Table 2. Association of Psoriasis with Alleles of HLA-C and CDSN.

| Gene and Haplotype Number | Corresponding Allele(s)* | Haplotype Sequenceb | Frequencyc | T:NT (%T)d | TDT*e |
|---------------------------|--------------------------|---------------------|------------|----------|-------|
| HLA-C                     |                          |                     |            |          |       |
| 1                         | *01(0201–04, 06–13), *02(05), *08(12), *12(0301–07, 09, 11–13,15), *14(0202), and *16(01–0401, 08) | GCCTCCG           | 0.1142     | 12:34(26.1) | 1.0 x 10^-3 |
| 2                         | *01(05), *02(0201–04, 06–16), *03(14), *05(01–14), *07(07, 16), *08(0101–14), *12(0201–0203, 08, 14 and 16–18), *15(0201–17, 16(06–07), and *17(0104) | GCCACCG           | 0.1399     | 35:45(43.8) | 2.6 x 10^-1 |
| 3                         | *03(0201–13, 16, and 18–29) | GCCACTG           | 0.1655     | 19:49(27.9) | 3.0 x 10^-4 |
| 4                         | *0315                     | GCAACTG           | 0.007      | 0.20(0)   | 4.9 x 10^-1 |
| 5                         | *03(17), *14(0201, and 0203–07N, 08) | GCCCTGT           | 0.0536     | 3:22(12.0)  | 1.0 x 10^-4 |
| 6                         | *04(010101–0104, 0401, 05, 08–10, 12–14, and 16–21) | GAAATGT           | 0.0326     | 1:14(6.7)  | 1.0 x 10^-3 |
| 11                        | *06(02–13)                | CCATCCG           | 0.3893     | 137:23(85.6)| 2.0 x 10^-10 |
| 12                        | *07(0101–03, 05–06, 08–10, 13–15, and 17–30, 32N-39) | GCAACCG           | 0.0909     | 10:28(26.3)| 3.0 x 10^-3 |
| 13                        | *07(0401–0402 and 11–12) | CCAACCC           | 0.007      | 2.25(50.0) | 1.0 |
| CDSN                      |                          |                     |            |          |       |
| 1                         | TTT                       |                     | 0.1994     | 41:88(31.5)| 2.0 x 10^-5 |
| 2                         | TTC                       |                     | 0.4662     | 116:41(73.9)| 1.7 x 10^-9 |
| 4                         | TCG                       |                     | 0.1227     | 25:44(36.2)| 2.2 x 10^-4 |
| 6                         | TCT                       |                     | 0.2117     | 49:57(46.2)| 4.4 x 10^-1 |

*HLA-C allele designations follow the classification scheme of release 2.14.1 (Aug 2006) of the IMGT/HLA Sequence Database maintained by the HLA Informatics Group of the Anthony Nolan Research Institute. CDSN allele designations follow the classification scheme of Romphruk et al.[49].

†Haplotype for seven coding SNPs of HLA-C (mRNA positions 213, 218, 341, 361, 387, 459, and 540) and three missense polymorphisms of CDSN (mRNA positions 632, 649, and 714), in 5'→3' orientation.

‡Haplotype frequency, based on 889 founder chromosomes genotyped in 228 pedigrees for HLA-C and CDSN.

§For the multi-allelic TDT.

All P values are uncorrected for multiple testing.

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evaluated which of the HLA-Cw6 or CDSN*TTC allele is more likely to be the primary PSORS1 risk allele, by analyzing the recombinant haplotypes that have segregated the HLA-Cw6 and CDSN*TTC risk alleles in the 163 families as well as additional 65 affected sib pairs. After discarding 101 (10.2%) phase unknown haplotypes, the 889 founder haplotypes of the HLA-Cw6 and CDSN*TTC alleles from the 228 pedigrees were analyzed by TDT test (Table 4). Of the 889 founder haplotypes, HLA-Cw6/ CDSN*TTC and HLA-Cw6/CDSN*TTC alleles are both common with 32.6% and 52.6% frequency, respectively. As expected, the HLA-Cw6/CDSN*TTC haplotype is strongly associated with risk for psoriasis (T:NT = 129:34, p = 9.9 x 10^-6), whereas the HLA-Cw6/CDSN*TTC haplotype is clearly not associated (T:NT = 50:117, p = 2.2 x 10^-4). The HLA-Cw6/CDSN*TTC recombinant haplotype is also common (12.9%) and is surely not associated with risk for psoriasis (T:NT = 29:57, p = 0.0025). The HLA-Cw6/CDSN*TTC recombinant haplotype is rare (1.8%) so that its association with risk cannot be determined (Table 4). This result strongly indicates that the CDSN*TTC allele itself does not confer any risk without the presence of the HLA-Cw6 allele and further suggests that the PSORS1 gene is likely to be located nearby HLA-C than CDSN within the 369-kb critical region.

To further refine the location of the PSORS1 gene in Chinese population, we then performed an exclusion analysis within the 369-kb critical region (between HLA-B and M6S187), as demonstrated in Nair et al’s study [14]. We first mapped the breakpoints of ancestral recombination between the HLA-Cw6 and CDSN*TTC alleles in the 163 families. By analyzing the genotypes of 7 markers (HLA-Cw6, CDSN*TTC and 5 microsatellite markers) within the 369-kb region, 103 recombinant haplotypes between HLA-Cw6 and CDSN*TTC loci were identified (among the 699 founder chromosomes) in the 163 families, including 7 HLA-Cw6/CDSN*TTC haplotypes and 98 HLA-Cw6/CDSN*TTC haplotypes (Table 6). Of the observed 105 recombinant haplotypes, 32 (30.5%) of the recombination breakpoints are mapped to the 48-kb region between HLA-C and M6S172, and 67 (63.8%) are mapped to the 92-kb region between M6S172 and C1_3_2. Only 6 (5.7%) recombination breakpoints are mapped to the 7-kb interval between C1_3_2 and CDSN. Hence, the recombination events between HLA-C and CDSN loci appear to be unevenly distributed and are largely limited to the 140-kb interval between HLA-C and C1_3_2 in the Chinese population. We then performed an exclusion analysis within the 369-kb critical region by identifying the maximum portion of the HLA-Cw6/
CDSN*TTC risk haplotype that is retained by the non-risk HLA-Cw6 / CDSN*TTC recombinant haplotypes. As shown in Table 7, the HLA-Cw6 / CDSN*TTC recombinant haplotypes that carry the C1_3_2-C2_4_5 portion of the HLA-Cw6 / CDSN*TTC risk haplotype are clearly not associated with risk to psoriasis (T:NT = 28:47, p = 0.028). It is also true for the HLA-Cw6 / CDSN*TTC recombinant haplotypes that carry the C1_3_2-C2_4_5 portion of the HLA-Cw6 / CDSN*TTC risk haplotype that is retained by the non-risk HLA-Cw6 / CDSN*TTC recombinant haplotypes that carry the C1_3_2-C2_4_5 portion of the HLA-Cw6 / CDSN*TTC risk haplotype (T:NT = 27:45, p = 0.034). For the HLA-Cw6 / CDSN*TTC recombinant haplotypes that carry the M6S172-CDSN portion of the HLA-Cw6 / CDSN*TTC risk haplotype, our data suggested an under-transmission of the recombinant haplotypes (T:NT = 11:20, p = 0.11), but the evidence is not statistically significant. Therefore, our results provided significant evidence for supporting the exclusion of the C1_2_6-C2_4_5 interval from the 369-kb critical region of the PSORS1 locus. Our result also provides suggestive evidence to further exclude the M6172-C1_2_6 interval, but statistical evidence for this exclusion is moderate and needs to be confirmed by further study.

**Combined Linkage and Association Analysis of the HLA-Cw6 Allele**

Finally, we evaluated the contribution of the HLA-Cw6 risk allele to the linkage evidence observed at the PSORS1 locus. We performed a LAMP analysis in the 163 families by using the HLA-Cw6 and CDSN*TTC alleles as well as the 7 microsatellite markers. As expected, the LAMP analysis revealed highly significant evidences for both linkage (LOD = 5.19, p = 2.6×10⁻⁵) and association (LOD = 45.88, p = 7.2×10⁻⁴⁰), and both the evidences are mapped to the HLA-Cw6 locus. After controlling for the effect of the HLA-Cw6 Allele, the CR results are shown in Table 3. This analysis supports the genetic evidence for the HLA-Cw6 allele, but demonstrates that further genetic evidence for the HLA-Cw6 allele is required before this allele can be excluded from the candidate gene list.
PSORS1 is a major contributor to the linkage evidence observed at the B57 HLA-Cw7-B58 what has been found by previous studies in other populations. The present in the Chinese population.

B13-Cw1 associated alleles of the 5 marker loci as well as identified a 369-kb critical region for with psoriasis. Allele comparison among all the risk haplotypes has likely derived from two known ancient risk haplotypes (14).

Discussion

We have done the first fine mapping analysis of the PSORS1 locus in Chinese population. Our single-locus association analysis provides strong supporting evidence for the PSORS1 locus in a Chinese population and reveals a similar association pattern across the PSORS1 region to that demonstrated in various populations. Our haplotype-based association analysis indicates that all the risk haplotypes within the PSORS1 locus are HLA-C6b positive and likely derived from two known ancient risk haplotypes (HLA-C6b-B57 and HLA-C6b-B13) in the Chinese population, consistent with what has been found by previous studies in other populations. The HLA-C6b-B58 haplotype, which was found to be positively associated with psoriasis in a Sardinian population [10], was not present in the Chinese population. B13-C6a1 and B13-C6a3 haplotypes are present in our Chinese samples, but the sample size of these two haplotypes was too small to test for association with psoriasis. Allele comparison among all the risk haplotypes has identified a 369-kb critical region for PSORS1 where all the risk-associated alleles of the 5 marker loci as well as HLA-C and CDSN loci are carried by the identified risk haplotypes, reflecting the extensive LD across the PSORS1 region in the Chinese population as reported in other populations. The 369-kb critical region identified in Chinese population is very similar to the 300-kb critical region identified in a western population by similar methods [14].

Within the critical region, HLA-C and CDSN are the two leading candidates for the PSORS1 gene both with strong supporting evidence. HLA-C6b allele is the most significant marker for psoriasis risk prediction and has been indicated to be involved in the psoriasis-related immune response [19]. CDSN is the second extensively investigated candidate and codes corneodesmosin involved in keratinocyte cohesion and desquamation [48]. To evaluate which of the two genes is more likely to be the candidate, we performed the association analysis of the recombinant haplotypes that carry only one of the two risk alleles of the two genes. The frequencies of these two risk alleles in Chinese population are 39% and 47%, respectively, which are slightly higher than their frequencies in Caucasian population (21% and 23%, respectively) [14]. The frequency of the recombinant haplotypes of the HLA-C6b and CDSN alleles is also higher in Chinese psoriatic families (14.7%) than in Caucasian psoriatic families (2.7%) [14]. The majority of the recombinant haplotypes are HLA-C6b / CDSN+ alleles (12.9%) that are clearly not associated with disease phenotype, suggesting that without the HLA-C6b allele, CDSN does not confer any risk. This result is further supported by our conditional association analysis where only the HLA-C6b allele shows independent effect from the genotypes of the surrounding marker loci including the CDSN allele. Therefore, our results have clearly excluded CDSN as the PSORS1 susceptibility gene, which is consistent with the conclusion from Nair et al’s study in Caucasian population [14].

We then attempted to further narrow down the location of the PSORS1 gene within the 369-kb critical region by mapping the recombination breakpoints and performing an exclusion analysis, as illustrated in Nair et al’s study [14]. The common region of the recombination breakpoints between HLA-C and CDSN loci in Chinese population (HLA-C to C1_3.2) is partially overlapped with the one observed in Caucasian population (M6S169 to CDSN) where over 90% of recombination breakpoints were mapped [14]. In Caucasian population, most of the recombination breakpoints between HLA-C and CDSN loci are located within the region telomeric to C1_2_6 (M6S111) (39.5%), whereas in Chinese population, most of the recombination breakpoints are located within the region centromeric to C1_2_6 (about 86.7%). Our results can exclude the C1_2_6-C2_4_5 interval from the 369-kb critical region of PSORS1 with confidence. For the HLA-C6b / CDSN alleles recombinant haplotypes that carry the M6S172-C2_4_5 portion of the HLA-C6b / CDSN alleles risk haplotype, our result also indicates an under-transmission of the haplotypes (T:NT = 11:20, p = 0.11), but the evidence is not statistically significant. Therefore, we can narrow down the PSORS1 locus to the 172-kb region between HLA-B and C1_2_6 and potentially to the interval between HLA-B and M6S172, if the exclusion of the M6S172-C2_4_5 interval can be confirmed by further study in Chinese population. The more centromeric location of the recombination breakpoints in Chinese population (than in Caucasian population) allows us to narrow down the location of the PSORS1 gene into a smaller region than Nair et al’s study [14].

Within our conservatively mapped critical region of the PSORS1 between HLA-B and C1_2_6, there are only two known genes HLA-C and HCG27. It has been confirmed by many studies, including our present study, that the HLA-C6b allele is a strong

### Table 4. Association of Psoriasis with Recombination Haplotypes of HLA-Cw6 and CDSN*TTC Alleles.

| haplotype        | Frequency | TDT       |
|------------------|-----------|-----------|
|                  |           | T:NT (%T) | p^2      |
| HLA-Cw6/CDSN*TTC | 0.3262    | 129.34(79.1) | 9.9 x 10^-14 |
| HLA-Cw6/CDSN*TTC | 0.0180    | 5.4(55.6)  | 7.4 x 10^-1  |
| HLA-Cw6/CDSN*TTC | 0.1294    | 29.57(33.7) | 2.5 x 10^-3  |
| HLA-Cw6/CDSN*TTC | 0.5264    | 50.117(29.9) | 2.2 x 10^-7  |

^a Frequency of founder haplotypes are based on 889 chromosomes genotyped in 228 pedigrees.

^b For the biologic TDT.

^c Uncorrected exact binomial P value for TDT.

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### Table 5. Results of Conditional Association Analysis.

| locus  | Single marker analysis | Omnibus test excluding everything else | Omnibus test after controlling for locus |
|--------|------------------------|--------------------------------------|----------------------------------------|
| D5S273 | 1.9 x 10^-4            | 0.79                                 | –                                      |
| HLA-C  | 1.9 x 10^-4            | 9.9 x 10^-4                          | 0.14                                   |
| M6S172 | 2.9 x 10^-4            | 0.08                                 | –                                      |
| C1_2_6 | 3.1 x 10^-4            | 0.36                                 | –                                      |
| C1_3_2 | 1.9 x 10^-3            | 0.95                                 | –                                      |
| CDSN   | 3.9 x 10^-3            | 0.23                                 | 0.03                                   |
| C2_4_4 | 3.9 x 10^-3            | 0.39                                 | –                                      |
| C2_4_5 | 7.4 x 10^-4            | 0.08                                 | –                                      |
| M6S187 | 4.3 x 10^-1            | 0.39                                 | –                                      |

Empirical p-value from 1000 permutations were provided.
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haplotypes by Nair et al [14] in Caucasian population indicated sequence analysis of two risk haplotypes and eight non-risk to be the primary susceptibility gene for psoriasis. Direct susceptibility allele for psoriasis. But, there is limited evidence for susceptibility gene of the provided strong supporting evidence for region. Therefore, our recombination mapping analysis has identify any evidence of known microRNAs within this critical region. However, there is no supporting evidence for them to be functional as either coding or regulatory sequences. Searching in the UCSC Genome Browser (May 2006 version) also failed to find any conserved non-coding sequences within the critical region. Besides these two known genes, there are a few ESTs as well as a few non-coding sequences within the critical region. Therefore, our recombination mapping analysis has provided strong supporting evidence for HLA-C to be the primary susceptibility gene of the PSORS1 locus.

Table 6. 105^a Recombinant Haplotypes between the Risk Alleles of HLA-Cw6 and CDSN^*TTC.

| Haplotype Type | cluster | Alleles^b |
|----------------|---------|-----------|
|                |         | HLA-B     | HLA-C | M6S172 | C1_2_6 | C1_3_2 | CDSN | C2_4_4 | C2_4_5 | Number |
| Cw6+/CDSN^*TTC^c | 1       | 13/57    | 2     | 1   | 1/4    | 2     | TTC   | 2     | 3     |         |
| Cw6+/CDSN^*TTC- | 1       | 13       | 2     | 1   | 1/4    | 2     | -     | -     | -     | 1       |
|                 | 2       | 13/57    | 2     | 1   | 1/4    | -     | -     | -     | -     | 2       |
|                 | 3       | -        | 2     | 1   | -      | -     | -     | -     | -     | 2       |
|                 | 4       | 13       | 2     | -   | -      | -     | -     | -     | -     | 2       |
| Cw6-/CDSN^*TTC+ | 1       | -        | -     | -   | TTC    | 2     | 3     | 3     |       |         |
|                 | 2       | -        | -     | -   | TTCC   | 2     | 3     | 7     |       |         |
|                 | 3       | -        | -     | 1/4 | TTCC   | 2     | 3     | 52    |       |         |
|                 | 4       | -        | 1     | 1/4 | TTCC   | 2     | 3     | 29    |       |         |
|                 | 5       | -        | -     | -   | -      | TTCC  | -     | 2     |       |         |
|                 | 6       | -        | -     | -   | TTCC   | 2     | -     | 1     |       |         |
|                 | 7       | -        | 4     | 2   | TTCC   | 2     | -     | 3     |       |         |
|                 | 8       | -        | 1     | 1   | 2      | TTCC  | 2     | 4     |       |         |

^aOf the 699 founder chromosomes from 163 pedigrees.
^bShow all the risk alleles within the 369-kb critical region carried by all the Cw6+/CDSN^*TTC+ risk haplotypes
^cBold alleles are the risk alleles within the 369-kb critical region of the Cw6

Table 7. Association Analysis of the HLA-Cw6^-/CDSN^*TTC^- Recombinant Haplotypes Carrying Different Portions of the HLA-Cw6^-/CDSN^*TTC^- Risk Haplotype.

| Test region^c | number | Frequency^d |
|---------------|--------|-------------|
|               | T:NT (%T)^e | chi | p^f |
| CDSN-C2_4_5   | 91     | 0.1302      | 28.49/36.3 | 5.7 | 1.7x10^-2 |
| C1_3_2-C2_4_5 | 88     | 0.1259      | 28.47/37.3 | 4.8 | 2.8x10^-2 |
| C1_2_6-C2_4_5 | 81     | 0.1159      | 27.45/35.7 | 4.5 | 3.4x10^-2 |
| M6S172-C2_4_5 | 29     | 0.0415      | 11%/35.5   | 2.6 | 1.1x10^-3 |

^cTo be conservative, the smallest possible portion of the extended risk haplotype carried by HLA-Cw6^-/CDSN^*TTC- recombinant haplotypes were used to determine whether a recombinant haplotype qualified for assessing the exclusion of the test region from the PSORS1 candidate interval.
^dFrequency of HLA-Cw6^-/CDSN^*TTC- recombinants carrying the test region; results are based on 699 founder chromosomes in 163 pedigrees.
^eFor the biallelic TDT.
^fUncorrected exact binomial P value for TDT.

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HLA-Cw6 is a major contributor to the linkage and association evidence observed at the PSORS1 locus, but may not be the only risk allele within the PSORS1 locus. The importance of the HLA-Cw6 allele is demonstrated by the fact that after controlling for the effect of the HLA-Cw6 allele, we could no longer identify significant association evidence at the PSORS1 locus by the family-based test, and the supporting evidence for linkage to the PSORS1 locus has also been dramatically reduced. However, the HLA-Cw6 allele cannot fully explain all the linkage evidence, because the linkage evidence for the PSORS1 locus is still significant after controlling for the effect of the HLA-Cw6 allele. This result can be explained by two possibilities. The HLA-Cw6 is not the only risk allele, and there are additional risk allele(s) within the region. Alternatively, the HLA-Cw6 is only a marker allele and therefore indirectly associated with disease phenotype through LD with an unidentified causal allele. Although we can detect residual linkage evidence after controlling the effect of the HLA-Cw6 allele, we could not identify independent association evidence from the HLA-Cw6 allele in our family-based association study. This could be because our conditional family-based association analysis does not have sufficient power for detecting the residual association evidence due to the moderate number of informative families for family-based association test. It is very interesting to see that the HLA-Cw6 can only partially explain the observed linkage evidence at the PSORS1 locus, but the result needs to be confirmed by more studies.

Our fine mapping result is only partially consistent with the ones from previous studies. The minimal candidate region of PSORS1 mapped by our study overlaps the regions mapped in an American Caucasian population [14] and a Jewish population [11], but is different from the ones mapped by other studies in Caucasian, Japanese and Sardinian psoriatic populations [9,10,12] (Fig 2). This difference could be due to the existence of different risk haplotypes in other populations. For example, the HLA-Cw7-B58 recombinant was found to be positively associated with psoriasis in Sardinian samples but not present in our Chinese samples. The
difference could also be due to the employment of different methodologies for fine mapping analysis. Another explanation is that there might be different susceptibility alleles in different populations. Giving the fact that psoriasis is a chronic inflammatory skin disorder and that many genes within the PSORS1 region are known to be involved in autoimmune response and skin development, it is not implausible that there might be more than one gene within this region to play a role in the pathogenesis of psoriasis. It is therefore possible that different ethnic populations might carry different susceptibility alleles from different genes and that the results from the fine mapping studies done in different populations are not always consistent with each other due to the genetic heterogeneity of the PSORS1 locus. Further studies will be needed to confirm this hypothesis.

Supporting Information

Table S1  Clinical details of all pedigrees.

Supporting Information

Table S2  Results of single-marker association study.

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Author Contributions

Conceived and designed the experiments: SY WH JL XZ. Performed the experiments: XF ZW LS YL KZ. Analyzed the data: XF JL. Contributed reagents/materials/analysis tools: SY MG YR WD YS. Wrote the paper: XF JL XZ.
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