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Association analyses identify six new psoriasis susceptibility loci in the Chinese population

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COMPETING INTERESTS STATEMENT

The authors declared no competing financial interests.

AUTHOR CONTRIBUTIONS

X.-J.Z. conceived of this study and obtained financial support. X.-J.Z., S.Y. and L.-D.S. designed the study. H.C., Z.-X.W., A.-P.Z. and P.-G.W. participated in the design and were responsible for sample selection, genotyping and project management. J.T.E., A.F., A.M.B., J.-H.X., Q.-X.Z., F.-R.Z., X.-M.P., X.-Q.Y., J.-Z.Z., A.-E.X., R.-N.W., L.-M.X., M.W., G.-S.L., Z.-M. L., H.-M.G., W.-Z.Y., Cheng Zhang, B.-Q.Y., Y.-Y.S., S.-S.L., Y.L., J.-H.J., C.-T.L., R.-X.C., X.J., P.Z., W.-M.S., J.T., H.-Q.Z., L.S., J.C., L.-J.Z., B.T., F. H., Q.Q., X.-P.P., A.-M.Z., L.-M.S. and J.-L.L. conducted sample selection and data management, undertook recruitment, collected phenotype data, undertook related data handling and calculation, managed recruitment and obtained biological samples. S.-M.Z., H.-Y.W., X.F., G.C., P.L., J.Z., H.-S.Z., T.T., and S.-K.S. performed genotyping analysis. Y.-Q.R., Chi Zhang, R.P.N., P.E.S., E.B., J.-J.L., F.-Y.Z., and W.-D.D. undertook data processing, statistical analysis and bioinformatics investigations. C.A.H. performed analyses and designed genotyping assays for the 254 nuclear families, which was performed by L.P. and A.M.B. All the authors contributed to the final paper, with X.-J.Z., S.Y., L.-D.S., H.C., Z.-X.W., J.T.E, P.E.S., A.M.B., A.F., A.-P.Z., and P.-G.W. playing key roles.

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Abstract

We extended our previous GWAS for psoriasis with a a multistage replication study including 8,312 cases and 12,919 controls from China as well as 3,293 cases, 4,188 controls from Germany and the USA, and 254 nuclear families from the USA. We identified 6 new susceptibility loci associated to psoriasis in Chinese, containing candidate genes *ERAP1*, *PTTG1*, *CSMD1*, *GJB2*, *SERPINB8*, *ZNF816A* ($P_{Combined}<5\times10^{-8}$) and replicated one locus 5q33.1 *(TNIP1/ANXA6)* previously reported ($P_{Combined}=3.8\times10^{-21}$) in European studies. Two of these loci showed evidence for association evidence in the German study, at *ZNF816A* and *GJB2* with $P=3.6\times10^{-3}$ and $P=7.9\times10^{-3}$, respectively. *ERAP1* and *ZNF816A* were preferentially associated with Type I (early onset) psoriasis in Chinese Han population (test for heterogeneity $P=6.5\times10^{-3}$ and $P=1.5\times10^{-3}$, respectively). Comparisons with previous GWAS of psoriasis highlight the heterogeneity of disease susceptibility between Chinese and European populations. Our study identifies new genetic susceptibility factors and suggests new biological pathways in psoriasis.

Psoriasis is a chronic inflammatory hyperproliferative skin disease affecting up to 3% of the population, with considerable ethnic variation. Family-based analyses and population-based epidemiological studies have confirmed the genetic basis of psoriasis[1] and suggested a genetic influence upon its age at onset[2]. In addition to earlier linkage analyses, genome-wide and candidate gene association studies have implicated many genomic regions in the pathogenesis of psoriasis[3–6], not all of which have been confirmed. However, these genetic signals do not fully account for the observed variation in genetic liability to psoriasis, suggesting that additional genetic factors remain to be discovered. Furthermore, differences in the results of similarly-powered GWAS of psoriasis in Chinese[5] and European[4] populations suggest the existence of allelic and/or locus heterogeneity between different populations.

To explore additional susceptibility factors for psoriasis, we performed a multistage association study based on our first-stage GWAS (including our initial GWAS samples reported in ref. 5 [5] and an additional 95 healthy controls, see **Online Methods)**, followed by replication in 6 independent cohorts consisting of Chinese Han (Replications 1 and 2), Chinese Uygur (Replication 3), as well as European population from Germany (Replication 4) and the United States (Replications 5 and 6). We further genotyped SNPs from previously

reported loci in European population in the samples of Replication 2 to investigate the possibility of genetic heterogeneity of disease susceptibility between different populations. The experimental design is summarized in Figure 1.

In Replication 1, 61 SNPs from 51 regions were selected in the GWAS dataset (see **Online Methods**), and genotyped in an independent sample of 4,610 cases and 5,373 controls from Chinese Han population (Supplementary Table 1). Combined analysis (GWAS and Replication 1) showed that 4 SNPs met the genome-wide significant threshold ($P<5.0\times10^{-8}$): rs3762999 at 5q33.1 (*TNIP1*/*ANXA6*), $P_{Combined}=1.1\times10^{-12}$, OR=1.24; rs999556 at 5q33.1 (*TNIP1*/*ANXA6*), $P_{Combined}=4.3\times10^{-13}$, OR=1.24; rs2431697 at 5q33.3 (*PTTG1*), $P_{Combined}=1.1\times10^{-8}$, OR=1.20; rs3751385 at 13q12.11 *(GJB2*), $P_{Combined}=1.7\times10^{-10}$, OR=0.85.

After applying standard quality-control procedures as described elsewhere[5], 20 SNPs with $P<0.05$ from Replication 1 were genotyped in another independent cohort of 2,024 cases and 5,495 controls of Chinese Han (Replication 2, Supplementary Table 1). In the joint association analysis using samples from GWAS, Replication 1 and Replication 2, 9 SNPs annotated in 7 loci reached genome-wide significance: rs151823 at 5q15 (*ERAP1*), $P_{Combined}=9.3\times10^{-9}$; rs999556/rs3762999 at 5q33.1 (*TNIP1*/*ANXA6*), $P_{Combined}=3.8\times10^{-21}$ and $P_{Combined}=4.6\times10^{-18}$; rs2431697 at 5q33.3 (*PTTG1*), $P_{Combined}=1.1\times10^{-8}$; rs10088247/rs7007032 at 8p23.2 (*CSMD1*), $P_{Combined}=4.5\times10^{-9}$ and $P_{Combined}=3.8\times10^{-8}$; rs3751385 at 13q12.11 (*GJB2*), $P_{Combined}=8.6\times10^{-8}$ ($P_{GWAS+Replication1}=1.7\times10^{-10}$); rs514315 at 18q22.1 (*SERPINB8*), $P_{Combined}=5.9\times10^{-9}$; and rs9304742 at 19q13.41 (*ZNF816A*), $P_{Combined}=2.1\times10^{-9}$ (Table 1). All of these 9 SNPs showed consistent association evidence independently in each replication panel and in the combined analysis of Chinese Han. The association at rs3751385, however, showed significant heterogeneity ($I^2=50\%$) between the GWAS/replication 1 and 2 samples (Supplementary Table 2). Consequently, the analysis of the combined GWAS+R1+R2 samples (under a random effect model) yielded less significant association ($P_{Combined}=8.57\times10^{-8}$) than the analysis of the GWAS+R1 samples ($P_{Combined}=1.7\times10^{-10}$). The association at rs3751385 in the GWAS+R1 samples satisfied the genome-wide criteria for significance, although the association in the GWAS+R1+R2 samples was barely below the genome-wide significance. In addition, SNP rs2431697 reached genome-wide significance ($P=1.11\times10^{-8}$) in the combined results of GWAS and Replication 1, although its genotyping failed in Replication 2. Association results of the other 52 SNPs are summarized in Supplementary Table 3.

Of the 9 SNPs, two (rs999556 and rs3762999) in strong linkage disequilibrium (LD) with each other ($r^2=0.97$) were located in the same region (*TNIP1*/*ANXA6)* as the SNP rs17728338, which is associated with psoriasis in European population[4]. Multivariate logistic regression analysis showed that the association of rs999556 and rs3762999 was dependent upon association at rs17728338 in our GWAS dataset, providing evidence that all three SNPs detect the same disease association in the Chinese Han population. To address this question in European population, we performed multivariate logistic regression analysis using two European GWAS samples that are, in part, subsets of replication 4 and 5. The results showed that of these three SNPs, only SNP rs17728338 was strongly associated with psoriasis in the European population ($P=1.7\times10^{-8}$), SNP rs999556 was not associated ($P=0.96$), SNP rs3762999 was also not associated ($P=0.062$), and the association with SNPs rs999556 and rs3762999 was dependent upon the association with rs17728338. Thus although LD in this region is apparently more extensive in Chinese than in European population, SNP rs17728338 is the best known tag for the *TNIP1/ANXA6* susceptibility locus for psoriasis in both populations.

To further assess the impact of the 6 new loci on genetic risk for psoriasis in diverse populations, we analyzed genotyping data from four additional cohorts: Chinese Uygur (Replication 3: 539 cases, 824 controls), Germany (Replication 4: 823 cases, 1,840 controls) and the U.S. (Replication 5: 2,470 cases, 2,348 controls and Replication 6: 254 nuclear families described elsewhere[7–9]). Interestingly, the C allele of rs9304742 in the *ZNF816A* region showed a consistent protective effect among Chinese Han ($P=2.1\times10^{-9}$, OR=0.88), Chinese Uygur ($P=1.7\times10^{-3}$, OR=0.77), and Germans ($P=7.9\times10^{-3}$, OR=0.84); suggestive evidence for a protective effect of the A allele of rs11084211 (which is in strong LD with the C allele of rs9304742) was also seen in the U.S. cohort from Replication 5 ($P=1.6\times10^{-2}$, OR=0.90) (Table 2). In addition, rs151823 at 5q15 (*ERAP1*) showed consistent association ($P=2.9\times10^{-5}$, OR=0.69) in the Chinese Uygur sample, and rs3751385 at 13q12.11 (*GJB2*) showed consistent association ($P=3.6\times10^{-3}$, OR=0.79) in the German sample. However, in the combined analysis of the Germany and US case-control samples (see **Online Methods**), none of these newly-associated SNPs conferred significant risk for psoriasis, although it was not possible to test association with *ZNF816A* (Table 2). Indeed, all the six loci showed significant heterogeneity between the Chinese and Caucasian samples ($I^2>0.40$, Supplementary Table 4).

Because the *PTTG1* SNP rs2431697 is located fairly close to *IL12B* (within ~1.1 Mb), we also carried out a multivariate logistic regression analysis conditioning on the genetic effect of both rs7709212 and rs3213094 within the *IL12B* locus in our initial GWAS, and found that rs2431697 still showed significant association in the Chinese Han population ($P=2.7\times10^{-3}$). This suggests that effects of both loci are independent. In contrast, no association was seen for *PTTG1* in the European studies (Table 2), whereas *IL12B* is strongly associated[4].

In an attempt to uncover susceptibility genes underlying each of the association signals, we investigated the patterns of recombination and LD around the associated SNPs and identified the gene(s) located within each region of LD (Supplementary Fig. 1). Of these 9 SNPs, 7 were within 6 genes or LD blocks at 5q15 (*ERAP1*), 5q33.3 (*PTTG1*), 8p23.2 (*CSMD1*), 13q12.11 (*GJB2*), 18q22.1 (*SERPINB8*), 19q13.41 (*ZNF816A*), where a single gene was found within the LD block harboring the association. The remaining two SNPs located at 5q33.1, where two genes (*TNIP1* and *ANXA6*) were identified in the LD region. *TNIP1* has been considered as a susceptibility gene for psoriasis in European populations due to its important role in working downstream of TNF-α to negatively regulate NF-κB[4] and the fact that its encoded protein binds to the product of another psoriasis gene (*TNFAIP3*). However, *ANXA6* might also be implicated in the pathogenesis of psoriasis via early and late stages of epidermal growth factor receptor (*EGFR*) downregulation[10].

To further explore the potential biological implications of the other six newly discovered susceptibility genes (*ERAP1*, *PTTG1*, *CSMD1*, *GJB2*, *SERPINB8*, *ZNF816A*), we performed a literature search. *ERAP1* (Endoplasmic reticulum aminopeptidase 1) is an interferon-γ-induced aminopeptidase with several proposed biological functions in the endoplasmic reticulum, including the trimming of peptide antigens to optimal length for binding to MHC class I molecules[11]. Furthermore, *ERAP1* is also associated with ankylosing spondylitis, another major Class I-associated autoimmune disease, in both Caucasian and Chinese populations[12,13]. Given that *HLA-Cw6* is much more strongly associated with Type I than with late-onset (Type II) psoriasis in both Chinese and European population[14], it is of interest that *ERAP1* was preferentially associated with Type I psoriasis in the combined Chinese Han datasets (rs151823, $P=6.5\times10^{-3}$, OR=0.88, Supplementary Table 5).

PTTG1 (Pituitary tumor transforming gene) encodes a multifunctional protein with roles in the control of mitosis, cell transformation, DNA repair, and gene regulation[15], with in vitro

angiogenic activity[16], at least in part through the regulation of *VEGF*[17], which in turn has been reported to be overexpressed psoriasis plaques[18]. Notably, the *TNFAIP3, TNIP1,* and *PTTG1* regions are now genetically implicated in both psoriasis and systemic lupus erythematosus (SLE)[4,19,20].

CSMD1 (CUB and Sushi multiple domains 1) is a tumor suppressor gene expressed in areas of regenerative growth such as skin and epithelial cells[21]. Higher expression levels of *CSMD1* correlated with tumor differentiation, suggesting that it could contribute to the pathogenesis of psoriasis by influencing keratinocyte differentiation[22].

Connexins form gap junctions, which play an important role in regulating homeostasis and differentiation in many tissues[23]. Encoded by *GJB2* (Gap junction protein beta 2), connexin 26 is highly up-regulated in psoriasis lesions[24] and a transgenic mouse overexpressing *GJB2* in suprabasal keratinocytes exhibits several features of psoriasis[25].

SERPINB8 (Serpin peptidase inhibitor clade B member 8) is one of a family of serine protease inhibitors and is up-regulated in psoriasis lesions[26]. It has been suggested that this family of protease inhibitors contributes to the pathophysiology of psoriasis and other common inflammatory skin diseases[27,28].

ZNF816A (Zinc finger protein 816A) encodes a zinc-finger protein. Zinc finger domains function as specific modules for protein recognition, and have multiple regulatory functions, such as the recognition of RNA and proteins[29]. Furthermore, *ZNF816A* belongs to the same functional class of proteins as *ZNF313* (Zinc finger protein 313), which was recently identified as a novel psoriasis susceptibility gene[30]. Like *ZNF313, ZNF816A* appears to be associated with Type I (early onset) psoriasis in the combined Chinese Han datasets (rs9304742, $P=1.5\times10^{-3}$, OR=0.85, Supplementary Table 5).

Along with genome-wide exploration, we further investigated 15 SNPs from 8 loci reported to be associated with psoriasis in European populations[4] in Replication 2 (see **Online Methods**). In addition to *TNIP1* rs17728338 highly associated with psoriasis in the combined Chinese Han data (GWAS and Replication 2: 3,163 cases and 6,722 controls), only three genes, *IL23R, IL13* and *TNFAIP3,* showed suggestive evidence for association ($P_{Combined}=4.2\times10^{-5}$, OR=1.23 for *IL23R;* $P_{Combined}=1.0\times10^{-3}$, OR=0.90 for *IL13* and $P_{Combined}=3.7\times10^{-3}$, OR=1.16 for *TNFAIP3*). No nominal associations were observed for the remaining 8 SNPs within *IL1RN, TSC1, IL23A*/*STAT2* and *SMARCA4* (Supplementary Table 6). In most cases, the minor alleles and their allele frequencies (Supplementary Table 6) were similar between the European and Chinese Han populations, suggesting genetic heterogeneity of psoriasis within the two ethnic groups. However, it is possible that the power of the sample was not sufficient to detect risk SNPs with a low minor allele frequency in the Chinese Han population, as is likely to be the case for rs2066808 and rs12983316 (MAF<0.05, Supplementary Table 6). It is also possible that differences in patterns of linkage disequilibrium account for the ethnic diversity, since some of the associated variants identified in European population were not sufficiently tagged by any SNP screened in our GWAS.

This study was designed to maximize statistical power in a cost-effective manner by adopting a multistage analysis strategy in several large Chinese and European population samples, resulting in the identification of six novel susceptibility loci. Our findings increase the number of genetic risk factors of psoriasis, some of which have also been implicated in other autoimmune diseases. Our results also highlight new and plausible biological pathways in psoriasis, suggest additional genetic factors that may contribute to its age at onset, and provide insight into genetic heterogeneity of psoriasis across different ethnic populations. Further studies using large samples drawn from diverse ethnic populations will

be required to provide a more comprehensive understanding of the global genetics of psoriasis.

ONLINE METHODS

Subjects

To maximize the power of the study, we included data from our previously published GWAS in psoriasis, as well as data from 95 newly genotyped controls (totally 1,139 psoriasis cases and 1,227 healthy controls). Replication 1 (4,610 cases and 5,373 controls) and Replication 2 (2,024 cases and 5,495 controls) samples were recruited from the Chinese Han population through collaboration with multiple hospitals in China. Replication 3 Chinese Uygur consisted of 539 cases and 824 matched controls, obtained from Xinjiang Uygur Autonomous Region in China. The clinical diagnosis of all subjects for GWAS and Replication 1–3 were confirmed by at least two dermatologists. We collected clinical information from the subjects through a full clinical checkup, and additional demographic information from both the cases and controls through a structured questionnaire as used previously[5,19]. All controls were clinically assessed without psoriasis, other autoimmune disorders, systemic disorders, and family history of autoimmune disorders (including first-, second- and third-degree relatives). Informed consent was obtained from all the participants. The study was approved by each institutional ethical committee and conducted according to Declaration of Helsinki principles. Replication 4 (823 cases and 1,840 controls) from Germany was provided by Prof Andre Franke. Replication 5 (2,470 cases, 2,348 controls) from the U.S. was provided by Prof. James T. Elder and Replication 6 (254 nuclear families with two siblings with psoriasis) from the U.S. was provided by Prof Anne Bowcock[7–9]. All the controls and cases for each replication cohort were sampled from the same locality and the same population to assure minimal population stratification effects for each replication.

SNP selection for replication

We adopted two approaches to select SNPs for our Replication 1 study. As a first approach, we reanalyzed our initial GWAS data after the addition of 95 newly genotyped controls (1,139 cases and 1,227 healthy controls). We excluded all SNPs with MAF < 0.05 both in cases and controls, with HWE test p-values < 0.01 in controls, or that were genotyped for replication in our previous study. We then selected the 21 top SNPs with an association $P < 10^{-5}$. As a second approach, we increased our power to identify promising SNPs for further replication by incorporating 3,946 cases with other immune related diseases (SLE[19], leprosy[31], vitiligo and atoptic dermatitis) as “pseudo-controls”. When combined with the initial GWAS data used in the first approach, this yielded 1,139 cases and 5,173 controls, from which we selected the 40 top SNPs with an association $P < 10^{-5}$. We note that all 6,312 samples are from the Chinese Han population and were genotyped using the same Illumina Human 610-Quad BeadChips. This same strategy for improving the power of a GWAS analysis was also used in our published study on SLE[19], and a similar strategy has been employed by other groups, such as deCODE[32] and WTCCC[33]. We emphasize that these 3,946 “pseudo-controls” were only used in the process of SNP selection and were not included in the replication study or in the final joint association analysis of the combined samples. In total, these two approaches provided 61 SNPs for Replication 1.

In Replication 2, 35 SNPs were genotyped, These included 1) 20 SNPs with P <0.05 in replication 1; 2) 15 SNPs from 8 loci previously found to be associated with psoriasis in the European population (2 SNPs from each locus, one with the same SNP identification as previously reported in European population, and one with the highest P value in our initial GWAS excluding confirmed *TNIP1* region) to investigate the heterogeneity of disease susceptibility between Chinese and European populations.

Genotyping and quality control in GWAS

Our GWAS dataset comprised of 620,901 SNPs and CNV probes genotyped in 1,139 psoriasis cases and 1,132 controls, we added additional genotype data of 95 healthy controls (Table 1) in the current study. The Cochran-Armitage trend test was conducted to calculate the genotype-phenotype association as described previously[5]. Quantile-quantile plot showed an excess of significant associations after removal of MHC SNPs. Population structure analysis indicated a minimal overall inflation of the genome-wide statistical results due to population stratification (λ_{GC}=1.078) (Supplementary Fig. 2a–c).

Genotyping and quality control in the replication study

Genotyping analyses in Replication 1–3 (including replication for Chinese Uygur) were conducted by using Sequenom MassArray system (San Diego, USA) and Biosystems TaqMan assays (USA) at Anhui Medical University, Hefei, China. Approximately 15ng of genomic DNA was used to genotype each sample. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom) at the Key Laboratory of Dermatology (Anhui Medical University), Ministry of Education, China, following manufacture's instructions. The DNAs were amplified by multiplex PCR reactions, and the PCR products were then used for locus-specific single-base extension reaction. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrograms were analyzed by the MassARRAY TYPER software (Sequenom). Genotyping of Replication 4 and 5 was done using TaqMan assays (Applied Biosystems) at the Christian-Albrechts-University Kiel in Kiel, Germany and the University of Michigan in Michigan, U.S.A. Quality control was performed in each dataset separately using PLINK version 1.05[34]. In each case-control cohort (Replication 1–5), we excluded SNPs with call rate <95%, low minor allele frequency (MAF) (<0.05) or deviation from HWE proportions (P<0.01) in the controls. Genotyping of Replication 6 conducted at Washington University School of Medicine in Saint Louis, U.S.A. was performed with the Sequenom MassArray system. Primer sequences are available upon request. Quality control of Replication 6 was described elsewhere[6]. The cluster plots from the Illumina, Sequenom and TaqMan analyses were checked to confirm their good quality.

Statistical analysis

95 additional healthy controls were incorporated into a re-analysis of the GWAS dataset described previous[5]. The quantile-quantile plot and genomic control were calculated using the statistical analysis program R (http://www.r-project.org/), which evaluated the overall significance of the genome-wide association results and the potential impact of population stratification. All the statistical results were reported without genomic control correction, as the minimal impact of population stratification to be found. The remaining samples were subsequently assessed for population outlier and stratification using a principal component analysis (PCA) based approach[5,35]. First, all samples were analyzed together with the 206 reference samples from the International HapMap Project: 57 Yoruba in Ibadan, Nigeria (YRI), 44 Japanese in Tokyo, Japan (JPT), 45 Han Chinese in Beijing, China (CHB) and 60 CEPH (Utah residents with ancestry from northern and western Europe) (CEU). No population outliers were detected. Afterwards, we carried out PCA for all the remaining case and control samples. After quality control, we analyzed genotype data of 494,902 SNPs in 1,139 cases and 1,227 controls in GWAS stage.

We conducted Cochran-Armitage trend test in five replication samples (Replication 1–5) respectively. Heterogeneity test (I^2 and p-values of Q statistic) between different groups was performed using the method described by Higgins and Thompson[36]. The extent of heterogeneity was assessed by using the I^2 index[37]. In general, I^2 less than 30% is

considered as no heterogeneity, I^2 value of 30 to 50% is considered as moderate heterogeneity, and I^2 value above 50% is considered as strong heterogeneity. Therefore, we set the threshold of I^2 to be 30% in our analysis. If I^2 is less than 30%, the fixed effect model (Mantel-Haenszel) was used to combine the result of different cohorts[38]; otherwise, the random effect model (DerSimonian-Laird) was used[39]. The combined analyses were separately performed in Chinese Han (GWAS + Replication 1, Replication 1+ Replication 2 or GWAS + Replication 1 + Replication 2) and European populations (Replication 4 + Replication 5). Since nuclear families with affected sibling pairs (Replication 6) were genotyped, we estimated the kinship coefficient by the program KinInbcoef[40], and then we performed a case-control association test by a quasi-likelihood score test described by Thornton T and McPeek MS[41]. We estimated the allele frequency from a linear unbiased model, and the odds ratios were obtained by the formula $g(1-f)/(f(1-g))$ where g is the estimated allele frequency in cases and f is the estimated allele frequency in controls. All P values from the replication analysis were reported without correction for multiple testing. Multiple logistic regression analyses were used to test association patterns within 5q33.3 (*PTTG1* and *IL12B*), and within 5q33.1 (*TNIP1/ANXA6*). Recombination plots of discovered susceptibility loci were generated using the information from the HapMap project. To test sub-phenotypes specific related SNPs, we used the logistic regression analyses restricted to cases (case-only analyses) with subclinical phenotypes as the outcome variable[31].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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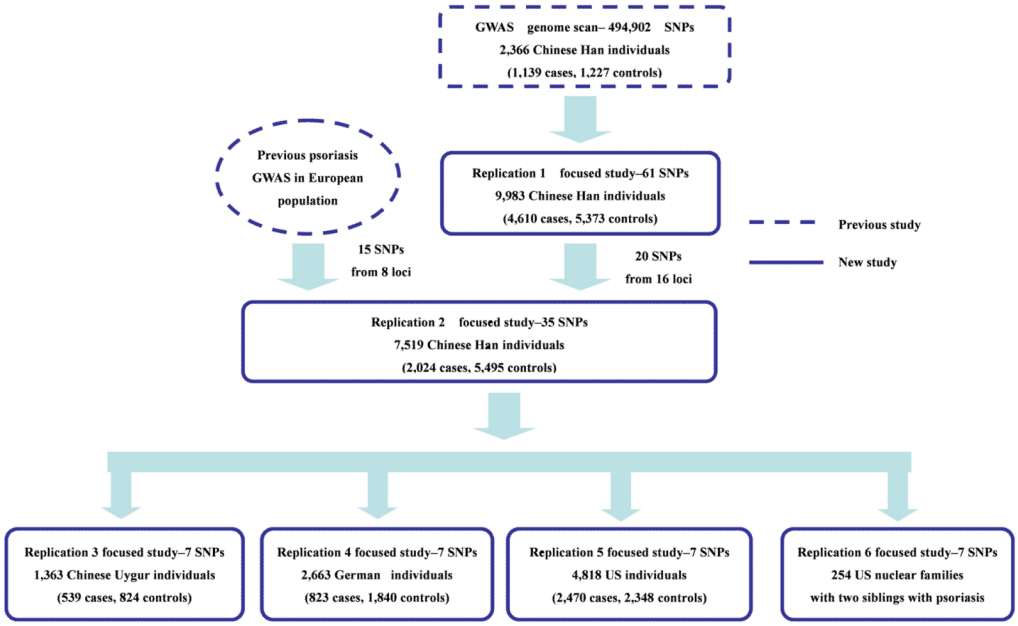

Figure 1. Experimental design

We performed a multiple stage replication study to identify risk loci for psoriasis in a larger cohort of Chinese Han (Replication 1 and 2), Chinese Uygur (Replication 3), the Germany (Replication 4) and the United States populations (Replication 5 and 6) based on our GWAS, as described in the Online Methods. We also genotyped 15 SNPs from loci previously reported in European population in the samples of Replication 2 to investigate the genetic heterogeneity of disease susceptibility between Chinese and European populations.

Table 1

Association evidence for 9 SNPs at 7 loci in GWAS, replication and combined studies in Chinese Han

SNP	Chr	GENE	Allele	GWAS				Replication 1				Replication 2				Combined(GWAS+R1+R2)	
				Allele Frequency		OR	P^*	Allele Frequency		OR	P^*	Allele Frequency		OR	P^*	OR	$P^\#$
				Case	Control			Case	Control			Case	Control			(95% CI)	
rs151823	5q15	*ERAP1*	*C*	0.4661	0.5073	0.85	3.93E-03	0.479	0.5018	0.91	1.25E-03	0.4718	0.508	0.87	7.50E-05	0.89 (0.85–0.92)	9.32E-09
rs3762999	5q33.1	*TNIP1/ANXA6*	*G*	0.2638	0.2278	1.22	4.63E-03	0.266	0.2258	1.24	8.16E-11	0.3043	0.2483	1.32	6.26E-11	1.23 (1.18–1.29)	4.55E-18
rs999556	5q33.1	*TNIP1/ANXA6*	*A*	0.2788	0.2384	1.24	1.93E-03	0.2748	0.2343	1.24	5.95E-11	0.2921	0.2384	1.32	1.87E-10	1.25 (1.20–1.31)	3.83E-21
rs2431697	5q33.3	*PTTG1*	*C*	0.2212	0.1834	1.27	1.28E-03	0.2031	0.1765	1.19	2.31E-06	failed	failed	failed	failed	1.20 (1.13–1.28)	1.11E-08
rs7007032	8p23.2	*CSMD1*	*C*	0.2208	0.1724	1.36	2.54E-05	0.1997	0.1846	1.10	7.66E-03	0.1986	0.1766	1.16	2.86E-03	1.16 (1.10–1.22)	3.78E-08
rs10088247	8p23.2	*CSMD1*	*C*	0.2243	0.1695	1.42	2.16E-06	0.2016	0.1842	1.12	1.89E-03	0.2056	0.1826	1.16	1.52E-03	1.17 (1.11–1.23)	4.54E-09
rs3751385	13q12.11	*GJB2*	*C*	0.4636	0.5106	0.83	1.26E-03	0.4871	0.5266	0.85	4.40E-08	0.4943	0.5171	0.91	1.41E-02	0.87 (0.84–0.91)	8.57 E-08
rs514315	18q22.1	*SERPINB8*	*C*	0.2221	0.2808	0.73	3.88E-06	0.2391	0.2595	0.90	9.14E-04	0.2301	0.2567	0.87	1.43E-03	0.87 (0.83–0.91)	5.92E-09
rs9304742	19q13.41	*ZNF816A*	*C*	0.3057	0.3608	0.78	7.55E-05	0.3243	0.3471	0.90	7.85E-04	0.3272	0.3547	0.88	1.99E-03	0.88 (0.84–0.92)	2.11E-09

*P values from the Cochran-Armitage trend test;

#P values from the joint analysis using either fixed effect model or random effect model (see Online Methods)

Table 2

Association evidence for 7 SNPs at 6 loci in replications of Chinese Uygur and European populations.

SNP	Chr	GENE	Allele	Replication 3 (Chinese Uygur)				Replication 4 (Germany)				Replication 5 (USA)				Combined (Replication 4+Replication 5)				Replication 6 (USA) **			
				Allele Frequency		OR	*P**	Allele Frequency		OR	*P**	Allele Frequency		OR	*P**	Allele Frequency		OR	*P#*	Allele Frequency		OR	*P*
				Case	Control			Case	Control			Case	Control			Case	Control			Case	Control		
rs151823	5q15	*ERAP1*	*C*	0.683	0.7571	0.69	2.92E-05	0.9173	0.9176	1	9.71E-01	0.9151	0.9232	0.9	1.11E-01	0.9155	0.9210	0.92	1.85E-01	0.8959	0.8913	1.05	8.00E-01
rs2431697	5q33.3	*PTTG1*	*C*	0.3874	0.3902	0.99	8.83E-01	0.4274	0.4403	0.95	3.87E-01	0.4405	0.4293	1.04	2.89E-01	0.4372	0.4343	1.01	8.98E-01	0.4221	0.4182	1.02	7.00E-01
rs7007032	8p23.2	*CSMD1*	*C*	0.2406	0.2444	0.98	8.22E-01	failed	failed	failed	failed	0.2876	0.2971	0.96	3.24E-01	NA1	NA1	NA1	NA1	0.2563	0.2574	0.99	9.40E-01
rs10088247	8p23.2	*CSMD1*	*C*	0.2415	0.235	1.04	6.96E-01	0.2783	0.2932	0.93	2.77E-01	0.2713	0.2805	0.95	3.07E-01	0.2731	0.2861	0.95	1.44E-01	0.2476	0.2459	1.01	9.30E-01
rs3751385	13q12.11	*GJB2*	*C*	0.6991	0.6987	1	9.82E-01	0.8147	0.8475	0.79	3.55E-03	0.8022	0.8071	0.97	5.03E-01	0.8053	0.8251	0.88	2.05E-01	0.8182	0.8321	0.91	5.80E-01
rs514315	18q22.1	*SERPINB8*	*C*	0.2968	0.3168	0.91	2.71E-01	0.2854	0.2926	0.97	5.96E-01	0.3075	0.3172	0.95	2.94E-01	0.3105	0.3355	0.96	2.41E-01	0.3316	0.3141	1.08	2.50E-01
rs9304742	19q13.41	*ZNF816A*[1]	*C*	0.3177	0.3764	0.77	1.70E-03	0.3209	0.3594	0.84	7.93E-03	0.3404	0.3638	0.9	1.62E-02	NA2	NA2	NA2	NA2	0.3285	0.2896	1.20	6.00E-02

*P values from the Cochran-Armitage trend test,

**Data from family samples analyzed using the similar statistic method by Thornton T and McPeek MS (See Online Method).

#P values from the joint analysis using either fixed effect model or random effect model (see Online Methods)

[1]SNP rs9304742 could not be genotyped in Replication 5 and association results come from the A allele of SNP rs11084211 having a strong LD with rs9304742 (r^2=0.88) in CEU.

NA1: no combined results were available for SNP rs7007032 failing to genotype in Replication 4.

NA2: no combined analysis was attempted because two different SNPs were typed for ZNF816A in Replication 4 and 5.