ARTICLE

Genome-wide Association Analysis of Psoriatic Arthritis and Cutaneous Psoriasis Reveals Differences in Their Genetic Architecture

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Psoriasis vulgaris (PsV) is a common inflammatory and hyperproliferative skin disease. Up to 30% of people with PsV eventually develop psoriatic arthritis (PsA), an inflammatory musculoskeletal condition. To discern differences in genetic risk factors for PsA and cutaneous-only psoriasis (PsC), we carried out a genome-wide association study (GWAS) of 1,430 PsA case subjects and 1,417 unaffected control subjects. Meta-analysis of this study with three other GWASs and two targeted genotyping studies, encompassing a total of 9,293 PsV case subjects, 3,061 PsA case subjects, 3,110 PsC case subjects, and 13,670 unaffected control subjects of European descent, detected 10 regions associated with PsA and 11 with PsC at genome-wide (GW) significance. Several of these association signals (*IFNLR1*, *IFIH1*, *NFKBIA* for PsA; *TNFRSF9*, *LCE3C/B*, *TRAF3IP2*, *IL23A*, *NFKBIA* for PsC) have not previously achieved GW significance. After replication, we also identified a PsV-associated SNP near *CDKAL1* (rs4712528, odds ratio [OR] = 1.16, $p = 8.4 \times 10^{-11}$). Among identified psoriasis risk variants, three were more strongly associated with PsC than PsA (rs12189871 near *HLA-C*, $p = 5.0 \times 10^{-19}$; rs4908742 near *TNFRSF9*, $p = 0.00020$; rs10888503 near *LCE3A*, $p = 0.0014$), and two were more strongly associated with PsA than PsC (rs12044149 near *IL23R*, $p = 0.00018$; rs9321623 near *TNFAIP3*, $p = 0.00022$). The PsA-specific variants were independent of previously identified psoriasis variants near *IL23R* and *TNFAIP3*. We also found multiple independent susceptibility variants in the *IL12B*, *NOS2*, and *IFIH1* regions. These results provide insights into the pathogenetic similarities and differences between PsC and PsA.

Introduction

Psoriasis vulgaris (PsV [MIM: 177900]) is a chronic, immune-mediated disease of the skin characterized by inflammation and epidermal hyperplasia, with a prevalence of 0.2%–6.5%, depending upon the population of origin.[1] Approximately 15%–30% of people with psoriasis will eventually develop psoriatic arthritis (PsA [MIM: 607507]), an often debilitating musculoskeletal condition that is usually seronegative for rheumatoid factor.[2] PsV

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and PsA pose increased risk for cardiovascular, autoimmune, metabolic, and psychiatric conditions.[3–6]

Both PsV and PsA have a strong genetic component. Based on twin and family studies in populations of European descent, estimates of heritability for PsV range from 50% to 90%,[7–12] with recurrence rates in siblings (λ_s) and first-degree relatives (λ_1) of 4–19.[13–16] The genetic contribution to PsA appears even larger, with estimates of 80%–100% for heritability[13,15] and of 30–49 for λ_s and λ_1.[13,15–17] A series of large-scale genome-wide association studies (GWASs) and genome-wide (GW) meta-analyses during the past decade have identified nearly 70 genetic loci harboring PsV risk variants.[18–32] In contrast, only three small to modestly sized GWASs for PsA have been published,[19,33,34] which, together with analyses of candidate loci in larger samples and recently published Immunochip array studies, have identified 13 regions associated with PsA at GW significance ($p \leq 5 \times 10^{-8}$): near *HLA-B* (MIM: 142830) and *HLA-C* (MIM: 143840) in the major histocompatibility complex (MHC)[19,21,33,35–45] and also near *IL12B* (MIM: 161561),[21,33,45] *IL23R* (MIM: 607562),[45] *IL23A* (MIM: 605580),[45] *TNIP1* (MIM: 607714),[21,45,46] *TRAF3IP2* (MIM: 607043),[25,33,40,45] *CSF2* (MIM: 138960),[45] *FBXL19* (MIM: 609085),[24] *REL* (MIM: 164910),[34] *RUNX3* (MIM: 600210),[47] *TYK2* (MIM: 176941),[45] *NOS2* (MIM: 163730),[48] and *PTPN22* (MIM: 600716).[48]

There are no published GWASs of cutaneous-only psoriasis (PsC), which we define as the presence of skin symptoms without PsA for at least 10 years. Candidate studies of PsC have identified seven associated genetic regions achieving GW significance: near MHC genes *HLA-C*, *HLA-B*, *CCHCR1* (MIM: 605310), and *TNF* (MIM: 191160),[21,33,37,40,42,49–53] and also near *IL12B*,[21] *TNIP1*,[21] *TNFAIP3* (MIM: 191163),[21] *IL23R*,[21] *NOS2*,[24] and *IL13* (MIM: 147683).[21]

It is reasonable to hypothesize that the observed differences in phenotype, prevalence, and heritability of PsA and PsC stem at least in part from differences in their underlying genetic architecture. Differences in strength of association with PsC and PsA have been repeatedly observed for the MHC, including a stronger association of *HLA-C*06* with PsC[42] and a stronger association of *HLA-B*27* with PsA,[41] both differences reaching GW significance. There is a paucity of well-powered studies formally comparing association for PsA versus PsC outside of the MHC region. A scan of the literature finds only ten non-MHC regions with at least a nominally significant ($p < 0.05$) difference in the strength of association for these two subphenotypes of psoriasis—near *IL13*,[54–57] *CSF2* (MIM: 601267),[45] *CCR2*,[58] *IFNG* (MIM: 147570),[54] *IL12B*,[21,46] *ZNF816*,[46] *IL23R*,[21] *FBXL19*,[24] *PTPN22*,[48] and the *KIR* gene cluster.[59]

To address the need for more powerful and comprehensive genetic studies of PsA and PsC, we first carried out a large GWAS of PsA, involving 1,430 PsA case subjects and 1,417 unaffected control subjects. We then combined results of this PsA GWAS with five published studies of psoriasis association—three GWASs and two targeted studies, comprising a discovery meta-analysis of 3,061 PsA case subjects, 3,110 PsC case subjects, 9,293 PsV case subjects, and 13,670 unaffected control subjects. For markers with promising association signals in this discovery analysis, a large partially overlapping sample of up to 13,857 individuals was genotyped, allowing both independent replication and validation of the quality of imputed genotypes in the discovery sample. We used stepwise conditional regression to discover additional PsA, PsC, and PsV risk variants that were independent of the primary index signal in each region. We then devised a bootstrap procedure to compare the relative strength of PsA and PsC associations for all strongly associated primary and secondary variants detected by the discovery and replication analyses, as well as for most variants previously reported as differentially associated with PsA versus PsC. Finally, we investigated potential functional consequences of our findings.

Material and Methods

Samples

All PsV case subjects were diagnosed by a dermatologist; a large subset of these individuals was also evaluated for PsA by a rheumatologist. Individuals affected with PsV for 10 or more years without developing any signs of PsA were classified as PsC. For all studies, each PsA and PsC case subject was also classified as a PsV case subject; the remaining PsV case subjects were PsA-negative individuals affected with psoriasis for less than 10 years or people for whom a diagnosis of PsA was either unavailable or uncertain. Control subjects were 18 years of age or older and unaffected with PsV or PsA. All participating individuals provided written informed consent and were recruited according to the protocols approved by the institutional review board of each institution.

For the PsA GWAS, 1,526 PsA case subjects and 1,508 unaffected control subjects were recruited by four collection centers—Michigan, Toronto, Newfoundland, and the National Psoriasis Foundation. Samples for the other five studies in the discovery meta-analysis have been described previously. Three have GW coverage, referred to here as the Collaborative Association Study of Psoriasis (CASP),[21] Kiel,[25] and Genizon[25,34] GWAS. The other two are targeted genotyping studies: a deep follow-up study of the CASP GWAS (CASP-DFU) designed to fine-map and replicate association signals from this earlier GWAS[60] and the Psoriasis Association Genetics Extension (PAGE) Immunochip-based study.[27] For these five published studies, case and control subjects were recruited from local populations at each of 17 collection centers in 4 countries—the United States, Canada, Estonia, and Germany. The published PAGE Immunochip study was extended with an additional 395 unaffected control subjects from the United States and 568 PsA case subjects from the United States, Canada, New Zealand, Australia, and 11 European countries. For our replication/validation sample, we genotyped up to 13,857 individuals from six collection centers in the United States, Canada, and Sweden (7,004 PsV case subjects, 2,554 PsA case subjects, 1,921 PsC case subjects, and 6,830 unaffected control subjects) that partially overlapped the six studies in the discovery set. All samples used in this study were of self-reported white European ancestry.

Genotyping and Quality Control

Samples for the PsA GWAS were genotyped with the Illumina HumanOmni1-Quad BeadChip array (Illumina). Genotypes were obtained for 1,020,596 autosomal SNPs. We removed SNPs with a call rate < 95%, a HWE p in controls $< 1 \times 10^{-7}$, or a minor allele frequency (MAF) < 0.01. Samples were removed if they had substantial non-European admixture, were duplicates or first- or second-degree relatives of other samples, had a genotype call rate < 98%, had outlier inbreeding coefficients ($F < -0.0225$ or $F > 0.0285$), or were part of unresolvable sample swaps determined by X chromosome genotypes.

Population outliers for the PsA GWAS were determined by principal components analysis (PCA), using SNPs passing the aforementioned quality-control filters that were further filtered to exclude those with a MAF < 0.05 and those occurring in known regions of psoriasis association. After linkage disequilibrium (LD)-based pruning with version 1.7 of Plink[61] to quasi-independence (maximum pairwise LD-$r^2 = 0.20$), 103,699 SNPs remained. Genotypes for these SNPs were combined with HapMap2 genotypes for samples from the YRI, CEU, JPT, and CHB reference populations and subjected to PCA, and individuals lying farther than 1/10 of the distance between CEU and JPT/CHB/YRI when plotted on the first two PCA axes were considered to have substantial non-European admixture.

Array-based genotyping and subsequent quality control measures for the CASP GWAS, Kiel GWAS, Genizon GWAS, CASP-DFU, and PAGE Immunochip were similar to those employed for the PSA GWAS and have been described in detail previously.[21,25,27,34,60] We used Plink[61] and RelativeFinder[62] to determine genetic relatedness of samples among all six discovery studies, removing all but one of each cluster of duplicated samples or first- or second-degree relatives, which ensured that meta-analysis was restricted to unrelated individuals.

We genotyped 25 markers to validate and replicate promising signals from the discovery analysis. Genotyping was performed by Taqman, Snapshot, or length polymorphisms assays (Life Technologies), or as part of a custom set on the Affymetrix Axiom Biobank Plus Array (Affymetrix), or both. We were unable to design successful genotyping assays for three of the 25 markers (those near *CAMTA1* [MIM: 611501], *NFKBIZ* [MIM: 608004], and *LINC00701*); for *NFKBIZ* we instead genotyped the second most significantly associated marker (rs4683946) in the region. Subsequent validation analyses based on this genotyping found that 7 of the 23 follow-up markers had questionable imputation quality. For one of these (rs8018217), we substituted a marker that was more reliably imputed in the discovery analysis (rs3168891), which is in near-perfect LD ($r^2 = 0.985$) with the primary signal, and which also happened to be genotyped in the replication/validation set as part of the standard content of the exome array. Samples that failed to genotype for 3 or more of the 23 markers or that were not of self-reported European ancestry were excluded from downstream analysis. Samples were also excluded if they had two or more discrepant genotypes among the 11 markers typed twice by different methods. After sample filtering, genotyping success exceeded 95% and HWE test p values were >0.05 for all 23 markers.

Imputation

Version 1.0.16.b of MaCH[63] was used to pre-phase haplotypes based on genotype data for each of the six discovery studies. Release 2012-05-29 of minimac[64] was used to impute SNP and indel genotypes based on phased chromosomal haplotypes from the 379 individuals in the EUR subpopulation of release 3, phase 1 of the 1000 Genomes Project.[65] The following criteria were used to flag markers with poor imputation despite passing our predicted imputation quality threshold ($r^2 \geq 0.3$): (1) a substantially (ratio > 1.10 or < 0.91) and significantly (adjusted p < 0.05) different MAF for imputed and experimental genotypes, (2) empirical imputation quality substantially ($\geq$0.1 r^2 units) worse than predicted, and (3) strength of association substantially (ratio of log(OR) values > 1.1 or < 0.91) and significantly (p < 0.05) different when comparing association results from meta-analysis of imputed and assayed genotypes.

Association Tests

We used logistic regression to test for association of variants with the binary case-control phenotype. Variant genotypes were coded as imputed allele dosages. To control for population stratification in array-based studies, we used coordinates from the top ten dimensions of PCA or multidimensional scaling analysis as covariates in the regression model for each individual study. Geographic cohort covariates were also included for the PsA GWAS, CASP GWAS, and PAGE Immunochip studies, as well as when analyzing the replication and validation markers. To test for associations that were independent of the primary signal in a region, we utilized stepwise forward logistic regression. The regression model in this case included imputed allele dosages of the most significantly associated variant as an additional covariate. Dosages of the most significantly associated variant in the region when conditioning upon the primary signal were then included as a new covariate in the next round of stepwise analysis, providing its significance exceeded the threshold set to correct for multiple testing ($p \leq 1 \times 10^{-6}$, based on the number of LD-independent tested variants within all analyzed regions and phenotype comparisons combined). To test for epistasis between pairs of variants, imputed allele dosages of the two tested variants and a multiplicative dosage interaction term were included in the logistic regression model along with cohort and PCA covariates.

Inverse variance-weighted fixed-effects meta-analysis was used to combine association results across datasets. For the initial six-study discovery analysis, all variants for which at least one study had good imputation quality ($r^2 \geq 0.3$)[63] were tested; no minimum MAF criterion was imposed. For meta-analysis of stepwise regression results and generation of association meta-p-values needed for construction of 95% Bayesian credible sets, the analyses for a region were restricted to those discovery studies with good coverage in the region, and only those markers well imputed in all included studies were tested. Heterogeneity of ORs across studies was tested with Cochran's Q test.

We utilized QQ plots and the genomic inflation factor (λ_{GC}) to test for residual effects of population stratification not fully controlled for by the inclusion of PCA and cohort covariates in the regression model. All variants in regions of known association at the time of the analysis[27] were excluded, and variants were also LD-pruned with version 1.7 of Plink[61] (window size = 50, step = 5, pairwise r^2 threshold = 0.2).

A posteriori power of detecting significant ($\alpha = 0.05$) association for markers in the replication sample was determined with the Genetic Power Calculator.[66] For power analysis, estimates of ORs and their 95% confidence intervals were based on results from the discovery meta-analysis that had been adjusted for ascertainment bias (winner's curse).[67]

Linkage Disequilibrium

We computed D′ and r^2 measures of LD for pairs of variants using genotypes of 379 individuals of European ancestry (EUR subpopulation) in release 3 of phase 1 of the 1000 Genomes Project.[65] Blocks of LD in regions of association were delineated by the D′ confidence interval method[68] based on all SNPs with MAF $\geq$ 0.05 in the same EUR subpopulation. Version 1.7 of Plink was used for all LD calculations.[61]

Phenotypic Variance Explained

For the non-MHC risk variants detected by this study, which are all biallelic, we used a liability threshold model to compute separately for each variant the percent variance of liability for disease explained.[69] Relative risk was approximated by the log-additive OR, frequency of the risk allele in the underlying population was estimated as a weighted average of its frequency in PsV case subjects and unaffected control subjects (weights = 0.02 and 0.98, respectively) of our combined discovery set, and population prevalences for PsV, PsA, and PsC were assumed to be 0.02, 0.003, and 0.017 based on published data.[1] For regions with multiple risk variants, we adjusted values for any interdependencies due to LD by first computing the variance explained by the correlated SNPs as a group[69] and then partitioning this total explained variance into non-overlapping variances for each SNP by the method of Pratt.[70] For the MHC, which contains several correlated multiallelic polymorphisms that influence risk for psoriasis, we applied a multiallelic extension of the biallelic method[69] to ORs derived from a full regression model containing all MHC risk variants and their multiple alleles that were identified by stepwise regression analysis of our six discovery studies.[44]

Comparison of Effect Sizes for PsA and PsC Associations

We used both parametric and bootstrap methods to compare effect sizes from meta-analysis of PsA case versus control subjects and of PsC case versus control subjects. With the exception of the PsA GWAS, the unaffected control subjects are shared by all discovery studies in these two meta-analyses, so the two meta-ORs are dependent random variables. A test statistic for the difference in two dependent ORs can be formulated as

$$\frac{(\beta_{PsA} - \beta_{PsC})^2}{V_{PsA} + V_{PsC} - 2\rho_{PsA,PsC}\sqrt{V_{PsA}}\sqrt{V_{PsC}}},$$

where β_{PsA} and β_{PsC} are the log ORs for PsA-affected versus control individuals and PsC-affected versus control individuals, V_{PsA} and V_{PsC} are the variances of these log ORs, and $\rho_{PsA,PsC}$ is the correlation of the two log ORs. Under the null hypothesis of no difference in the two effects, this test statistic follows a χ^2 distribution with 1 degree of freedom. Although the log ORs and their variances for the six discovery studies can be estimated by fixed-effects meta-analysis, there is no analytic approximation for the correlation of the ORs. As expected, simulations show that the correlation is nearly always positive but that its value varies substantially, especially in response to the relative and absolute magnitudes of the observed effect sizes. Setting the last term in the denominator of the test statistic to zero, equivalent to assuming independence of the two ORs, affords a rapid albeit conservative parametric test.

As an alternative, we devised a bootstrap procedure. For each of the six discovery studies, we separately sampled with replacement PsA case subjects, PsC case subjects, and unaffected control subjects, maintaining the same sample sizes as in the original studies. Association tests for PsA case versus control subjects and PsC case versus control subjects were then run for each individual study, followed by fixed-effects meta-analysis for each of the two phenotype comparisons, using the same logistic regression models applied to the observed data. Sampling and testing were repeated 50,000 times. A χ^2 test statistic was computed by dividing the square of the difference in the two observed log meta-ORs by the variance of their difference, where the latter is computed from the 50,000 bootstrap estimates of the difference. This standard bootstrap approach[71] yielded very similar p values to other bootstrap methods (percentile, bias-corrected). To validate the bootstrap approach, we inspected all final and intermediate bootstrapped estimates for 100,000 iterations of 52 independently associated markers. For all markers, the bootstrapped distribution for the difference in log ORs was normal (Lilliefor's test $p > 0.05$) and bias was negligible (mean and median bias = 0.0001 and 0.0000, respectively). Furthermore, for all markers and each phenotype comparison, bootstrapped and parametric association results for the individual studies and the meta-analysis were very similar.

Bayesian Credible Sets

Using a Bayesian approach, for selected association signals we identified the credible set of markers that were 95% likely, based on posterior probability, to contain the causal disease-associated variant. Although the concept was based on the approach of Maller et al.,[72] our method used a Bayes factor derived from more recent work[73] that explicitly considers meta-analysis. Input for a signal consisted of meta-ORs and their standard errors for all markers within 500 kb of the most significantly associated variant in the six-study discovery analysis.

Analysis of RNA Expression

We used the Wilcoxon rank-sum test to test for differences in RNA expression levels, derived by RNA-seq, between 92 punch biopsies of lesional skin from psoriatic individuals versus 82 biopsies of normal skin from unaffected control subjects, as described previously.[74] To declare a gene as differentially expressed, we required a 2-fold or greater difference in median RPKM-normalized transcript levels, with a rank-sum test $p < 10^{-6}$ (corresponding to a family-wise error rate < 0.025 for all genes with detectable expression in at least half of the lesional or normal skin biopsies).

For analysis of possible eQTL variants, RNA levels were determined for all gene models in Ensembl v.74 for the same 92 lesional and 82 normal skin biopsies, using version 1.3.3 of Tophat[75] for alignment and version 2.1.1 of Cufflink[76] for ab initio transcript assembly, as described previously.[77] All 92 lesional samples and all but two of the 82 normal samples were genotyped on the Affymetrix Axiom Biobank Plus Array, which contains a GWAS backbone panel of 260,000 SNPs in addition to exome and custom content. After quality control, high-quality genotypes for 586,178 SNPs were available for GW imputation using all populations of release 1 of phase 3 of the 1000 Genomes Project. All Cufflink-normalized RNA values < 0.1 were treated as zero. Only those genes with RNA expression > 0 in at least 20% of the normal or 20% of the lesional samples were analyzed. Before analysis, we first applied a square-root transformation for variance stabilization, then standardized separately within lesional and normal groups to a mean of zero and standard deviation of one. Merlin-offline, distributed as part of version 1.1.2 of Merlin,[78] was used to test

for significant correlation of transcript levels with imputed allele dosages of variants; inverse normalization was applied to the expression levels, and the top two principal components and an indicator variable for lesional/normal skin were included as covariates in the association model.

The RNA expression data used in these analyses are available at NCBI's Gene Expression Omnibus (GEO accession number GSE63979). Details of the generation of these data by RNA-seq, QC metrics, and validation of normalized expression values are presented elsewhere.[74,77]

Annotation Analysis

For associated variants, the UCSC Table Browser[79] was used to extract PhastCons, PhyloP, and Gerp++ conservation scores, as well as location within CpG islands, from hg19 reference assembly tracks phastCons100way, phyloP100wayAll, allHg19RS_BW, and cpgIslandExt, respectively. Version 2014-11-12 of ANNOVAR[80] was used to determine positions of variants relative to RefSeq genes. Version 3 of HaploReg[81] was used to determine how many cell types among the ENCODE and Roadmap reference epigenomes have promoter histone marks, enhancer histone marks, or DNase I hypersensitivite sites overlapping a variant, as well as ChIP-seq evidence of transcription factors that bind to the interval containing the variant, and which transcription factor binding sites motifs are changed by a variant. Version 1.1 of RegulomeDB[82] was used to obtain scores of how likely it is that a variant has regulatory function, and version 1.2 of CADD[83] provided scores of variant deleteriousness. The overlap of associated variants with chromatin states of 33 cell types relevant to psoriasis, which were derived from a 15-state segmentation model based on five chromatin marks, was based on results of analyses generated by the Roadmap Epigenomics Project.[84]

Results

PsA GWAS

Application of quality control filters to the PsA GWAS yielded high-quality genotypes at 791,217 SNPs for 1,430 PsA case subjects and 1,417 control subjects. Demographic details of the post-filtering sample collection are shown in Table S1. After imputation, genotypes for 11,532,644 SNPs and 918,976 indels were available for downstream analysis. QQ plots and genomic inflation factors (1.013–1.025) for four different MAF bins of the imputed variants indicated good control of population stratification (Figure S1).

Five regions in the PsA GWAS were associated at GW significance (Figure S2, Table S2). These were all known risk regions for PsA, and the strongest signals were near *HLA-B* (rs36058333, OR = 2.13, $p = 1.3 \times 10^{-29}$), *IL12B* (rs918520, OR = 1.50, $p = 9.3 \times 10^{-10}$), *TRAF3IP2* (rs33980500, OR = 1.74, $p = 4.2 \times 10^{-9}$), *TNIP1* (rs8177833, OR = 1.75, $p = 3.7 \times 10^{-8}$), and *TYK2* (rs35251378, OR = 1.41, $p = 3.5 \times 10^{-8}$). Five additional regions surpassed a suggestive threshold of significance ($p = 1 \times 10^{-6}$), four of which (near *TNS1* [MIM: 600076], *ATP10D*, *PTPRN2* [MIM: 601698], *CTNNA3* [MIM: 607667]) have no previously reported risk variants for PsV or PsA.

Meta-analysis of Six Discovery Studies

To increase power and extend our analysis to include PsC case subjects, we combined the results of the PsA GWAS with those of five published genetic studies of psoriasis in people of European descent. Three of these studies have GW coverage; the other two use targeted genotyping arrays. As shown in Table 1, the four GWASs combined provided a discovery set of 4,007 PsV, 1,946 PsA, and 1,363 PsC case subjects as well as 4,934 unaffected control subjects. The two targeted studies provided an additional 5,286 PsV, 1,115 PsA, and 1,747 PsC case subjects and 8,736 control subjects for a subset of the genome. The five additional datasets were imputed to the same reference panel as the PsA GWAS. Fixed-effects meta-analysis was used to test for association of the six discovery studies combined. The four phenotype comparisons tested were PsV case versus unaffected control subjects, PsA case versus unaffected control subjects, PsC case versus unaffected control subjects, and PsA case versus PsC case subjects. The PsA GWAS was excluded from the latter two meta-analyses because it did not include any PsC case subjects. For each of the four phenotype comparisons, QQ plots of p values from the meta-analyses indicated good control of population stratification (Figure S3), as did genomic inflation factors for the six individual studies as well as for the meta-analyses (Table S3).

The Manhattan plots in Figure 1 label all regions with GW significant association detected by the four meta-analyses (see Tables S4–S7 for detailed results). Figure 1A shows 26 GW significant regions for the test of PsV case versus unaffected control subjects; all harbor known PsV susceptibility variants, including two near *CAMK2G* (MIM: 602123) and *NFKBIZ* that were recently published as psoriasis associated[32] based on a sample that has a large overlap with the discovery sample of this study. Figure 1B shows ten regions with GW significant association for PsA. Seven of these were previously known (near *IL23R*, *REL*, *TNIP1*, *IL12B*, *HLA-C*, *TRAF3IP2*, and *TYK2*) and the other three were newly established at GW significance—*IFNLR1* ([MIM: 607404] rs7540214, OR = 1.40, $p = 1.6 \times 10^{-8}$), *IFIH1* ([MIM: 606951] rs1990760, OR = 1.22, $p = 1.8 \times 10^{-8}$), and *NFKBIA* ([MIM: 164008] rs12883343, OR = 1.22, $p = 2.6 \times 10^{-9}$). Twelve regions with GW significant association were identified by meta-analysis of PsC cases (Figure 1C), including six known PsC signals (near *IL13*, *TNIP1*, *IL12B*, *HLA-C*, *TNFAIP3*, and *NOS2*) and a candidate locus near *RGS6* ([MIM: 603894] rs12895275, OR = 1.65, $p = 2.0 \times 10^{-8}$). In addition, five known PsV signals were newly established as associated with PsC at GW significance—*TNFRSF9* ([MIM: 602250] rs4908742, OR = 1.22, $p = 2.2 \times 10^{-8}$), *LCE3C/B* ([MIM: 612615, 612614] rs10888503, OR = 1.25, $p = 1.8 \times 10^{-11}$), *TRAF3IP2* (rs9481169, OR = 1.39, $p = 5.7 \times 10^{-11}$), *IL23A* (rs11575231, OR = 1.48, $p = 7.9 \times 10^{-9}$), and *NFKBIA* (rs61251127, OR = 1.25, $p = 1.3 \times 10^{-8}$). Meta-analysis of PsA case versus PsC case subjects identified only two

Table 1. Characteristics of the Six Studies in the Discovery Meta-analysis

Study	No. Individuals					No. Genotyped SNPs[a]	No. Imputed Variants[b]	
	PsV	PsA	PsC	Controls	Total		SNPs	Indels
PsA GWAS	1,430	1,430	0	1,417	2,847	791,217	11,532,644	918,976
CASP GWAS	1,352	344	695	1,389	2,741	438,609	10,708,247	865,325
Kiel GWAS	464	33	269	1,135	1,599	504,637	10,730,120	872,971
Genizon GWAS	761	139	399	993	1,754	489,501	10,525,123	863,572
CASP-DFU	1,563	133	575	1,141	2,704	7,592	207,911	18,001
PAGE Immunochip	3,723	982	1,172	7,595	11,318	168,360	1,654,147	141,025
All	9,293	3,061	3,110	13,670	22,963	–	–	–

Abbreviations are as follows: PsV, psoriasis vulgaris; PsA, psoriatic arthritis; PsC, cutaneous psoriasis.
[a]After applying all quality control measures.
[b]Restricted to variants with minimum acceptable imputation quality (MaCH-$r^2 \geq 0.3$).

GW significant peaks (Figure 1D). One is a well-known signal near *HLA-C* and *HLA-B* (rs1050414, OR = 1.53, $p = 7.4 \times 10^{-11}$). The second is a previously unreported association near *LOC100505817* on chromosome 18 (rs4891505, OR = 1.63, $p = 6.7 \times 10^{-9}$), which is an uncharacterized long non-coding RNA.

The discovery meta-analysis found nominal ($p \leq 0.05$) association at all previously reported GW-significant signals for PsV, PsA, and PsC in populations of European ancestry, and association at $p \leq 1 \times 10^{-5}$ for most of them (76%, 77%, and 100%, respectively) (Table S8). In contrast, just 23% of the PsV-associated regions detected only in Chinese populations reached even nominal association in our study.

Validation and Replication of Candidate Signals

The discovery analyses uncovered 25 regions with no published psoriasis variants at the time of analysis that were at least suggestively associated ($p < 1 \times 10^{-6}$) with PsV, PsA, or PsC (Tables S4–S7). In order to replicate these promising association signals in an independent sample, and also to validate their imputed dosages in the discovery sample, we genotyped up to 13,857 individuals of European descent that partially overlapped the discovery set. Depending upon the phenotype comparison being tested, the imputation quality of a marker across the six discovery studies, and the method of assay, this yielded a set of 2,615–8,415 retyped individuals for validation of imputation quality and of 2,602–7,779 newly typed individuals for independent replication.

Samples from four of the discovery studies (CASP GWAS, PsA GWAS, CASP-DFU, and PAGE Immunochip) were retyped for the 23 successfully assayed replication markers. We assessed reliability of imputation (Figures S4–S6; see Material and Methods for more details) and found that 7 of the 23 replication markers (near *BHLHE40* [MIM: 604256], *USP49*, *JAK2* [MIM: 147796], *ZNF462*, *MBIP* [MIM: 609431], *RGS6*, and *APOBEC3G* [MIM: 607113]) might have unreliable imputation quality in at least one study of the discovery analysis (Table S9).

After exclusion of poorly imputed markers for which we could find no well-imputed substitute, the newly typed samples provided an independent replication of 17 association signals. Association statistics for these markers for the discovery set, the replication set, and the two sets combined are given in Table 2 (see Table S10 for full results for all 25 suggestively associated signals). Only two of the markers (rs4712528 near *CDKAL1* [MIM: 611259] and rs2675669 near *CAMK2G*) were significantly associated in the replication set, which pushed the evidence of their association with PsV to GW significance in the combined analysis (OR = 1.16, $p = 8.4 \times 10^{-11}$ for *CDKAL1*; OR = 1.13, $p = 3.9 \times 10^{-11}$ for *CAMK2G*). Neither of these SNPs showed significant heterogeneity of association across studies in the combined analysis (Cochran's Q test p = 0.54 and 0.42, respectively). The *CAMK2G* region has recently been shown to be associated with psoriasis based on a sample that has substantial overlap with the one used in this study,[32] but to our knowledge GW-significant psoriasis association for a *CDKAL1* variant has not been previously described. The association peak for *CDKAL1* lies fully within the gene, near its 5′ end (Figure S7). Two SNPs that were associated at GW significance in the discovery stage (near *NFKBIZ* and *LOC100505817*) were no longer associated at this level in the combined analysis.

Conditional Association Analysis to Identify Secondary Susceptibility Variants

We next investigated whether we could detect additional susceptibility variants independent of the best signal in any of the regions attaining suggestive significance ($p = 1 \times 10^{-6}$) in our discovery analysis. To this end, for each of the six discovery studies, we utilized stepwise forward logistic regression. We excluded the MHC region from our analysis, because we have recently published a fine-mapping analysis of this region for PsV, PsA, and PsC associations based on the same six discovery sets of this study.[44] For each region, all markers within 500 kb of the primary signal were candidates for conditional analysis.

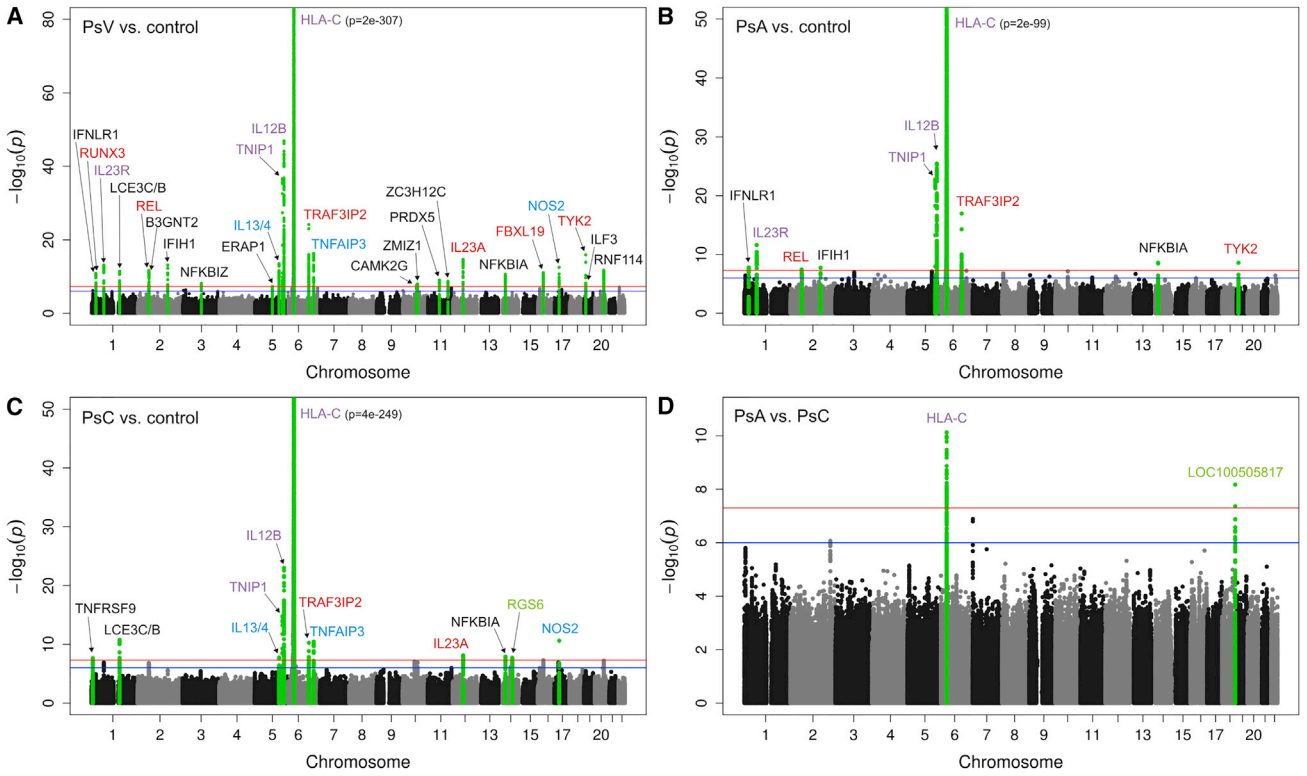

Figure 1. Manhattan Plots of Association Results for Meta-analysis of Six Discovery Studies
Each circle represents the $-\log_{10}(p)$ of the imputed variants, including SNPs and indels. Thresholds of suggestive ($p = 1 \times 10^{-6}$) and GW ($p = 5 \times 10^{-8}$) significance are delineated with blue and red lines, respectively. All chromosomal regions achieving GW significance are highlighted in green and labeled with their leading candidate susceptibility gene. Font color of the candidate genes indicates published GW significant associations with psoriasis and its cutaneous and joint subphenotypes: purple indicates known PsA, PsC, and PsV association, red indicates known PsA and PsV association, cyan indicates known PsC and PsV association, black indicates known PsV association, and green indicates possible new psoriasis association. We tested association for four binary phenotypes: PsV case versus control subjects (A), PsA case versus control subjects (B), PsC case versus control subjects (C), and PsA case versus PsC case subjects (D).

The results of meta-analysis of qualifying studies are shown in Table S11 and Figures S8–S13. Variants independent of the primary signal were detected in four susceptibility regions, containing candidate genes *IL23R*, *IL12B*, *NOS2*, and *IFIH1*. Multiple secondary signals upstream of *IL12B* were associated with PsV, PsA, and PsC, yielding, respectively, a total of five, four, and three independent variants in this region for these three phenotypes. Two PsV-associated conditional signals were observed for *IL23R*, the first near the 5′ end and the second near the 3′ end of the gene. A single secondary signal near *NOS2* was associated with PsV, and another secondary signal upstream of *IFIH1* was associated with PsA. A comparison of unconditional and full model association results for these four regions reveals that the identified signals within a region are not fully independent of each other. Because of both positive and negative LD among the variants, several show either much weaker or much stronger association in the full model than when tested in isolation.

Epistasis of Psoriasis Susceptibility Variants

We tested for statistical interaction among pairwise combinations of all known or newly established primary plus all secondary risk variants reaching adequate statistical significance in the discovery analysis ($p \leq 1 \times 10^{-7}$ for primary signals; $p \leq 1 \times 10^{-6}$ for secondary signals). For PsV, positive epistasis was observed between the risk alleles of the primary MHC variant (rs12189871) and the *ERAP1* (MIM: 606832) variant (rs30377) (interaction OR = 1.14; $p = 4.6 \times 10^{-6}$) and the risk alleles of the MHC variant and the *LCE* variant (rs10888503) (OR = 1.26; $p = 6.9 \times 10^{-10}$), confirming previous reports of epistasis for these two pairs of loci.[23,27,85,86] No pairs of PsA or PsC risk variants showed significant interaction after correction for multiple testing.

Comparison of Strength of Association with PsA and PsC

Genetic association with PsA and PsC case subjects was directly compared in the discovery meta-analysis (Figure 1D). This analysis necessarily omitted nearly half of the PsA case subjects because they were from the PsA GWAS, which had no PsC case subjects. We thus turned to a conservative parametric test of the ratio of the ORs from the meta-analyses of PsA case versus control subjects and PsC case versus control subjects. As shown in Figure S14, the increase in power due to inclusion of all PsA case subjects yielded a peak MHC signal (rs2523615,

Table 2. Selected Results for Previously Unreported Regions of Psoriasis Association

						Discovery (6-Study Meta)			Replication			Combined (Meta)		
Marker	Chr	hg19 Position[a]	Alleles (Risk/Nonrisk)	Nearby Gene	Phenotype Comparison[b]	No. Cases/ Controls	OR	p	No. Cases/ Controls	OR	p	No. Cases/ Controls	OR	p
rs13396545	2	215,672,833	A/G	*BARD1*	PsA-PsC	516/1,363	1.59	9×10^{-7}	1,663/1,101	0.93	0.4	2,179/2,464	1.17	0.01
rs4685408	3	16,996,035	G/A	*PLCL2*	PsV-ctl	9,293/13,670	1.12	4×10^{-7}	799/1,438	1.08	0.2	10,092/15,108	1.12	2×10^{-7}
rs4683946	3	101,615,826	G/T	*NFKBIZ*	PsV-ctl	7,730/12,529	1.19	1×10^{-8}	1,949/2,440	1.05	0.4	9,679/14,969	1.16	7×10^{-8}
rs4683946	3	101,615,826	G/T	*NFKBIZ*	PsA-ctl	2,928/12,529	1.27	2×10^{-7}	162/2,440	1.21	0.2	3,090/14,969	1.27	9×10^{-8}
rs72622783	4	32,577,723	T/C	—	PsA-ctl	1,946/4,934	2.78	3×10^{-7}	893/4,789	1.08	0.7	2,839/9,723	1.91	3×10^{-5}
rs113633694	4	68,949,589	T/C	*TMPRSS11F*	PsA-ctl	1,602/3,545	7.13	5×10^{-7}	1,168/5,443	0.88	0.6	2,770/8,988	1.63	0.02
rs4712528	6	20,678,430	C/G	*CDKAL1*	PsV-ctl	9,293/13,670	1.14	2×10^{-7}	2,223/3,185	1.22	6×10^{-5}	11,516/16,855	1.16	8×10^{-11}
rs4725242	7	1,629,781	C/T	*PSMG3*	PsA-PsC	1,154/1,840	1.46	1×10^{-7}	1,596/1,140	1.04	0.5	2,750/2,980	1.23	4×10^{-5}
rs56677333	8	6,750,801	C/A	*DEFB1*	PsA-ctl	1,946/4,934	1.59	2×10^{-7}	343/2,996	0.99	1.0	2,289/7,930	1.45	3×10^{-6}
rs7922314	10	64,538,279	C/T	*ADO*	PsC-ctl	3,110/12,253	1.42	9×10^{-8}	342/2,889	0.91	0.6	3,452/15,142	1.33	2×10^{-6}
rs12356475	10	67,761,720	C/T	*CTNNA3*	PsA-ctl	1,946/4,934	1.38	8×10^{-8}	873/4,744	1.08	0.3	2,819/9,678	1.25	1×10^{-6}
rs2675669	10	75,653,872	T/C	*CAMK2G*	PsV-ctl	9,293/13,670	1.13	2×10^{-8}	2,223/3,191	1.16	3×10^{-4}	11,516/16,861	1.13	4×10^{-11}
rs149014202	13	29,436,518	T/C	*SLC46A3*	PsA-ctl	33/1,135	297	2×10^{-7}	1,768/4,936	1.84	0.1	1,801/6,071	3.49	0.001
rs3168891	14	36,789,729	T/G	*MBIP*	PsV-ctl	4,007/4,934	1.35	1×10^{-5}	3,789/3,990	0.93	0.4	7,796/8,924	1.14	0.009
rs35144987	14	62,725,664	T/–	*LINC00643*	PsC-ctl	2,535/11,112	1.24	8×10^{-7}	1,222/5,648	1.02	0.7	3,757/16,760	1.15	4×10^{-5}
rs17052344	17	21,717,543	A/G	*FAM27L*	PsC-ctl	2,535/11,112	1.24	1×10^{-7}	1,220/5,625	1.08	0.2	3,755/16,737	1.18	4×10^{-7}
rs4891505	18	71007958	A/C	*LOC100505817*	PsA-PsC	1,498/2,535	1.63	7×10^{-9}	2,101/1,221	1.04	0.7	3,599/3,756	1.30	1×10^{-5}
rs6088273	20	30045393	A/G	*DEFB123*	PsV-ctl	7,730/12,529	1.14	9×10^{-7}	3,234/3,804	1.02	0.6	10,964/16,333	1.09	2×10^{-5}

Regions in table had no published reports of GW significant association with PsV, PsA, or PsC at time of analysis (February 2014). Abbreviations are as follows: chr, chromosome; PsV, psoriasis vulgaris; PsA, psoriatic arthritis; PsC, cutaneous psoriasis; ctl, unaffected control; OR, odds ratio.

[a]For insertions or deletions of the reference sequence, position of first base before the insertion point or of first base of the deleted sequence is shown, respectively.

[b]Binary phenotype tested for association: PsA-ctl, PsA case versus unaffected control subjects; PsC-ctl, PsA case versus unaffected control subjects; PsV-ctl, PsV case versus unaffected control subjects; PsA-PsC, PsA case versus PsC case subjects; PsA-PsC+ctl, PsA case versus combination of PsC case and unaffected control subjects.

OR = 1.91, $p = 9.9 \times 10^{-28}$) 17 orders of magnitude more significant than the best MHC signal from the PsA versus PsC meta-analysis. The signal in *LOC100505817* on chromosome 18, which was GW significant in the meta-analysis of PsA versus PsC case subjects but failed independent replication, does not reach suggestive significance in this analysis.

We devised a bootstrap procedure to compare ORs from the PsA versus control and PsC versus control subject meta-analyses. Although the bootstrap approach remedies the modestly conservative nature of the parametric test (Figure S15), it is too computationally demanding for GW analysis. We instead first applied the bootstrap test to the four non-MHC regions with $p < 1 \times 10^{-5}$ from GW analysis using the parametric test. The significance of association increased for the best variant in each of the four regions, but still failed to reach suggestive ($p = 1 \times 10^{-6}$) significance.

We then applied the bootstrap test to all known and newly established psoriasis signals with strong evidence of association ($p \leq 1 \times 10^{-7}$) in the discovery analysis, as well as to all secondary signals uncovered in the conditional analysis. The most significantly associated variant for each of the four phenotype comparisons that met the aforementioned criteria was tested. Nine variants, comprising five LD-independent groups, had an FDR-adjusted $p < 0.05$ for a test of the null hypothesis of equal strength of association with PsA and PsC (Table 3; Table S12 has results for all 59 tested variants). Three of these groups were variants whose PsV risk allele was more strongly associated with PsC than PsA—rs4908742 near *TNFRSF9* (OR = 1.19, p = 0.00020; Figures 2A–2C), rs10888503 near *LCE3C/B* (OR = 1.15, p = 0.0014; Figures 2D–2F), and four SNPs (rs1050414, rs12189871, rs13214872, and rs13210419) near *HLA-C* (OR = 1.34–1.80, $p = 5.5 \times 10^{-9}$–1.7×10^{-21}). The other two groups were variants whose PsV risk allele was more strongly associated with PsA than PsC—two SNPs (rs12044149 and rs4655683) upstream of *IL23R* (OR = 1.20 and 1.14, p = 0.00018 and 0.0075, respectively) and SNP rs9321623 (OR = 1.17, p = 0.00022), which lies 230 kb upstream of *TNFAIP3*.

The PsA-specific signals upstream of *IL23R* and *TNFAIP3* are of particular interest, as they are independent of all published PsV associations. Figures 3A–3C clearly show two LD-independent peaks of association for *IL23R*—the PsA-specific peak near the 5′ end of the gene, and a peak associated with both PsA and PsC near the 3′ end of the gene. Similarly, Figures 3D–3F illustrate two independent peaks of association for the *TNFAIP3* region—the PsA-specific peak well upstream of the gene, and a peak within *TNFAIP3* that is associated with both subphenotypes. The published PsV associations for each of these two regions are in LD with the second but not first peak of association.

Finally, we attempted to confirm previous reports of non-MHC variants exhibiting significantly different association with PsA versus PsC. As shown in Table S13, we applied the bootstrap test to 10 of 12 such variants (the other two, a copy-number variant affecting *KIR2DS2* [MIM: 604953] and a microsatellite within *IFNG*, were not in our discovery set). We could confirm only two of the previous findings, for rs2201841 near *IL23R* (OR = 1.10, p = 0.035) and for rs2476601 in *PTPN22* (OR = 1.16, p = 0.041). The original report for rs2201841 was based on a subset of the discovery samples in the current study; this SNP is not in LD with either of the PsA-specific variants uncovered here ($r^2 < 0.1$), but is in moderate LD ($r^2 = 0.62$) with rs116238258, the second conditional PsV signal we detected in the *IL23R* region. Variants mapping near *CCR2*, *CSF2*, *IL13*, *IL12B*, *FBXL19*, and *ZNF816* showed no evidence of differential association with PsA versus PsC. Non-confirmation of the PsA specificity of the *CSF2* association was unexpected given the robustness of the original report.[45] Although we were able to confirm the existence of a PsA signal near *CSF2* that is independent of association with nearby *IL13*, we also detected comparable *IL13*-independent association with PsC (Figure S16). Our inability to confirm putative PsA-specific signals at *IL13* itself (Table S12, Figure S16) is also noteworthy given the multiple positive reports for three different *IL13* SNPs.[54–57]

Disease Liability for Regions with Multiple Risk Variants

Table S14 lists, for each independent non-MHC risk variant for PsV, PsA, or PsC identified by this study, an LD-adjusted percent of variance in disease liability explained. The total proportion of disease liability explained by all non-MHC variants combined summed to 7.80%, 4.49%, and 4.91% for PsV, PsA, and PsC, respectively. These figures increased to 14.39%, 9.22%, and 12.10% when the contribution of the MHC region[44] was also considered.

Table S14 includes variants in 29 distinct genomic regions. Sixteen of these regions contained only one independent signal, eight regions had two signals that were in substantial pairwise LD ($r^2 = 0.46$–1.00), and five regions (*IL12B*, *IL23R*, *NOS2*, *IFIH1*, and *TNFAIP3*) had two or more signals, at least one pair of which were in low pairwise LD ($r^2 < 0.3$). Figure 4 displays disease liability plots for the five regions with at least two independent signals. Plotting disease liabilities allowed a more meaningful comparison of relative risk for multiple variants in a region than did association statistics such as p values or ORs. These plots also allowed unified display for primary and conditional signals of different disease phenotypes, as well as visual representation of LD and spatial relationships of the risk variants with each other and with candidate genes. Spatially, the risk variants in Figure 4 aggregated into one (*NOS2*), two (*IL23R*, *TNFAIP3*, *IFIH1*), or four (*IL12B*) distinct clusters. The number of LD-independent clusters per region varied from two to four based on the D′ measure and from two to five based on the r^2 measure. Most of the 19 plotted variants fell outside of the leading candidate gene for the region, but six were intronic (*IL23R*, *TNFAIP3*, *NOS2*, and *IFIH1*) and two exonic (*IFIH1*).

Table 3. Selected Results for Comparative Strength of Association of PsA versus PsC for Established Psoriasis Variants

Candidate Gene	Rnd[a]	Marker	Chr	Position (hg19)[b]	Index Marker for:[c]				Alleles[d]	RAF[e]	PsA versus Control[f]		PsC versus Control[f]		PsA-ctl versus PsC-ctl[g]	
					PsV ctl	PsA ctl	PsC ctl	PsA PsC			Meta-OR	Meta-p	Meta-OR	Meta-p	OR	p
TNFRSF9	0	rs4908742	1	8,245,030			X		A/C	0.3487	1.024	0.53	1.223	2.2×10^{-8}	0.837	2.0×10^{-4}
IL23R	0	rs12044149	1	67,600,686		X			T/G	0.2525	1.296	2.5×10^{-12}	1.084	0.023	1.196	1.8×10^{-4}
IL23R	1	rs4655683	1	67,611,613	X				A/G	0.3368	1.319	7.8×10^{-14}	1.162	1.6×10^{-5}	1.135	0.0075
LCE3C/B	0	rs10888503	1	152,593,549	X		X		T/C	0.6413	1.085	0.018	1.247	1.8×10^{-11}	0.870	0.0014
HLA-C	0	rs1050414	6	31,239,506				X	G/C	0.1150	2.333	1.5×10^{-70}	4.020	6.5×10^{-225}	0.580	1.7×10^{-21}
HLA-C	0	rs12189871	6	31,251,924	X				T/C	0.1429	2.631	5.6×10^{-86}	4.462	6.6×10^{-248}	0.590	2.2×10^{-19}
HLA-C	0	rs13214872	6	31,255,008		X			G/C	0.5621	2.416	2.3×10^{-99}	3.231	1.2×10^{-206}	0.748	5.5×10^{-9}
HLA-C	0	rs13210419	6	31,266,977			X		A/G	0.0929	2.778	1.8×10^{-84}	5.004	3.5×10^{-249}	0.555	5.7×10^{-21}
TNFAIP3	0	rs9321623	6	137,958,265		X			C/T	0.5461	1.201	5.9×10^{-8}	1.025	0.44	1.172	2.2×10^{-4}

Abbreviations are as follows: Chr, chromosome; RAF, risk allele frequency; PsV, psoriasis vulgaris; PsA, psoriatic arthritis; PsC, cutaneous psoriasis; ctl, control; OR, odds ratio.

[a]Round of stepwise conditional association analysis: 0, unconditional analysis; 1, first round stepwise conditional; 2, second round stepwise conditional, etc.

[b]For insertions or deletions of the hg19 human reference sequence, position of first base before the insertion point or of first base of the deleted sequence is shown, respectively.

[c]An X indicates, for each of the four analyzed phenotype comparisons (PsV case versus control subjects, PsA case versus control subjects, PsC case versus control subjects, PsA case versus PsC case subjects), that the marker is either the most strongly associated variant in the region with a $p \leq 1 \times 10^{-7}$ (unconditional meta-analysis) or the best associated independent locus with a $p \leq 1 \times 10^{-6}$ at the specified round of the stepwise conditional meta-analysis.

[d]Risk and nonrisk alleles based on results of association analysis of PsV case subjects and unaffected control subjects.

[e]Risk allele frequency for unaffected controls.

[f]For regions with a single independently associated variant, OR and p value are for a fixed-effects meta-analysis of PsA or PsC case versus unaffected control subjects where no other loci are included as conditioning covariates; for regions with multiple independently associated variants, OR and p value are for fixed-effects meta-analysis where the logistic regression model includes the other independent risk variants in the region as conditioning covariates.

[g]OR and nominal p value for a bootstrap test of the equality of the log(OR)s from meta-analyses of PsA versus control and PsC versus control subjects for the six study discovery sample; p values ≤ 0.05 after FDR adjustment are indicated in the final column.

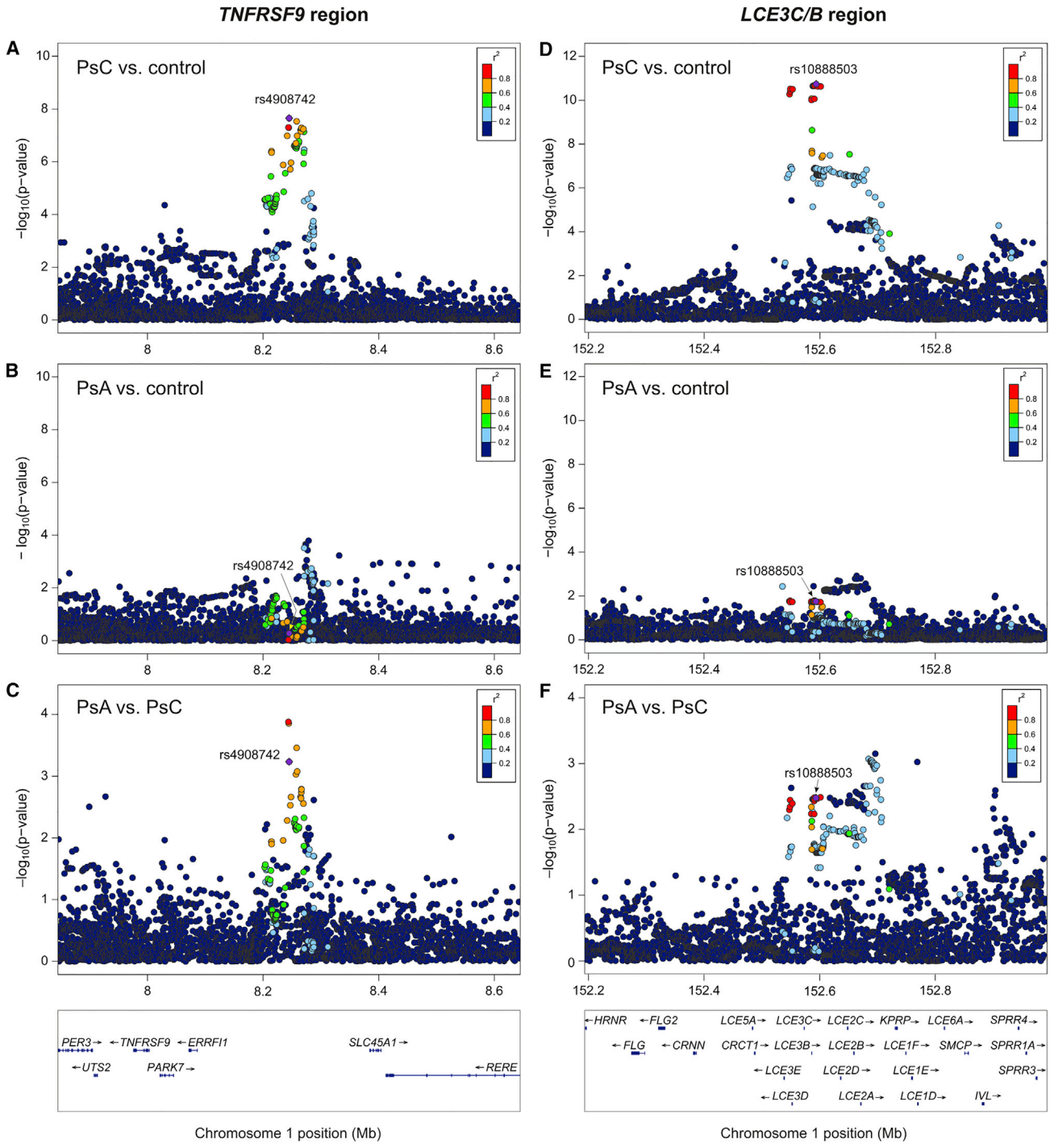

Figure 2. Comparison of PsC and PsA Associations for the *TNFRSF9* and *LCE3C/B* Regions
Negative log10-transformed p values from meta-analyses of association for the six discovery datasets are shown for 800-kb regions encompassing genes *TNFRSF9* (A–C) and *LCE3C* and *LCE3B* (D–F). For each region the SNP with the most significant association for the comparison of PsC case subjects with unaffected control subjects is labeled. The color of each symbol denotes the strength of LD (r^2 coefficient) with the index SNP. (A) and (D) Shown are association results for PsA case versus control subjects (A, D), for PsC case versus control subjects (B, E), and for a conservative parametric test of the difference in log(OR) values from the PsA versus control and PsC versus control subjects (C, F) comparisons. The bottom two panels give the physical positions of known genes in each region.

Functional Annotation

We investigated potential functional consequences of associated variants for eight regions where this study has enhanced our knowledge of the genetics of psoriasis and PsA: *CDKAL1* (PsV variant), *TNFRSF9* and *LCE3C/B* (PsC-specific association), *IL23R* and *TNFAIP3* (PsA-specific associations independent of known PsV variants), and *IFIH1*, *IL12B*, and *NOS2* (secondary PsV, PsC, and PsA signals independent of previously reported risk variants).

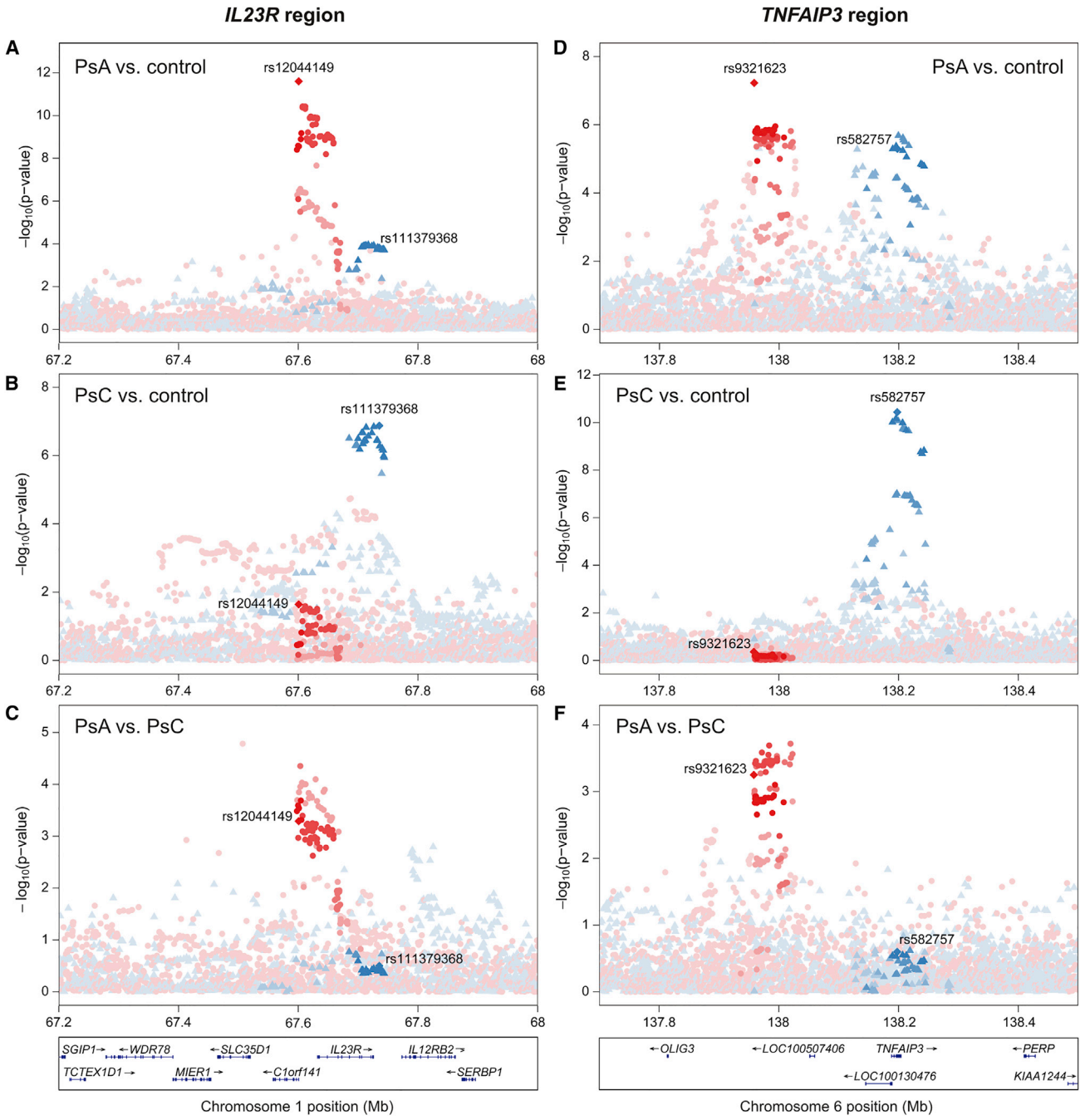

Figure 3. Comparison of PsA and PsC Associations for the *IL23R* and *TNFAIP3* Regions
Negative log10-transformed p values from meta-analyses of association for the six discovery datasets are shown for 800-kb regions encompassing genes *IL23R* (A–C) and *TNFAIP3* (D–F). For each region two reference SNPs are labeled—the red and blue diamonds denote the variant that was most significantly associated for a test of PsA case versus control subjects or PsC case versus control subjects, respectively. For all other plotted variants, the color and shape of the symbol denotes which index SNP the variant is in highest LD with (red circles for the PsA index, blue triangles for the PsC index), and the intensity of the color indicates the magnitude of the pairwise LD r^2 coefficient. Shown are association results for PsA case versus control subjects (A, D) and for PsC case versus control subjects (B, E). Results for a conservative parametric test of the difference in log(OR) values from the PsA versus control and PsC versus control comparisons shown in (C) and (F). The bottom two panels give the physical positions of known genes in each region.

Dendrograms for LD-based hierarchical clustering (Figure S17) show that 7 of the 22 associated variants detected by this study in these regions were not in strong LD ($r^2 \geq 0.7$) with published variants for any genetic trait. These include the primary PsV signal near *TNFRSF9*, one of the secondary *IL23R* PsV signals, the primary PsV and secondary PsA signals in the *IFIH1* region, the primary PsV, PsA, and PsC as well as one of the secondary PsV signals

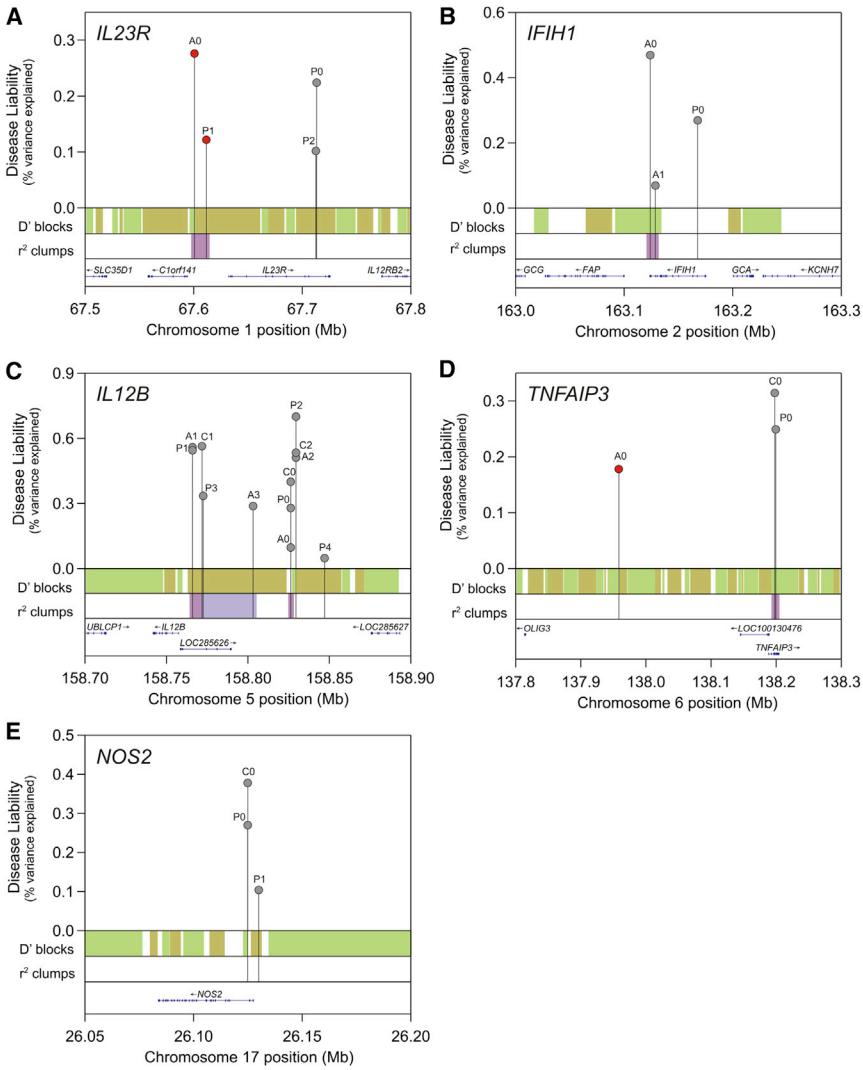

Figure 4. Liability Plots for Five Regions with Multiple Independent Psoriasis Variants

Liability plots for five genomic regions—near *IL23R* (A), *IFIH1* (B), *IL12B* (C), *TNFAIP3* (D), and *NOS2* (E)—with multiple independent variants associated with psoriasis or its cutaneous and joint subphenotypes. Percent variance in disease liability explained by each variant was computed and adjusted for LD dependencies with other variants in the region by the method of Ho et al.[69] The label above each plotted symbol indicates both the associated phenotype (P = PsV, A = PsA, C = PsC) and the round of conditional association analysis (0 = unconditional, 1 = first round conditional, 2 = second round conditional, etc.). Grey fill for a plotted symbol indicates that the association ORs for the variant in the six-study meta-analysis of PsA case versus unaffected control subjects and PsC case versus unaffected control subjects were not significantly different; red fill indicates a significantly larger OR for the PsA versus control comparison. LD blocks, shown with two alternating shades of green, were delineated by the D′ confidence interval method[68] using genotypes for all SNPs with MAF $\geq$ 0.05 in the 379 individuals of European ancestry in phase 1 of the 1000 Genomes Project.[65] The bands for the r^2 clumps, shown in two shades of purple when necessary, encompass clusters of markers with an average pairwise r^2 LD coefficient $\geq$ 0.3.

near *IL12B*, and the secondary PsV signal near *NOS2*. Of the remaining 15 risk variants, all but the *CDKAL1* SNP and the PsA-specific signal upstream of *TNFAIP3* were in strong LD with previously reported variants for PsV or PsA; other diseases sharing these variants or variants in strong LD with them include Crohn disease (MIM: 266600), ulcerative colitis (MIM: 266600), inflammatory bowel disease (MIM: 266600), ankylosing spondylitis (MIM: 106300), multiple sclerosis (MIM: 126200), type 1 diabetes (MIM: 222100), selective IgA deficiency (MIM: 137100), systemic lupus erythematosus (MIM: 152700), and vitiligo (MIM: 606579). All of these immune-mediated diseases have been reported to share susceptibility signals with psoriasis.[27,87,88]

Overlap of association signals with various functional features is presented in Table S15. Because the most significantly associated variants are not necessarily causative, for each signal we computed a Bayesian credible set that had a 95% posterior probability (PP) of including the causal disease-associated variant.[72,73] To accommodate uncertainty about which variant drives an association signal, we annotated by computing weighted averages of each feature over all variants in the credible set, using PPs as weights. Most of the association signals in the eight regions were intronic or intergenic. Only the two independent PsA association signals in *IFIH1* had a PP > 0.50 of being exonic; the index variants for these signals are missense SNPs with a high likelihood (PP = 0.79 and 0.97) of being causal. Another variant likely to influence protein expression is a 32-kb indel spanning *LCE3C* and *LCE3B*, which is in complete LD with the index SNP for this region and is known to be associated with psoriasis.[85,89] Many of the association signals fell within regions bearing evidence of regulatory function. The strongest such evidence was for the PsA-specific signal near *IL23R*, which was in a region with promoter histone marks, enhancer histone marks, and DNase I hypersensitive sites for a weighted average of 40, 11, and 12 different cell types, respectively, among 127 reference epigenomes. The evidence for the index variant of this signal, rs12044149, was even more striking: 67, 15, and 19 cell types, respectively, where the SNP was located within chromatin having promoter histone marks, enhancer histone marks, and DNase I hypersensitive sites; location within a CpG island; and a 2b ("likely to affect binding") RegulomeDB score. Another association signal with

multiple lines of evidence for regulatory function was the likely causative SNP for the best PsV and PsC association with *NOS2* (rs28998802), which alters binding motifs for seven transcription factors (TFs), is scored as likely to affect TF binding, and contains DNase I hypersensitive sites in 11 cell types.

Figure S18 shows overlap of association signals in the eight regions of interest with chromatin states for 33 cell types relevant to psoriasis and PsA. As before, the PsA-specific signal near *IL23R* appears to be located within an important regulatory region, showing promoter-proximal activity in many relevant cell types, including monocytes, neutrophils, mononuclear cells, most T helper cells, $CD8^+$ T cells, fibroblasts, keratinocytes, melanocytes, mesenchymal stem cell-derived cells, and osteoblasts. The strongest secondary *IL12B* signal for PsV and PsA (P1/A1) shows enhancer activity in NK cells, in all three keratinocyte cell lines and in T regulatory cells, and also in activated T helper cells. The primary PsV signal for *IFIH1*, which is intronic, is in a region of transcriptional activity for most cell types, but exhibits enhancer activity in memory T helper and T regulatory cells. In contrast, both *NOS2* signals are located in regions that are silenced by Polycomb group proteins in the majority of relevant cell types.

Regulatory mechanisms often affect RNA expression. Accordingly, we examined differential expression in psoriasis lesions versus skin from unaffected individuals for all genes within 500 kb of the index signal in each of the eight regions of interest, as well as evidence that the associated regions contain *cis*-eQTLs for any of these genes (Table S16). Most of the plausible candidate genes in these eight regions showed a substantial (fold-change [FC] > 2 or < 0.5) and significant ($p < 1 \times 10^{-6}$) difference in RNA expression between lesional and normal skin. Expression of the inflammation-related genes *IFIH1*, *IL12B*, *IL23R*, *NOS2*, and *TNFRSF9* was higher in lesional compared to normal skin (FC = 2.8, ∞, 4.4, 50, and 6.0, respectively). *TNFAIP3* was the only inflammation-related candidate gene showing no sign of differential expression. The gene-dense *LCE3C/B* region teems with plausible candidate genes; most (25/37) were dramatically up- or downregulated in psoriatic skin. After correction for multiple testing, only the PsV signal in the *LCE3C/B* region provided evidence for being an eQTL in skin tissue. The risk alleles for all 27 variants in the 95% Bayesian credible set for this locus showed a highly significant negative correlation with mRNA levels of both *LCE3C* and *LCE3B* ($p \leq 1 \times 10^{-24}$ and $p \leq 2 \times 10^{-12}$, respectively), and the peak eQTL variant for each gene (rs67488802 and rs10888503, respectively) was in the credible set. However, the risk alleles of the associated variants are all in very strong LD ($r^2 \geq 0.98$) with a 32-kb deletion spanning *LCE3C* and *LCE3B* and hence are simply markers for the absence of these two genes. The associated variants in this region also showed a significant ($p = 1.4 \times 10^{-5}$) positive correlation with *LCE3A* expression, but the peak eQTL for *LCE3A* (rs138035792, $p = 1.6 \times 10^{-6}$) is not in the 95% Bayesian credible set for the association signal.

Discussion

This study is a well-powered and comprehensive examination of the genetic associations of PsA and PsC. We found five independent variants that are differentially associated with these two subphenotypes, all in regions of known association with psoriasis.

A signal in the class I MHC between *HLA-C* and *HLA-B* exhibited by far the greatest degree of differential association in our analysis, with carriage of the PsV risk allele nearly halving the odds of developing PsA in addition to PsC. Previous work using the same six discovery samples of this study demonstrated that this differential association is driven by a glutamine residue at position 45 of HLA-B, which increased PsA versus PsC susceptibility more than any other classical HLA protein allele, amino acid, SNP, or indel in the region.[44] This amino acid is situated in the antigen binding groove of HLA-B, with potential influence on receptor cell-surface expression or antigen peptide binding or presentation.[44,90]

The PsA-specific association near *IL23R* was previously reported by us[91] and has been recently confirmed.[45] The index variant in both studies is the same (rs12044149), increasing the likelihood that this SNP is causal. However, although the confirmatory study demonstrated that this association is independent of previously reported psoriasis associations, our study demonstrates that the SNP itself is significantly more strongly associated with PsA than PsC. SNP rs12044149 lies in a region with promoter-proximal activity in diverse cell types, including many relevant to psoriasis (Table S15, Figure S18). We found no evidence that this SNP is a *cis*-eQTL for *IL23R* mRNA levels in skin tissue, but IL23R-expressing immunocytes likely to be of relevance to PsA (including IL17A-expressing $CD4^+$ and $CD8^+$ T cells[92]) are relatively minor components of normal and psoriatic skin.[93] This SNP is only 32 bp from the transcription start site (TSS) of uncharacterized protein-coding gene *C1orf141* (versus 31.5 kb from the TSS of *IL23R*), and its risk allele also shows a nominally significant ($p = 0.0036$) positive correlation with *IL12RB2* (MIM: 601642) mRNA levels in skin tissue, suggesting that its risk effect might be mediated via regulation of genes in this region other than *IL23R*. Our results also support the existence of at least three independent effects at the *IL23R* locus for psoriatic disease (Table S11, Figures 4 and S17).

We also discovered a PsA-specific variant (rs9321623) in the *TNFAIP3* region, which is LD independent of the best PsV and PsC associations. This SNP is one of a spatial cluster of seven (rs9321623, rs7769192, rs13192841, rs2327832, rs10499194, rs17264332, and rs6920220) associated with a variety of immune-mediated diseases, which

spans a 48-kb interval that is intergenic and situated more than 180 kb upstream of the TSS of *TNFAIP3*. However, only one of the risk variants for these other diseases (rs7769192, a secondary signal for multiple sclerosis[94]) is allied with the PsA variant (LD-r^2 = 0.85). When aggregated over all variants in the 95% Bayesian credible set, there is no compelling evidence that this PsA association signal lies in a regulatory region (Table S15, Figure S18). The index variant does, however, fall within an enhancer element in many cell types relevant to psoriasis and PsA, including $CD14^+$ monocytes, mesenchymal stem cells, fibroblasts, keratinocytes, and osteoblasts (data not shown). However, *TNFAIP3* is not differentially expressed in normal versus psoriatic skin, and the PsA-specific signal shows no evidence of being an eQTL for *TNFAIP3* in skin-derived transcriptomes (Table S16).

We established that a known psoriasis signal within a cluster of 19 LCE genes in the epidermal differentiation complex on chromosome 1q21.3[22,23,27] is more strongly associated with PsC than PsA. Published evidence for association of PsA with variants in the *LCE* region is mixed,[95–99] but a meta-analysis of 2,400 PsA case and 7,779 control subjects from five European countries found a nominally significant association (OR = 1.14, p = 0.00041).[95] We also found a nominally significant association of PsA with PsV index variant rs10888503 (OR = 1.085, p = 0.021), but the association with PsC was far stronger (OR = 1.247, $p = 1.8 \times 10^{-11}$). The index SNP is in perfect LD with a 32 kb deletion spanning *LCE3C* and *LCE3B* that has been shown to be associated with psoriasis to a degree equal to or better than any SNP surrogate.[85,89] We found a highly significant positive correlation between copy number and mRNA levels of *LCE3C* and *LCE3B* in skin tissue (Table S16). Expression of these two genes was strongly upregulated in psoriasis lesions compared to normal skin (72.8- and 12.5-fold increase, respectively), and they encode late cornified envelope structural proteins thought to be involved in skin barrier repair after injury or inflammation.[100] It has been theorized that lower levels of these LCE3 proteins due to the deletion impairs response of the skin barrier to exogenous agents, thereby facilitating the systemic skin inflammation characteristic of psoriasis.[101] It has also been demonstrated that the 32 kb indel possesses epidermal-specific developmental in vivo enhancer activity,[102] which might also play a role in the association with disease.

The final differential association we detected is tagged by SNP rs4908742, which lies between *ERRFI1* (MIM: 608069) and *SLC45A1* (MIM: 605763) on chromosome 1p36.23. This association signal appears to be specific to PsC (OR = 1.223, $p = 2.2 \times 10^{-8}$), with no detectable association to PsA (OR = 1.028, p = 0.53). Although this region contains susceptibility variants for several other autoimmune diseases, none of them are in LD with rs4908742 or the published[27] psoriasis variant (Figure S17). There are at least four plausible candidate genes in this region (*TNFRSF9*, *ERRFI1*, *SLC45A1*, and *RERE* [MIM: 605226]),[27] all of which are differentially expressed in both normal and psoriatic skin, especially *TNFRS9*, which is 6-fold upregulated, and *SLC45A1*, which is 2.4-fold downregulated in psoriasis lesions. The 95% Bayesian credible set for this locus contains 59 variants, and the PP for the index variant is only 0.091. Thus, further refinement of this PsC association signal is needed before the target or mechanism of action of the risk variant in this region can be determined.

Other than a nominally significant greater association with PsC than PsA for a known PsV variant within *IL23R*[21] and a recently published PsA signal in *PTPN22*,[48] we could not confirm any of the prior reports of differential association of these two subphenotypes with non-MHC genetic variants (Table S13) even though this study had greater power and used a more stringent definition of PsC than most prior reports.

Given the marked distinction in phenotype, heritability, and incidence of PsA and PsC, it is remarkable that we can detect or confirm only five differentially associated signals, all within known regions of PsV association. Much of this might stem from the fact that this study, though based on a large sample, is still underpowered compared to recently published case-control studies of PsV and other genetic traits.[103] The consequences of this disparity in power are also reflected by this study when comparing its greater effective sample size for PsV-control versus PsA-control and PsC-control comparisons (22,128 versus 10,604 and 9,996) with the resulting number of independent risk variants identified (40 versus 20 and 19, respectively) and the total percent of variance in phenotypic liability explained (14.4% versus 9.2% and 12.1%, respectively). Another limitation of this study is that two of the six discovery studies had targeted rather than GW coverage, which biases discovery to loci already implicated in psoriasis and other autoimmune diseases. It is likely that larger GWASs of PsA and PsC will uncover more variants specific to each subphenotype, aided by enhanced imputation resources such as the Haplotype Reference Consortium.

In addition to uncovering differential associations with PsA and PsC, we discovered GW significant association with PsA for three regions (near *IFNLR1*, *IFIH1*, and *NFKBIA*) and with PsC for five regions (*TNFRSF9*, *LCE3C/B*, *TRAF3IP2*, *IL23A*, and *NFKBIA*). This increases the list of firmly established PsA and PsC risk regions to 16 and 12, respectively.

We also substantiated a PsV susceptibility signal within the 5′ portion of the 700 kb *CDKAL1* gene. Nominal association of psoriasis to a SNP in this region has been noted previously.[104,105] The index SNP for this signal (rs4712528) is in strong LD with a risk variant for Crohn disease, but it is completely LD independent of a susceptibility variant for inflammatory bowel disease and a host of SNPs that increase risk for type 2 diabetes (MIM: 125853) and an array of other metabolic traits (Figure S17). *CDKAL1* was expressed at significantly lower levels in psoriatic lesions than in normal skin (FC = 0.77, $p = 3.7 \times 10^{-14}$), in keeping with an observation that *CDKAL1* transcription is

downregulated when immune cells expressing the gene (especially $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes) are activated.[105] We found no evidence that this signal is an eQTL for *CDKAL1* or that it maps to a regulatory feature, but the relatively low PP of the index variant (0.22) and the long interval spanned by the 95% Bayesian credible set (568 kb) indicate that deciphering the role of this locus in psoriasis will depend upon better refinement of the association signal.

Only 2 of 17 previously unreported associations were replicated, including only one of three signals achieving GW significance in the discovery analysis (Table 2). After adjusting discovery results for the well-known winner's curse[67] and correction for multiple testing, none of the 17 markers showed a significant difference between the ascertainment-adjusted OR of the discovery set and the observed OR of the replication set. Post hoc power tests indicated that only for rs4891505 in *LOC100505817* was there high confidence that power to replicate exceeds 80%, although the predicted power for the successfully replicated *CAMK2G* variant was respectable (43%). These results show that although effect sizes in the discovery and replication sets are similar for the candidate variants, the replication set is underpowered to replicate most of the true positives among them. Power to replicate *NFKBIZ* was also reduced by the need to substitute the second-best associated marker for the index variant (compare p for these two variants in Table S10). The PsA versus PsC signal in *LOC100505817* is probably a false positive, given its much reduced significance when including the PsA GWAS in the discovery analysis and its poor showing in the replication set.

Conditional association analysis revealed multiple independent risk variants in regions of known psoriasis association, several of which have not been reported previously (Table S11, Figures 4 and S17). Besides signals near *IL23R* and *TNFAIP3* that have already been discussed, we detected multiple variants for *IFIH1*, *NOS2*, and *IL12B*. Three independent associations were found for the *IFIH1* region—a PsV signal tagged by rs17715343 in this study and rs17716942 in previous studies;[23,27] a signal associated with both PsA (rs1990760) and PsV (rs211485) that also poses risk for type 1 diabetes, vitiligo, systemic lupus erythematosus, ulcerative colitis, and irritable bowel syndrome; and a PsA signal at rs3747517. The PsA signals at rs1990760 and rs3747517 are only 4.7 kb apart, but their risk alleles usually occur on different haplotypes ($r = -0.73$). The *IL12B* region harbors at least five independent risk variants for psoriatic disease. One cluster contains most of the previously reported associations for PsV and PsA (rs4921482, rs2546890, rs4379175, rs3213094, rs2082412, rs7709212, rs6887695, rs3212227, rs228831),[18,21,22,25,27,29,45,72] along with the strongest secondary PsV and PsA (rs62377586) and PsC (rs4921485) signals from this study, as well as risk SNPs for multiple sclerosis (rs2546890) and Crohn disease (rs6556412). A second group includes a secondary PsV, PsA, and PsC signal from this study, rs12188300, which is known to be associated with PsA[33] and PsV.[27] Three additional clusters contain variants not previously implicated in psoriasis susceptibility: a group with the best signal for PsV, PsA, and PsC (rs918520), a secondary PsV signal (rs6870256), and a group containing secondary PsV signal rs953861, secondary PsA signal rs1592975, plus SNPs associated with ankylosing spondylitis and Crohn disease. The *NOS2* region contains three independent psoriasis associations—rs28998802, the best PsV and PsC signal of this study as well in a large meta-analysis of PsV;[27] a known PsV signal tagged by rs4795067;[24] and rs2301369, a newly implicated secondary PsV signal.

Data mining suggests plausible functional consequences for some of the variants in the eight regions where this study has added to our knowledge of genetic susceptibility to psoriasis and its subphenotypes. These include variants that might mediate their risk via protein alterations (PsV-associated deletion of *LCE3B* and *LCE3C* and two independent PsA-associated missense SNPs in *IFIH1*), promoter-proximal regulation (PsA-specific *IL23R* SNP), enhancer-mediated regulation (PsV signal in *IFIH1*, strongest secondary PsV and PsA signal in *IL12B* region), and reversal of Polycomb repression (two independent *NOS2* variants). Given the great cost and labor involved in experimental follow-up studies, it is crucial to identify the causative variants driving the observed associations. This will require larger samples of affected case subjects that are densely genotyped or deeply sequenced for the entire genome. Correlation of the resulting finely mapped genetic information with transcriptomes and epigenomes of relevant cell types will aid prediction and therapy of psoriasis and its cutaneous and joint manifestations.

Accession Numbers

Genotypes for the PsA GWAS have been deposited in dbGaP under accession number phs000982.v1.p1.

Supplemental Data

Supplemental Data include 18 figures and 16 tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2015.10.019.

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes Project, ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release/20110521/
ANNOVAR, http://www.openbioinformatics.org/annovar/
CADD, http://cadd.gs.washington.edu/
dbGaP, http://www.ncbi.nlm.nih.gov/gap
Gene Expression Omnibus (GEO), http://www.ncbi.nlm.nih.gov/geo/
GWAS Catalog (28 August 2015 release), http://www.ebi.ac.uk/gwas/
HaploReg, http://www.broadinstitute.org/mammals/haploreg/haploreg.php
MACH, http://www.sph.umich.edu/csg/abecasis/MACH/
MERLIN, http://csg.sph.umich.edu/abecasis/Merlin/
Minimac, http://genome.sph.umich.edu/wiki/Minimac
OMIM, http://www.omim.org/
PLINK, http://pngu.mgh.harvard.edu/~purcell/plink/
RegulomeDB, http://RegulomeDB.org/
RelativeFinder, http://genome.sph.umich.edu/wiki/RelativeFinder
Roadmap Epigenomics Project, http://egg2.wustl.edu/roadmap/web_portal/
The Haplotype Reference Consortium, http://www.haplotype-reference-consortium.org/home
UCSC Genome Browser (February 2009 assembly, GRCh37/hg19), http://genome.ucsc.edu

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