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Genome-wide association analysis identifies three psoriasis susceptibility loci

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We carried out a meta-analysis of two recent psoriasis genome-wide association studies[1,2] with a combined discovery sample of 1,831 affected individuals (cases) and 2,546 controls. One hundred and two loci selected based on *P* value rankings were followed up in a three-stage replication study including 4,064 cases and 4,685 controls from Michigan, Toronto, Newfoundland and Germany. In the combined meta-analysis, we identified three new susceptibility loci, including one at *NOS2* (rs4795067, combined $P = 4 \times 10^{-11}$), one at *FBXL19* (rs10782001, combined $P = 9 \times 10^{-10}$) and one near *PSMA6-NFKBIA* (rs12586317, combined $P = 2 \times 10^{-8}$). All three loci were also associated with psoriatic arthritis (rs4795067, combined $P = 1 \times 10^{-5}$; rs10782001, combined $P = 4 \times 10^{-8}$; and rs12586317, combined $P = 6 \times 10^{-5}$) and purely cutaneous psoriasis (rs4795067, combined $P = 1 \times 10^{-8}$; rs10782001, combined $P = 2 \times 10^{-6}$; and rs12586317, combined $P = 1 \times 10^{-6}$). We also replicated a recently identified[3] association signal near *RNF114* (rs495337, combined $P = 2 \times 10^{-7}$).

Psoriasis vulgaris (PsV) is one of several immunologically mediated disorders whose genetic provenance is now coming into focus. Although the most evident cellular features of psoriasis are epidermal hyperplasia and altered keratinocyte differentiation, mounting evidence implicates both innate and acquired immunity in disease pathogenesis[4]. About a third of the individuals with PsV develop psoriatic arthritis (PsA), an inflammatory arthritis that is usually seronegative for rheumatoid factor[5]. Herein, the abbreviation PsC refers to individuals with purely cutaneous psoriasis (that is, PsV without PsA), and the general term 'psoriasis' refers to PsV unless otherwise specified. Individuals with psoriasis are at significantly greater risk of cardiovascular morbidity and mortality than the general population, especially younger individuals with more severe skin disease[6]. Psoriasis is also associated with inflammatory bowel disease and other autoimmune disorders[7].

Psoriasis is strongly influenced by genetic factors. The earliest reported genetic susceptibility factors for psoriasis map to the major histocompatibility complex (MHC)[8] and include haplotypes bearing *HLA-Cw6* (ref. 9). More recently, the MHC component of susceptibility has been fine mapped to or very near *HLA-Cw6* itself[10], and genome-wide association studies (GWAS) have identified nine additional regions outside the MHC with genome-wide levels of statistical significance: *IL12B*, *IL23R*, *IL23A*, *TNFAIP3*, *TNIP1*, *IL4-IL13*, *LCE3B-LCE3C*, *DEFB4* and *SPATA2-RNF114* (refs. 3,11,12). In this study, we report the discovery of three new genetic susceptibility loci for psoriasis, none of which have been definitively implicated in any complex genetic disorder to date. We also replicate a previous report[3] of association to the *SPATA2-RNF114* locus (combined $P = 2 \times 10^{-7}$) and extend the associated region at this locus to include two additional candidate genes.

We augmented published GWAS results from the Collaborative Association Study of Psoriasis (CASP)[1] by using genotype imputation[13] to combine the results of that study with those of an independent GWAS of psoriasis from Kiel, Germany[2] using either phase 2 HapMap or 1000 Genomes Project haplotypes as a reference (**Supplementary Table 1**). Only SNPs imputed with relatively high confidence (estimated r^2 between imputed and true genotypes >0.3) in both GWAS were considered for meta-analysis. Our discovery sample consisted of up to 1,831 cases and 2,546 controls with imputed genotypes for 2,502,313 (HapMap) or 7,456,344 (1000 Genomes Project) autosomal SNPs. Genomic control inflation factors for the *P* values from the meta-analysis of the CASP and Kiel GWAS were low ($\lambda = 1.061$ for HapMap and $\lambda = 1.054$ for 1000 Genomes Project imputations), and

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Table 1 Loci with strongest evidence of association with psoriasis in the combined sample

SNP[b]	Set[c]	Chr.	Pos (Mb)	Alleles risk/ nonrisk	Discovery samples (1,831 cases, 2,546 controls)[a] Frequency[d] Case	Discovery Control	Discovery OR (meta)	Discovery *P* (meta)	Replication samples (4,064 cases, 4,685 controls)[a] Frequency[d] Case	Replication Control	Replication OR (meta)	Replication *P* (meta)	Combined *P* (meta)	Notable nearby genes (relative position)[e]
rs4795067	B	17	23.13	G/A	0.397	0.354	1.20	5×10^{-5}	0.389	0.349	1.19	2×10^{-7}	4×10^{-11}	***NOS2* (intronic)**
rs10782001	B	16	30.85	G/A	0.394	0.347	1.22	1×10^{-5}	0.402	0.368	1.16	1×10^{-5}	9×10^{-10}	***FBXL19* (intronic)**
rs12924903	A	16	30.84	A/G	0.393	0.343	1.24	1×10^{-4}	0.384	0.349	1.16	2×10^{-6}	1×10^{-9}	***FBXL19* (–6.9 kb)**
rs12586317	C	14	34.75	T/C	0.783	0.742	1.25	1×10^{-5}	0.777	0.751	1.15	1×10^{-4}	2×10^{-8}	***NFKBIA*, *PSMA6***
rs1008953	B	20	43.41	C/T	0.809	0.770	1.27	1×10^{-5}	0.809	0.787	1.14	8×10^{-4}	1×10^{-7}	*SDC4* (–3.7 kb)
rs495337	C	20	47.96	G/A	0.610	0.573	1.17	7×10^{-4}	0.616	0.570	1.21	7×10^{-5}	2×10^{-7}	*RNF114* (silent)
rs12580100	B	12	54.73	A/G	0.898	0.868	1.33	2×10^{-6}	0.913	0.896	1.17	1×10^{-2}	1×10^{-6}	*RPS26* (–1.2 kb)

Chr., chromosome.
[a]The number of cases and controls that were genotyped and passed quality control for at least one of the 91 SNPs in the three replication sets. The actual numbers of cases and controls typed for each SNP varies and can be determined from the genotype counts in **Supplementary Table 2**. [b]SNPs attaining genome-wide significance and their notable nearby genes are indicated in bold. [c]Replication set for the SNP (see main text and **Supplementary Table 1** for more details). [d]Frequency of the risk allele. [e]Position of each SNP relative to notable nearby genes is given. +/– indicates whether the SNP is upstream (–) or downstream (+) of the transcription start site. SNPs within the gene are labeled as 'intronic' or 'silent'.

quantile-quantile plots (**Supplementary Fig. 1**) also indicated no substantial systematic differences between cases and controls due to cryptic relatedness, population substructure or other biases.

The replication sample consisted of up to 4,064 PsV cases and 4,685 controls from Michigan, Toronto, Newfoundland and Germany. All participating subjects gave informed consent and protocols were reviewed and approved by local institutional review boards. Ascertainment criteria for psoriasis have previously been described[10]. PsA was diagnosed according to the CASPAR criteria[14]. PsC was defined as the presence of skin lesions in the demonstrated absence of PsA at the time of recruitment in the study.

We selected 102 SNPs for replication based on their *P* value rankings either in a subset of 350 CASP PsA cases compared to normal controls (53 SNPs referred to as set A; **Supplementary Table 1**) or in the combined CASP-Kiel GWAS (49 SNPs referred to as sets B and C; **Supplementary Table 1**). Imputation quality of these SNPs was generally excellent. In the first-stage replication, we genotyped all selected SNPs in the large Michigan sample; 91 of these SNPs passed all quality control filters. In the second-stage replication, we typed all but one of the 51 quality control–filtered SNPs from set A of the PsA GWAS in the samples from Toronto and Newfoundland, which contained most of the PsA cases. SNPs with the most promising association results were then followed up with third-stage typing, which consisted of eight SNPs from sets B and C in the two Canadian samples and six SNPs from all three sets in the German sample. Statistical significance of association was assessed with an allelic χ^2 test for experimentally genotyped SNPs and an equal variance *t* test with allele dosages for imputed SNPs. Meta-analysis of association across cohorts incorporated both imputation quality and asymmetry in the number of cases and controls as well as the size of the cohort. See Online Methods for details of the quality control filters, imputation quality and meta-analysis.

We identified four SNPs at three loci for which association *P* values achieved genome-wide statistical significance ($P \leq 5 \times 10^{-8}$) in the combined discovery and replication samples. **Table 1** shows the results for these four SNPs along with three additional SNPs yielding a combined $P \leq 1 \times 10^{-6}$. Genotype counts for these seven SNPs are shown in **Supplementary Table 2**. Results for the remaining SNPs passing quality control are summarized in **Supplementary Table 3**, and association results for PsA compared to PsC are shown in **Table 2**.

One of the associated SNPs (rs4795067, combined $P = 4 \times 10^{-11}$, replication-stage OR = 1.19) mapped to an intron of *NOS2* (**Table 1**). As shown in **Figure 1**, the strongest imputed association signals from the CASP-Kiel GWAS clustered in a region of low linkage disequilibrium (LD) containing no other genes. *NOS2* encodes iNOS (inducible nitric oxide synthase), which is expressed by a subset of CD11c-positive, CD1c-negative, TNF-α (tumor necrosis factor-α)-producing inflammatory dendritic cells that is markedly expanded (30-fold) in psoriatic lesions[15]. Consistent with this, in lesional psoriatic skin we found a 1.54-fold overexpression of *NOS2* mRNA by microarray analysis (**Supplementary Table 4**) and a marked 16-fold overexpression by QRT-PCR (**Fig. 2** and **Supplementary Table 5**). However, neither rs4795067 nor any other SNP in this region was found to significantly

Table 2 Association of strongest replicated loci with psoriatic arthritis (PsA) and cutaneous psoriasis (PsC)

SNP	Chr.	Pos (Mb)	Alleles risk/ nonrisk	PsA versus control (1,747 PsA, 7,231 controls)[a] Frequencies[b]	PsA OR	PsA *P*	PsC versus control (3,394 PsC, 7,231 controls)[a] Frequencies[b]	PsC OR	PsC *P*	PsA versus PsC (1,747 PsA, 3,394 PsC)[a] Frequencies[b]	PsA/PsC OR	PsA/PsC *P*	Notable nearby genes
rs4795067	17	23.13	G/A	0.389/0.341	1.23	1×10^{-6}	0.391/0.348	1.20	1×10^{-8}	0.396/0.382	1.06	0.25	***NOS2***
rs10782001	16	30.85	G/A	0.412/0.358	1.26	4×10^{-8}	0.388/0.353	1.16	2×10^{-6}	0.412/0.385	1.12	0.022	***FBXL19***
rs12924903	16	30.84	A/G	0.412/0.352	1.29	4×10^{-9}	0.380/0.347	1.15	4×10^{-5}	0.403/0.378	1.11	0.058	***FBXL19***
rs12586317	14	34.75	T/C	0.786/0.751	1.22	6×10^{-5}	0.778/0.747	1.19	1×10^{-6}	0.785/0.780	1.03	0.60	***NFKBIA***
rs495337	20	47.96	G/A	0.615/0.573	1.20	2×10^{-3}	0.614/0.572	1.19	1×10^{-6}	0.615/0.618	0.99	0.86	*RNF114*
rs1008953	20	43.41	C/T	0.803/0.772	1.21	2×10^{-4}	0.792/0.769	1.15	2×10^{-4}	0.803/0.795	1.05	0.37	*SDC4*
rs12580100	12	54.73	A/G	0.882/0.867	1.15	0.029	0.893/0.866	1.29	1×10^{-6}	0.888/0.893	0.95	0.54	*RPS26*

Chr., chromosome.
[a]The number of cases and controls for the specified phenotype comparison that were genotyped and passed quality control for at least one of the 91 SNPs in the three replication sets. The actual numbers of cases and controls typed for each SNP varies and can be determined from the genotype counts in **Supplementary Table 2**. [b]Frequencies of the PsV risk allele for the two phenotypes being compared. Allele frequencies for the same phenotype (PsA, PsC or control) and the same SNP often differ among comparisons because of differing number of qualifying cohorts (for example, some cohorts have PsA but no PsC cases so qualify for PsA versus control but not PsA versus PsC or PsC versus control comparisons) and because variations in the proportions of each phenotype per cohort alter the relative cohort weights in the meta-analyses.

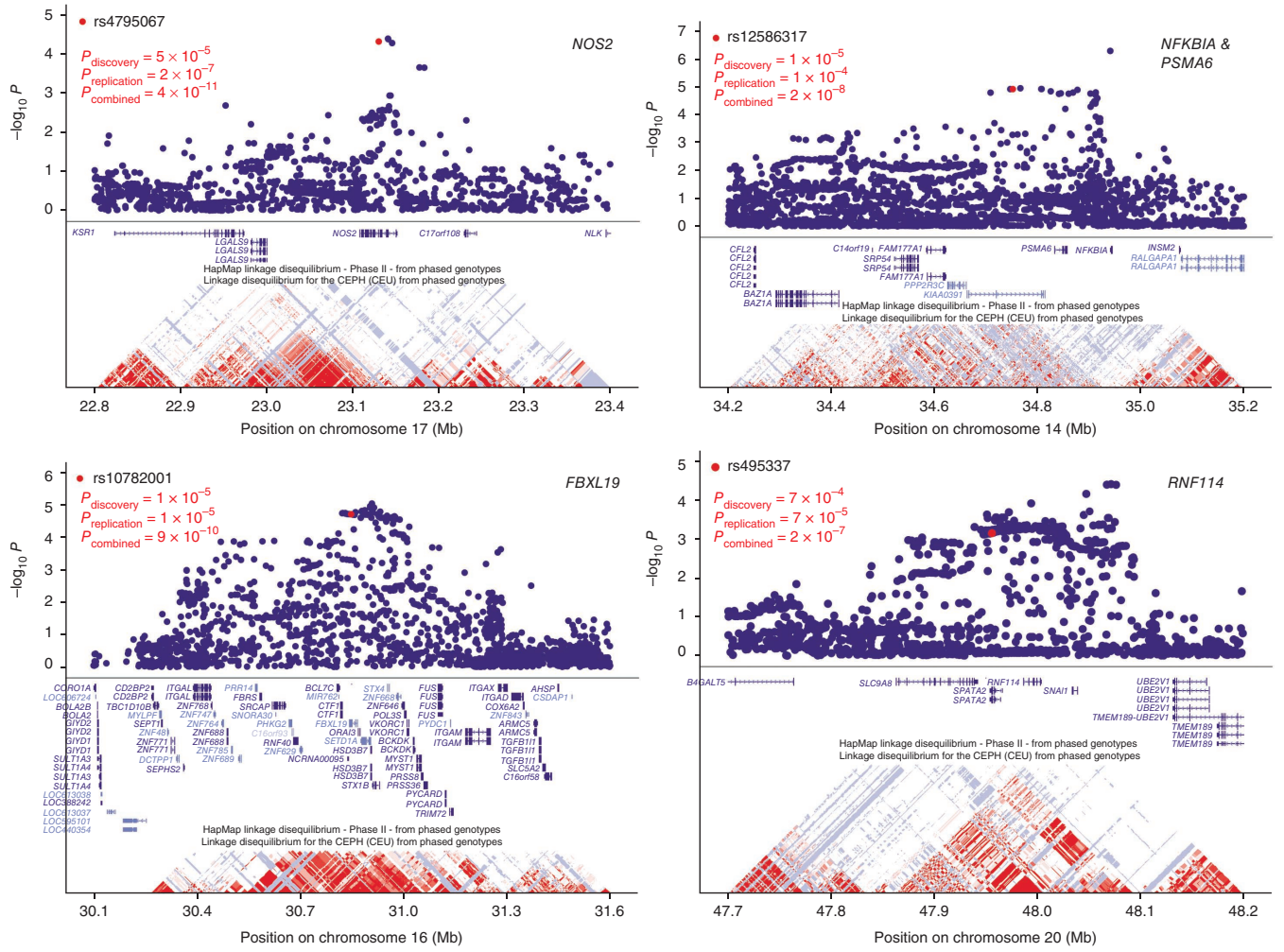

Figure 1 Evidence for psoriasis association in four genomic regions including the three new loci attaining genome-wide significance and the confirmed *RNF114* region. The upper portion of each plot depicts association *P* values for a meta-analysis of the CASP and Kiel discovery GWAS using 1000 Genomes Project–based imputation and the lower portion depicts RefSeq genes and LD plots from the phase 2 HapMap CEU sample. For each region, the most strongly associated replicated SNP is highlighted in red, as are its *P* values for the discovery, replication and combined samples.

regulate *NOS2* mRNA expression (that is, to function as a *cis*-acting expression quantitative trait locus (*cis*-eQTL)) as assessed by either microarray (**Supplementary Table 6**) or qRT-PCR assays (data not shown). Although this SNP attained genome-wide significance only for PsC in a subphenotype analysis, it also yielded a strong association with PsA (OR = 1.23, $P = 1 \times 10^{-6}$; **Table 2**). Notably similar to inflammatory dendritic cells in cutaneous psoriasis, cells infiltrating the sub-lining layer of diseased synovium express markedly higher levels of iNOS and TNF-α in PsA than in osteoarthritis or traumatic arthritis[16]. Although Online Mendelian Inheritance in Man (MIM163730) lists unconfirmed associations between *NOS2* variants and malaria, mycetoma, preterm delivery and Parkinson's disease, this is the first description of a genome-wide significant association between *NOS2* and any complex genetic disorder.

The next most significant SNP (rs10782001, combined $P = 9 \times 10^{-10}$, replication stage OR = 1.16) mapped to a large region of strong LD on chromosome 16p11.2 containing at least 31 genes (**Table 1** and **Fig. 1**). Both rs10782001 and rs12924903, which are in strong LD with each other ($r^2 = 1.0$ in the phase 2 HapMap European CEU population), attained genome-wide significance for PsA but not for PsC, despite the greater numbers of individuals with PsC ($n = 2{,}113$) relative to PsA ($n = 1{,}361$) (**Table 2**). Moreover, SNP rs10782001 was more strongly associated with PsA than with PsC at a statistically significant level (OR = 1.12, $P = 0.02$). Although any of the genes in the associated region could be responsible for the observed association signal, *POL3S* and *FBXL19* showed the strongest differential expression between psoriatic and normal skin (2.66-fold increase for *POL3S* and a 1.43-fold increase for *FBXL19* in lesional skin compared to control skin; **Fig. 1** and **Supplementary Table 4**). *POL3S* encodes a secreted serine protease about which little is known[17]. *FBXL19* is structurally related to *FBXL11*, an F-box family member recently shown to inhibit NF-κB (nuclear factor kappa light chain enhancer of activated B cells) activity by lysine demethylation[18]. Of the 68 human members of the F-box protein family, only three (FBXL10, FBXL11 and FBXL19) have zinc finger, plant homology domain and leucine-rich repeats in addition to the F-box[19]. FBXL10 and FBXL11 contain jumonji C domains known to be required for demethylase activity but FBLX19 does not[19]. Thus, FBXL19 might act as a dominant negative inhibitor of demethylase activity, thereby serving to activate NF-κB.

The third associated SNP (rs12586317, combined $P = 2 \times 10^{-8}$, replication stage OR = 1.15; **Table 1**) mapped to the vicinity of *NFKBIA*, *PSMA6* and *KIAA0391*. Imputation based on the 1000 Genomes Project led us to select this SNP for typing and revealed that the

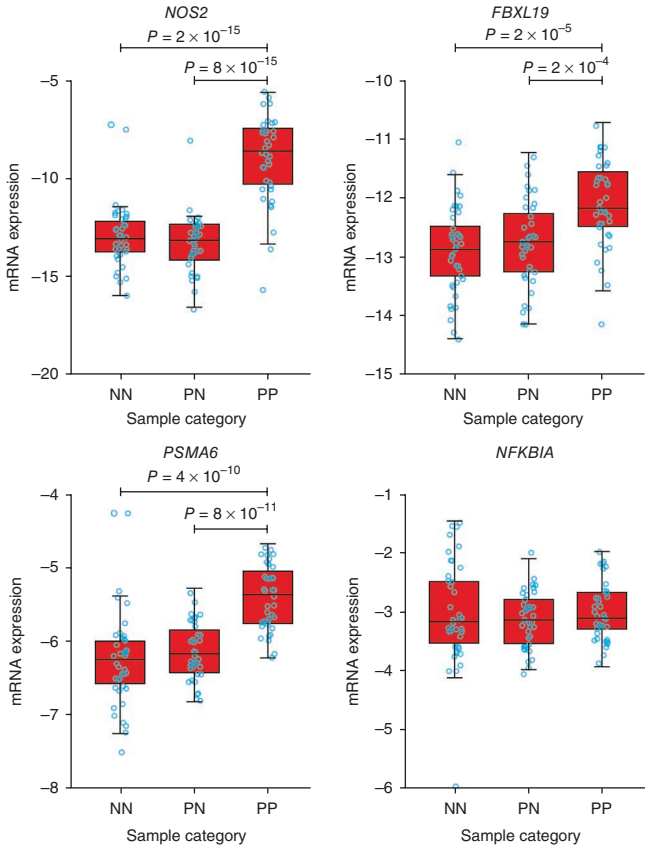

Figure 2 Expression data for notable candidate genes within the three psoriasis-associated regions. Log_2-transformed levels of mRNA assayed by qRT-PCR are shown for normal (NN), uninvolved (PN) and lesional psoriatic (PP) skin. Figures include both individual measurements (jittered scatterplots) and box plots summarizing the distribution. Bars and significance levels are shown for all pairwise comparisons of mean mRNA levels that are significant at a nominal ($P \leq 0.05$) level.

associated region extended to encompass *NFKBIA*, an observation that was not evident from HapMap-based imputation (**Fig. 1** and data not shown). Of these three genes, only *PSMA6* is overexpressed in psoriatic lesions (**Fig. 2** and **Supplementary Table 4**). *NFKBIA* and *PSMA6* are both attractive candidate genes for psoriasis susceptibility, as *NFKBIA* encodes IκB-α, an inhibitor of NF-κB signaling, and *PSMA6* encodes a proteosomal subunit involved in MHC class I antigen processing. Polymorphisms in both genes (MIM164008 and MIM602855) have been suggestively associated with susceptibility to various disorders including myocardial infarction, Graves' disease and inflammatory bowel disease. However, this is the first demonstration of a genome-wide significant association in this region for any complex genetic disorder.

Although they did not attain genome-wide significance, SNPs rs1008953 (combined $P = 1 \times 10^{-7}$, replication stage OR = 1.14) and rs12580100 (combined $P = 1 \times 10^{-6}$, replication stage OR = 1.17) were also of interest. rs1008953 resides 3.7 kb upstream of *SDC4*, which encodes syndecan-4, a cell-surface heparin sulfate proteoglycan that inhibits T-cell activation[20]. Expression of *SDC4* mRNA was markedly reduced in lesional psoriatic skin relative to both uninvolved and normal skin (**Supplementary Tables 4** and **5**). However, there are several other genes under the association peak, and several SNPs in the region function as *cis*-eQTLs for *SYS1*, *DBNDD2*, and *PIGT* expression (**Supplementary Table 6** and **Supplementary Fig. 2**). rs12580100 resides 1.2 kb upstream of *RPS26*, which resides in a region that has been implicated in susceptibility to type 1 diabetes[21] and which encodes a ribosomal protein subunit whose expression is under *cis*-acting genetic control in the liver[22]. rs12580100 lies only 300 kb from a confirmed association signal at rs2066808 near *IL23A* (ref. 1), but these association signals are at least partially independent because the two SNPs reside in different LD blocks (**Supplementary Fig. 2**) and show low pairwise LD ($r^2 = 0.23$), and a logistic regression model including both SNPs yielded significant partial association for each ($P = 0.016$ for rs12580100 and $P = 3.0 \times 10^{-4}$ for rs2066808). Of note, we found that *RPS26* is overexpressed in lesional psoriatic skin (**Supplementary Tables 4** and **5**) and confirm in skin that this region contains several highly significant eQTLs that clearly colocalize with the disease association signal for *RPS26* but not for *IL23A* (**Supplementary Table 6** and **Supplementary Fig. 2**).

Although also not reaching genome-wide significance, rs495337 (combined $P = 2 \times 10^{-7}$, replication stage OR = 1.21) confirms previous reports of association between psoriasis and the *SPATA2-RNF114* region[3]. However, the 1000 Genomes Project–based imputation demonstrated that the region of association extends farther than previously reported and encompasses *SLC9A8* and *SNAI1* (**Fig. 1**). Of these four genes, *RNF114* was the most strongly expressed in skin (**Supplementary Table 4**). None of the transcripts encoded by these four genes was increased in psoriatic lesions; one *SPATA2* probe detected a 23% decrease (**Supplementary Table 4**). We were unable to confirm a previous report of a *cis*-eQTL in this region[3], either by microarray (**Supplementary Table 6**) or qRT-PCR assays (data not shown); however, our gene expression sample was only about half the size of that used in the previous study.

Fine mapping studies are needed to identify causal variants in the three new susceptibility regions we have identified. Nevertheless, genes contained in these newly associated regions fit well with emerging concepts of psoriasis pathogenesis[12]. Given genetic, therapeutic and immunological results indicating that IL-23 produced by dendritic cells promotes the survival and expansion of pathogenic T-cells in psoriasis[12], it is noteworthy that one of our new regions contains *NOS2*, which is expressed by a massively expanded subset of TNF-α producing inflammatory dendritic cells in psoriatic lesions[15]. Moreover, given that *TNFAIP3* and *TNIP1* regulate NF-κB signaling and have been implicated in psoriasis susceptibility[1], the fact that *FBXL19* and *NFKBIA* regulate the same pathway further suggests the central importance of NF-κB signaling in psoriasis. Finally, when combined with the profound response of PsA to TNF-α blockers[4] and prior genetic results implicating *IL12B* and *TNIP1* in PsA susceptibility[1], the identification of a new PsA susceptibility region containing *FBXL19* suggests that this multicellular IL-23/IL-17/NF-κB signaling circuit may also be of critical importance to the development of joint disease in individuals with psoriasis.

URLs. MACH software, http://www.sph.umich.edu/csg/abecasis/mach/; HapMap 2 CEU phased haplotypes, http://hapmap.ncbi.nlm.nih.gov/downloads/phasing/2006-07_phaseII/phased/; August 2009 release of 1000 Genome Project phased data, http://www.sph.umich.edu/csg/abecasis/mach/download/1000G-Sanger-0908.html.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

R.P.N., P.E.S. and J.T.E. performed SNP selection, data analysis and prepared the figures and tables. R.P.N., T.T., P.E.S., P.R. and E.E. performed genotyping. P.E.S., Y.L. and J.D. performed genotype imputation and association analyses, and P.E.S., J.E.G., and J.D. performed the expression analyses. G.R.A. helped with statistical analyses and interpretation of results. R.P.N., T.T., J.J.V., R.I., M.W., S.W., B.E., C.G., H.E.W., H.W.L., P.R., M.K., U.M. and D.D.G. coordinated subject recruitment and collected phenotype data. J.T.E., G.G.K. and A.M.B. contributed genotypes and phenotypes from the CASP discovery GWAS. J.T.E. and P.E.S. drafted the manuscript; R.P.N., G.R.A., E.E., M.W. and A.F. edited the manuscript; and J.T.E. planned and supervised the study. All authors approved the final draft.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Genotyping. We determined genotypes for the discovery phase of the Collaborative Association Study of Psoriasis (CASP) on a Perlegen platform[1] and genotypes for the discovery phase of the Kiel GWAS on an Illumina HumanHap 550K array[2]. In Ann Arbor, follow-up genotyping was performed using either the TaqMan (Applied Biosystems) or the Sequenom MassArray (Sequenom) platforms. Additional follow-up genotyping was performed using the TaqMan platform in the samples from Kiel and the Sequenom platform in the samples from Newfoundland and Toronto.

Quality control filters. For the CASP and Kiel GWAS analyses, multiple quality control filters were applied to both the samples and the genotypes[1,2]. For the replication samples, we excluded SNPs with <95% genotyping call rates, with a Bonferonni-corrected Hardy Weinberg $P < 0.05$ or with cluster plots that did not show clear separation of the three genotype clusters. We also excluded samples with <95% genotyping call rate. For genotyping performed on the Sequenom platform, the sample filter was applied separately to each typing group (ranging from 15–23 SNPs in size). Sample and SNP filters were applied separately to each replication cohort.

Genotype imputation. We performed genotype imputation using a hidden Markov model algorithm implemented in MACH software version 1.0 as previously described[13,23]. For replication sets A and B, we used HapMap 2 CEU phased haplotypes[24], which serve as a good reference for populations of European descent[13,25]. For replication set C, we used phased haplotypes from the August 2009 release of the 1000 Genomes Project as the reference. Parameters for the hidden Markov model for the 1000 Genomes Project imputation were first estimated using a subset of 250 randomly sampled individuals, and then all individuals were imputed based on those parameters. For both imputations, a dosage score (ranging from 0 to 2) was computed at each SNP for each individual, which is the expected number of copies of a given allele conditional on the genotypes of directly assayed SNPs and integrating overall possible configurations of the phased reference haplotypes. Imputed dosages for the two GWASs were analyzed separately, and the association test results were then combined by meta-analysis using the weighting scheme described below.

The predicted imputation quality of the 91 SNPs selected for replication was excellent; the median MACH r^2 values were 0.9858 and 0.9688, respectively, for the HapMap and 1000 Genomes Project imputations of the CASP GWAS, and 0.9887 and 0.9841 for the two imputations of the Kiel GWAS. We also tested the accuracy of MACH's predicted estimates of r^2 between imputed and true genotypes. All 91 replication SNPs were genotyped for a large subset of the CASP GWAS samples (456 of 1,359 cases and 670 of 1,400 controls), and six of these SNPs yielding significance at or near genome-wide levels were also typed for a substantial subset of the Kiel GWAS samples (443 of 472 cases). A strong correspondence was seen between the observed and predicted squared correlation of imputed and experimentally determined genotypes for both the CASP GWAS (Pearson $r = 0.914$ for HapMap and $r = 0.937$ for 1000 Genomes Project imputations) and the Kiel GWAS ($r = 1.000$ for HapMap and $r = 0.999$ for 1000 Genomes Project).

Meta-analysis of association. Evidence for association was combined across cohorts using a method adapted from published recommendations[26]. Signed *z* scores were calculated directly from association test statistics, with the sign of each *z* score indicating the direction of effect in that cohort. *Z* scores were then summed across multiple cohorts using weights equal to the square root of the effective sample size in each cohort divided by the sum of effective sample sizes in all cohorts, which ensures that the squared weights sum to one. The effective sample size (*Ne*) was determined for each combination of cohort and SNP by adjusting the raw sample size for asymmetry in the number of cases and controls and by scaling with the imputation uncertainty when applicable, as follows:

$$Ne = \frac{4NaNu}{Na + Nu} \times \mathrm{MACH}\hat{r}^2$$

where *Na* and *Nu* are the number of affected cases and unaffected controls typed or imputed for the given SNP in the given cohort, and MACH $\hat{r}^2$ is an estimate of the squared Pearson correlation between the imputed genotype scores and the true genotypes for the given SNP. The first term on the right-hand side of the equation calculates the symmetric case-control sample size yielding the same non-centrality parameter, and hence power, under the null hypothesis for an allelic association test of a biallelic marker, as does the given asymmetric case-control sample size. Under the alternative hypothesis of association, both the non-centrality parameter and N_e depend upon not only N_a and N_u but also upon the genetic model (that is, the risk allele frequencies in cases and controls). However, for the effect sizes normally seen for complex disease loci (OR < 2.0), estimates of N_e under the null and the alternative differ only modestly for a wide variety of genetic models, total sample sizes and N_a to N_u sample allocations. The second term on the right-hand side of the equation down-weights the contribution of a GWAS when a particular SNP was poorly imputed.

Estimates of odds ratios and allele frequencies were summed across multiple cohorts using the square of the weights described above. Odds ratios were first log transformed and then back transformed after summation across cohorts.

Quantile-quantile plots and genomic control for meta-analysis of CASP and Kiel GWAS. Quantile-quantile plots and genomic control inflation factors for the *P* values from a meta-analysis of the CASP and Kiel GWAS were restricted to those SNPs with a MACH r^2 imputation quality of at least 0.3 for both studies. The genomic control variance inflation factor (λ) was computed using the bounded median method[27]. Quantile-quantile plots and genomic control λ values were determined both for all reliably imputed SNPs and after exclusion of SNPs within nine previously published regions of genome-wide significant association with psoriasis. For the MHC, the excluded interval was the 7.7-Mb extended region previously defined[28]. For regions harboring candidate loci *IL12B*, *IL23R*, *IL23A*, *IL13*, *TNIP1* and *TNFAIP3*, boundaries of the excluded intervals were chosen as ± 500 kb relative to the most strongly associated SNP as previously determined[1]. Boundaries were also chosen to be ± 500 kb relative to the most strongly associated SNP in the *RNF114* region[3] and ± 500 kb relative to the 32 kb deletion spanning the LCE3B and LCE3C genes on chromosome 1[29]. The published psoriasis locus that maps to a highly variable copy number variation near the β-defensin cluster on chromosome 8 was not excluded because there are no known SNPs in strong LD with this copy number variation[30].

For the 1000 Genomes Project–based imputations, a 210 kb region on chromosome 17p11.2 with multiple SNPs exhibiting strong but spurious association was also excluded for one of the quantile-quantile plots. These spurious associations are an artifact of a single SNP in the Kiel GWAS (rs4889730), which passed all quality control filters but was nevertheless unreliably typed as determined by *post hoc* inspection of the Illumina genotyping cluster plot. Removal of this SNP from the Kiel GWAS before re-imputation resulted in loss of all evidence of association (data not shown). Notably, this region showed no evidence of association in the CASP GWAS, nor could observed associations in the Kiel GWAS be replicated for rs1975974 and rs17052344 in the Michigan sample (**Supplementary Table 3**) or for rs17052344 in the German sample[2].

Measurement and analysis of gene expression. Six millimeter punch biopsies of normal skin (of unaffected individuals (74 subjects), as well as uninvolved skin and/or involved lesional skin of affected individuals (66 subjects), were obtained at the University of Michigan Department of Dermatology as previously described[31]. Involved skin biopsies were taken from psoriasis plaques, and uninvolved and normal skin were sampled from the buttocks at least 10 cm away from the nearest plaque. Study subjects did not use any systemic anti-psoriatic treatments for 2 weeks prior to biopsy or topical anti-psoriatic treatments for 1 week before biopsy. Informed consent was obtained from all subjects under protocols approved by the Institutional Review Board of the University of Michigan Medical School and the study was conducted according to the Declaration of Helsinki Principles. RNA from each biopsy was isolated using the RNeasy kit (Qiagen).

Samples for 64 normal skin biopsies and 58 paired affected and uninvolved skin biopsies were run on Affymetrix U133 Plus 2.0 arrays according to the manufacturer's protocol. The raw data were processed using the Robust Multichip Average (RMA) method, adjusting RMA expression values

to account for batch and gender effects[31]. Gene expression was further assessed by qRT-PCR, as described previously[31], for 39 normal skin, 38 uninvolved skin and 37 lesional skin biopsies. These qRT-PCR samples partially overlapped the microarray sample set (29 normal, 33 uninvolved and 29 affected skin samples common to both).

Two sample *t* tests were used to compare mean mRNA expression in skin from normal controls with that in lesional or non-lesional skin from individuals with psoriasis. For comparisons of mean expression in involved and uninvolved skin from affected individuals, paired *t* tests were used for microarray assays, as lesional and non-lesional skin was always sampled in pairs from each individual, but two-sample *t* tests were used for qRT-PCR assays because many of the affected individuals were biopsied for only lesional or non-lesional skin but not both. Log_2-transformed mRNA levels were used for all *t* tests.

For microarray data, we tested for *cis*-acting expression quantitative trait loci (*cis*-eQTL) separately in normal, uninvolved and involved skin. We used the score test in MERLIN (fastassoc option) to test *cis* associations between log_2-transformed mRNA levels of each probe (transcript) and SNPs in its *cis* candidate region, mapping from 1 Mb upstream of the transcription start site to 1 Mb downstream of the transcription end site. For genotyped SNPs, which were extracted from the CASP GWAS after quality control filtering, the observed number of copies of one allele was modeled. For imputed SNPs, which used HapMap2 CEU phased haplotypes as a reference, the dosage of one allele was modeled. Fifty-seven normal skin samples and fifty-three pairs of uninvolved and involved skin samples qualified for eQTL analysis, having both microarray expression profiling and genotype data. To increase power, a meta-analysis across independent skin samples (normal and uninvolved skin or normal and involved skin) was performed by combining sample size–weighted signed *z* scores for the individual skin types. Significance thresholds of 9×10^{-7}, 1.0×10^{-5} and 2.8×10^{-5}, corresponding to false discovery rates of approximately 0.01, 0.05 and 0.10 were used to assess evidence for *cis*-eQTLs.

For *cis*-eQTL analyses using qRT-PCR data, we used linear regression of mRNA levels against dosage of the risk allele. Combined analysis across independent skin types was performed by including skin type as an independent indicator variable in the regression model. Thirty-seven normal skin, thirty-seven uninvolved skin and 35 lesional skin samples qualified for analysis and had both qRT-PCR expression and genotype data.

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