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Expanding the Psoriasis Disease Profile: Interrogation of the Skin and Serum of Patients with Moderate-to-Severe Psoriasis

Mayte Suárez-Fariñas[1,3], Katherine Li[2,3], Judilyn Fuentes-Duculan[1], Karen Hayden[2], Carrie Brodmerkel[2] and James G. Krueger[1]

Psoriasis is a complex disease with an expanding definition of its pathological features. We sought to expand/refine the psoriasis transcriptome using 85 paired lesional and non-lesional samples from a cohort of patients with moderate-to-severe psoriasis vulgaris who were not receiving active psoriasis therapy. This new analysis identified 4,175 probe sets (representing 2,725 unique known genes) as being differentially expressed in psoriasis lesions compared with matched biopsies of non-lesional skin when the following criteria were applied: >2-fold change and false discovery rate <0.05. These probe sets represent the largest and most comprehensive set of genes defining psoriasis at the molecular level and within the previously unidentified genes, a link to functional pathways associated with metabolic diseases/diabetes and to cardiovascular risk pathways is identified. In addition, we profiled the serum of moderate-to-severe psoriatics compared with healthy controls to assess the overlap of overexpressed lesional genes with overexpressed systemic proteins. We identified linkage of functional pathways in lesional skin associated with metabolic diseases/diabetes and cardiovascular risk with those pathways overexpressed in the serum, suggesting a potential linkage between altered gene transcription in the skin and comorbidities commonly seen in patients with moderate-to-severe psoriasis.

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INTRODUCTION

A molecular definition of disease requires elucidation of genetic, genomic, metabolic, and proteomic disease elements. Disease profiles are then built upon multiple datasets and are influenced by many factors including disease severity, matrices profiled, concomitant medications, technical differences in collection, and patient characteristics.

The pathological definition of psoriasis has greatly expanded over several decades. Before 2000, application of conventional methods produced essential information about keratinocytes, blood vessels, and immune-related cells in psoriatic lesions, ultimately leading to the identification of ''unique'' psoriasis proteins, e.g., psoriasin (S100A7; Borglum *et al.*, 1995; Hardas *et al.*, 1996) and ψ-3 antigen (Mansbridge *et al.*, 1984). Starting in 2001, however, a more holistic definition of psoriasis pathology in diseased tissue could be pursued via a broad genomic approach comparing messenger RNA (mRNA) expression between psoriatic lesions and ''non-lesional'' background skin of the same patient.

The first list of psoriasis-associated genes (transcriptome), using an early Affymetrix platform, encompassed 159 genes (Oestreicher *et al.*, 2001). From these data, a process of ordering altered gene expression into functional pathways controlled by ''master'' cytokines or transcription factors was begun. Subsequent gene array studies identified varied dysregulated pathways encompassing many hundreds of genes (Zhou *et al.*, 2003; Gudjonsson, 2007; Yao *et al.*, 2008; Suárez Fariñas *et al.*, 2010). This growing list of differentially expressed genes (DEGs), or ''psoriasis transcriptome,'' has been driven by several factors, including: (1) development of higher-density arrays that now encompass all known human transcripts, (2) technological improvements in biochemical methods for determining complementary DNA transcripts, (3) assessment of larger patient cohorts, yielding greater statistical power after adjustments for multiplicity of testing, and (4) ongoing improvements in statistical methods for analyzing whole-genome transcripts. To date, Gudjonsson *et al.* (2010) have studied the largest set of psoriasis patients ($n = 58$) for transcriptome profiling, yielding identification of 1,326 DEGs.

[1]*Laboratory of Investigative Dermatology, Rockefeller University, New York, New York, USA and* [2]*Immunology & Biomarkers, Janssen Research & Development, Radnor, Pennsylvania, USA*

[3]*These authors contributed equally to this work.*

Correspondence: James G. Krueger, Laboratory of Investigative Dermatology, Rockefeller University, 1230 York Avenue, New York, New York 10065, USA. E-mail: James.Krueger@rockefeller.edu

Abbreviations: BMI, body mass index; CCL2, monocyte chemoattractant protein-1; COX, cytochrome c oxidase; CTLA, cytotoxic T-lymphocyte antigen; DEG, differentially expressed gene; FCH, fold change; FDR, false discovery rate; IPA, Ingenuity Pathway Analysis; mRNA, messenger RNA; NO, nitric oxide; RT-PCR, real-time reverse transcriptase PCR; TNF, tumor necrosis factor-α; TLR, Toll-like receptor

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In parallel with gene expression profiling, considerable effort has been made to define disease inheritance through conventional genetic methods and genome-wide association studies (Zhang *et al.*, 2009; Elder *et al.*, 2010; Ellinghaus *et al.*, 2010; Sun *et al.*, 2010). These studies have identified >20 risk alleles/genes contributing to the risk of acquiring psoriasis. However, little is currently known about the association between the risk alleles and altered gene function and/or associated biological processes.

Metabolic and proteomic profiling of psoriasis has been much more limited. Many publications focus on the measurement of a small number of circulating proteins with no systematic profiling of circulating/lesional proteins. However, a recent report that employed proteomics to profile lesional psoriatic skin (Piruzian *et al.*, 2010) confirmed production of keratin and S100 proteins previously known to be upregulated at the transcript level. Recent studies have shown that psoriasis comorbidities (e.g., cardio-metabolic) significantly impact patient health, and it has been hypothesized that inflammatory products could potentially be synthesized in the skin and released into systemic circulation by diffusion through cutaneous endothelium (Davidovici *et al*, 2010). Hence, we undertook a protein profiling effort for more than 90 serum proteins to understand the overlap between lesional overexpression of genes and systemically detectable proteins. In doing so, we also expanded/refined the psoriasis skin transcriptome to 4,175 transcripts in a cohort of 85 paired lesional and non-lesional samples obtained from patients with moderate-to-severe psoriasis vulgaris not receiving active psoriasis treatment. Understanding the ''mechanics'' of psoriasis, not only in the skin but also in the circulation, is important because many of the previously unidentified gene products from this analysis are linked to functional pathways associated with metabolic diseases/diabetes and to cardiovascular risk pathways, suggesting a potential linkage between altered gene transcription in the skin and comorbid diseases that are commonly seen in patients with moderate-to-severe psoriasis.

RESULTS

Patient cohorts

Baseline demographic characteristics for 89 psoriatic patients with skin biopsy samples, as well as 149 psoriatic patients and 162 healthy subjects with samples for serum protein analyses, are provided in Table 1. Of the 89 patients in the skin biopsy substudy, 62 contributed serum samples to the serum protein analyses. Baseline characteristics for the patients providing serum samples are summarized in Supplementary Table S1 online. Healthy subjects were more likely to be non-Caucasian and current smokers than psoriatic patients ($P<0.0001$). Among the patients with moderate-to-severe psoriasis, ~30% of their body surface area had psoriasis involvement, and the average baseline psoriasis area and severity index scores were ~21(±10.2) (Table 1).

The transcriptome of ''moderate-to-severe'' psoriasis plaques contains 4,175 differentially expressed transcripts

We identified 4,175 probe sets as being differentially expressed in psoriasis lesions versus non-lesional matched biopsies when defined by >2-fold change (FCH) and false discovery rate (FDR) <0.05. A heatmap of the DEGs is shown in Figure 1a. The top 50 genes over- or under-expressed in psoriatic lesions are listed in Tables 2a and 2b, respectively. All DEGs are listed in Supplementary Table S2 online. Tables 2a and 2b also specify whether identified genes are regulated in keratinocytes by the cytokines tumor necrosis factor-α (TNF), IL-17, or IFN-γ, which are key mediators of inflammation in psoriasis. The *S100A12* gene, a highly inflammatory molecule that binds to the receptor for advanced glycation end products, and is increased in inflammatory dendritic cells and keratinocytes in response to inflammatory cytokines such as IL-17, TNF, and IFN-γ (Nograles *et al.*, 2008; Zaba *et al.*, 2010) exhibited the largest increase in expression.

IL-17 and TNF are now known to exert additive and synergistic effects on keratinocytes to modulate gene expression (Chiricozzi *et al.*, 2011). Interestingly, 60% of the top 20 upregulated genes (Table 2a) have additive (A) or synergistic

Table 1. Baseline demographic characteristics of 89 psoriasis patients with lesional and non-lesional skin biopsy samples, as well as 149 psoriasis patients and 162 healthy control subjects included in serum protein analysis

	Psoriasis patients (*n*=89)	Psoriasis patients (*n*=149)	Healthy controls (*n*=162)
Age (years)	44.6 ± 13.1	46.3 ± 12.8	42.6 ± 14.0
Male	66 (77.5%)	114 (76.5%)	89 (59.3%)
Caucasian	75 (84.3%)	133 (89.3%)	29 (22.5%)*
Current smoker	36 (40.4%)	50 (33.6%)	68 (62.4%)*
Obese	47 (52.8%)	73 (49.3%)	35(32.1%)**
Body surface area with psoriasis (%)	30 ± 20.5	29.5 ± 18.6	—
Psoriasis area and severity index score	21.5 ± 10.8	21.3 ± 9.0	—
Psoriatic arthritis	19 (21.3%)	38 (25.5%)	—

Data shown are number (%) of patients or mean ± standard deviation.
*$P<0.0001$.
**$P=0.0026$.

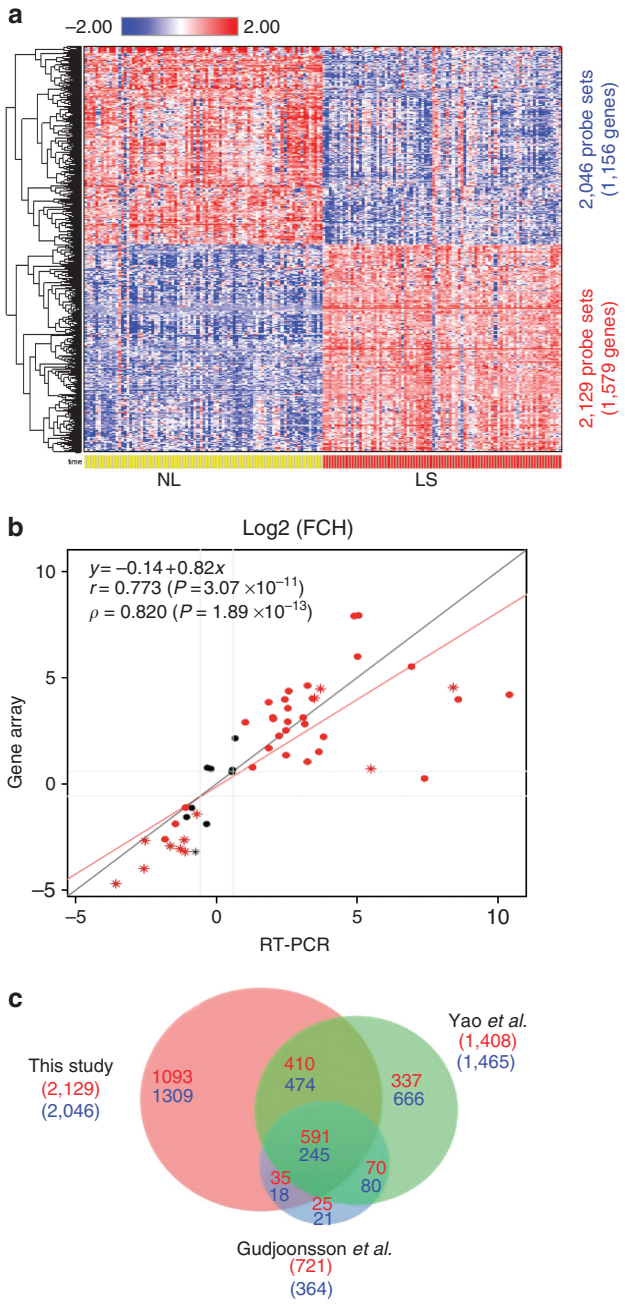

Figure 1. The transcriptome of moderate-to-severe psoriasis. (**a**) Heatmap of differentially expressed genes (DEGs). Unsupervised clustering of lesional (LS) versus non-lesional (NL) DEGs. (**b**) Scatter plot of the estimated fold change (log2 scale) by real-time reverse transcriptase PCR (RT-PCR, *x*-axis) and gene array (*y*-axis) for a selected group of 50 genes (see Table 3). Gray lines outline the two-fold change (FCH) regions. Stars and bullets represent two different low-density cards. Red shows confirmed genes. Classical linear regression analysis (red line) shows a slight compression of FCHs by gene array. Pearson's (*r*) and Spearman's (ρ) correlation values are presented along with *P*-values. (**c**) Venn diagram comparing genes (blue numbers: downregulated genes; red numbers: upregulated genes) identified in this study with those of two published studies employing the same Affymetrix HG U133 Plus 2.0 arrays using the same cutoff criteria (false discovery rate <0.05, FCH>2).

(S) responses to IL-17 and TNF, suggesting the particular importance of these cytokines in creating the ''top'' molecular profile of psoriasis. From comparing psoriatic DEGs with genes differentially regulated in human keratinocytes by cytokines, we find 231 genes of TNF signaling, 481 genes of IFN-γ signaling, 29 genes of IL-17 signaling, 10 genes of IL-22 signaling, and 144 genes that have additive or synergistic responses to IL-17 and TNF. Thus, $\geqslant$20% of the overall psoriasis transcriptome is concordant with gene alterations produced in cultured keratinocytes by defined cytokines. In addition, this gene set potentially reflects the direct effect of inflammatory cytokines on the disease phenotype.

Using gene array, we did not detect increased expression of many T-cell-produced cytokines, e.g., IFN-γ, IL-17 or IL-22, which consistently have been increased in psoriasis plaques, when measured by real-time reverse transcriptase PCR (RT-PCR) methods (Suárez Fariñas *et al.*, 2010). Hence, we also measured a set of disease-related cytokine mRNAs by RT-PCR in lesional and non-lesional tissues from this group of patients (Supplementary Table S3 online). As expected, we detected much greater expression of mRNAs encoding IL-23 (p40 and p19 subunits), IFN-γ, IL-17, and IL-22 in lesional versus non-lesional biopsies, with expression in psoriasis lesions ranging from 3.6-fold to 120-fold (*P*-values ranging from 10^{-11} to 10^{-29}) when measured by RT-PCR, but generally $<$2-fold on gene arrays. Hence, despite a large dynamic range to measure gene expression (16 logs), microarrays are unreliable for detecting quantitative differences in expression of these primary cytokine mRNAs generally found in low ($\sim$2 log values) levels (Suárez Fariñas *et al.*, 2010).

Low-density card confirmation

We performed extensive confirmation of 50 DEGs by quantitative RT-PCR (Table 3, Figure 1b), 42 of which were confirmed ($P<0.05$, FCH>2; note that seven genes were not confirmed, and one gene (*ADAM10*) was borderline). RT-PCR and gene array findings showed a high degree of correlation, with a Pearson's correlation coefficient of 0.773 ($P<10^{-11}$) for the magnitude of fold changes observed by these methods (Figure 1b). Linear regression analysis shows a slight compression of FCHs by gene array (18% reduction), a known feature of this technique (MAQC Consortium *et al.*, 2006).

Comparison with other psoriasis transcriptomes

To our knowledge, this study identified the largest set of DEGs in psoriasis vulgaris lesions, with more than 2,400 transcript differences that were not detected in two other recent studies employing transcriptional profiling of relatively large patient groups that also employed HGU133plus2 chips (Supplementary Table S4 online). Using a Venn diagram to plot the similarities/differences in mRNA expression between this study versus those reported by Gudjonsson *et al.* (2010) and Yao *et al.* (2008) (Figure 1c), we saw significant overlap of DEGs detected across the studies, with strong overlap within the most highly upregulated or downregulated genes

Table 2a. The top 50 unique genes upregulated in lesional skin compared with non-lesional skin biopsy samples obtained at baseline from 85 patients with moderate-to-severe psoriasis (FDR $P<0.05$ and FCH >2)

Probe set ID	Gene symbol	Gene title	Fold change (lesional vs. non-lesional)	Mean expression in skin samples		Cytokine regulation[1]
				Non-lesional	Lesional	
205863_at	*S100A12*	S100 calcium–binding protein A12	889.1	2.72	12.52	IL-17, TNF-α, IFN-γ, A
211906_s_at	*SERPINB4*	Serpin peptidase inhibitor, clade B (ovalbumin), member 4	740.44	3.76	13.30	IL-17, S
205513_at	*TCN1*	Transcobalamin I (vitamin B12–binding protein, R binder family)	400.17	2.95	11.60	
232220_at	*S100A7A*	S100 calcium–binding protein A7A	287.22	5.15	13.32	S
220664_at	*SPRR2C*	Small proline-rich protein 2C (pseudogene)	243.3	3.62	11.55	IL-17, A
205660_at	*OASL*	2′-5′-Oligoadenylate synthetase-like	238.28	3.15	11.04	IFN-γ
207602_at	*TMPRSS11D*	Transmembrane protease, serine 11D	199.6	2.89	10.53	S
1569555_at	*GDA*	Guanine deaminase	168.79	3.44	10.84	
210663_s_at	*KYNU*	Kynureninase (L-kynurenine hydrolase)	153.53	2.75	10.02	TNF-α, S
207367_at	*ATP12A*	ATPase, H+/K+ transporting, nongastric, α-polypeptide	148.59	3.93	11.15	A
220528_at	*VNN3*	Vanin 3	144.45	2.23	9.40	S
210413_x_at	*SERPINB3/SERPINB4*	Serpin peptidase inhibitor, clade B (ovalbumin), member 3/serpin peptidase inhibitor, clade B (ovalbumin), member 4	136.5	6.86	13.95	
206561_s_at	*AKR1B10*	Aldo-keto reductase family 1, member B10 (aldose reductase)	114.33	6.19	13.03	
202859_x_at	*IL8*	interleukin 8	105.05	2.82	9.54	IL-17, TNF-α, S
205783_at	*KLK13*	Kallikrein-related peptidase 13	94.62	4.16	10.73	
219554_at	*RHCG*	Rh family, C glycoprotein	74.17	5.51	11.72	IL-17, TNF-α, A
204470_at	*CXCL1*	Chemokine (C–X–C motif) ligand 1 (melanoma growth stimulating activity, alpha)	72.33	2.45	8.63	IL-17, TNF-α, IFN-γ, S
1554914_at	*PLA2G4D*	Phospholipase A2, group IVD (cytosolic)	66.91	2.75	8.81	
205476_at	*CCL20*	Chemokine (C–C motif) ligand 20	64.89	2.93	8.95	IL-17, TNF-α, S
219403_s_at	*HPSE*	Heparanase	63.46	6.14	12.13	
216258_s_at	*SERPINB13*	Serpin peptidase inhibitor, clade B (ovalbumin), member 13	61.38	3.25	9.19	
210164_at	*GZMB*	Granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	60.46	2.93	8.85	
232074_at	*PRSS27*	Protease, serine 27	56.4	4.33	10.15	
204733_at	*KLK6*	Kallikrein-related peptidase 6	51.71	6.79	12.48	
205844_at	*VNN1*	Vanin 1	50.72	4.41	10.08	
210038_at	*PRKCQ*	Protein kinase C, theta	48.3	2.54	8.14	
206912_at	*FOXE1*	Forkhead box E1 (thyroid transcription factor 2)	46.38	2.92	8.45	S
239430_at	*IGFL1*	IGF-like family member 1	46.06	4.77	10.30	
220322_at	*IL1F9*	Interleukin 1 family, member 9	45.99	7.82	13.34	IL-17, TNF-α, IFN-γ, A
209719_x_at	*SERPINB3*	Serpin peptidase inhibitor, clade B (ovalbumin), member 3	45.81	8.96	14.48	IFN-γ(−), S
212531_at	*LCN2*	Lipocalin 2	45.74	7.96	13.47	IL-17, TNF-α, IFN-γ(−), A

Table 2a continued in the following page

Table 2a. Continued

Probe set ID	Gene symbol	Gene title	Fold change (lesional vs. non-lesional)	Mean expression in skin samples		Cytokine regulation[1]
				Non-lesional	Lesional	
224204_x_at	*ARNTL2*	Aryl hydrocarbon receptor nuclear translocator-like 2	43.67	4.03	9.48	
213797_at	*RSAD2*	Radical *S*-adenosyl methionine domain containing 2	41.39	5.03	10.40	IFN-γ
233504_at	*C9orf84*	Chromosome 9 open reading frame 84	40.75	3.70	9.05	
227735_s_at	*C10orf99*	Chromosome 10 open reading frame 99	40.71	7.78	13.13	
203535_at	*S100A9*	S100 calcium–binding protein A9	40.71	9.25	14.60	IL-17, TNF-α, IFN-γ, A
226698_at	*FCHSD1*	FCH and double SH3 domains 1	39.37	5.42	10.72	
220249_at	*HYAL4*	Hyaluronoglucosaminidase 4	39.25	4.25	9.55	
202134_s_at	*WWTR1*	WW domain containing transcription regulator 1	36.95	4.69	9.89	TNF-α(−), IFN-γ(−)
206134_at	*ADAMDEC1*	ADAM-like, decysin 1	36.28	4.32	9.50	
241994_at	*XDH*	Xanthine dehydrogenase	35.21	3.41	8.55	IFN-γ
1553434_at	*CYP4Z2P*	Cytochrome P450, family 4, subfamily Z, polypeptide 2 pseudogene	34.49	2.22	7.33	
227609_at	*EPSTI1*	Epithelial stromal interaction 1 (breast)	34.32	3.77	8.87	
206008_at	*TGM1*	Transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-γ-glutamyltransferase)	34.05	5.62	10.71	TNF-α, IFN-γ, A
219691_at	*SAMD9*	Sterile α-motif domain containing 9	33.57	4.43	9.50	IFN-γ
240304_s_at	*TMC5*	Transmembrane channel-like 5	33.52	3.11	8.18	
221107_at	*CHRNA9*	Cholinergic receptor, nicotinic, α-9	32.35	3.19	8.21	
204465_s_at	*INA*	Internexin neuronal intermediate filament protein, alpha	31.67	3.85	8.83	
239586_at	*FAM83A*	Family with sequence similarity 83, member A	31.29	5.43	10.40	
209773_s_at	*RRM2*	Ribonucleotide reductase M2 polypeptide	31.19	5.71	10.67	IFN-γ(−)

Abbreviations: FCH, fold change; FDR, false discovery rate; TNF, tumor necrosis factor.
[1]Genes that are induced by IL-17, IFN-γ, and TNF-α in keratinocytes and yield a synergistic (S) or additive (A) effect between IL-17 and TNF on keratinocytes (Nograles *et al.*, 2008; Chiricozzi *et al.*, 2011).

using the same FDR (<0.05) and FCH (>2) cutoff criteria. As discussed previously (Suárez Fariñas *et al.*, 2005a; Suárez Fariñas and Magnasco, 2007), Venn diagrams often suggest overall results of studies are significantly discordant because arbitrary thresholds of differences in gene expression lead to ''absolute'' calls of a difference in expression. Using more recently published methods (Suárez Fariñas *et al.*, 2010), we calculated enrichment scores for the lists of Yao and colleagues (scores of 0.92 and 0.83 for upregulated and downregulated genes, respectively) and Gudjonsson and colleagues (scores of 0.94 and 0.89 for upregulated and downregulated genes, respectively; Supplementary Table S5 online). These results indicate very high concordance of the psoriatic genomic phenotype, as defined by gene expression, across the three studies. The present study has detected the largest number of DEGs, to our knowledge, that can be used to define ''moderate-to-severe'' psoriasis at the molecular level.

Expanded psoriasis transcriptome analysis

We utilized Ingenuity Pathway Analysis (IPA) to identify biological functions and pathways relevant to the psoriasis transcriptome and to more thoroughly understand genes uniquely identified in this study (Supplementary Table S4 online)

Within the ''unique'' set, IPA identified *cancer* as the most significantly enriched biological function ($P<10^{-12}$), followed by *endocrine system disease, gastrointestinal disease, genetic disorders, metabolic disease, and cardiovascular disease* ($P<10^{-7}$ in all cases; Supplementary Figure S1a online). The *genetic disorder* category includes several subcategories that were also enriched in this unique DEG subset, including *coronary artery disease* (162 genes, $P<10^{-7}$), and *Crohn's disease or inflammatory bowel disease* (140 genes, $P<10^{-4}$). The endocrine system and metabolic disease category also encompasses the previously

Table 2b. The top 50 unique genes downregulated in lesional skin compared with non-lesional skin biopsy samples obtained at baseline from 85 patients with moderate-to-severe psoriasis (FDR $P<0.05$ and FCH >2)

Probe set ID	Gene symbol	Gene title	Fold change (lesional vs. non-lesional)	Mean expression in skin samples		Cytokine regulation[1]
				Non-lesional	Lesional	
217059_at	*MUC7*	Mucin 7, secreted	−26.11	7.62	2.91	
241412_at	*BTC*	Betacellulin	−25.6	7.59	2.91	
205404_at	*HSD11B1*	Hydroxysteroid (11-β) dehydrogenase 1	−24.61	9.23	4.61	TNF-α, IFN-γ
205979_at	*SCGB2A1*	Secretoglobin, family 2A, member 1	−22.46	9.23	4.74	
229477_at	*THRSP*	Thyroid hormone responsive (SPOT14 homolog, rat)	−22.24	8.64	4.16	
204712_at	*WIF1*	WNT inhibitory factor 1	−21.11	9.21	4.81	
214053_at	*ERBB4*	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	−17.98	6.85	2.68	
227174_at	*WDR72*	WD repeat domain 72	−17.24	7.17	3.07	
205883_at	*ZBTB16*	Zinc-finger and BTB domain containing 16	−16.84	8.06	3.98	
208962_s_at	*FADS1/FADS3*	Fatty acid desaturase 1/fatty acid desaturase 3	−15.95	9.84	5.84	IFN-γ(−)
229151_at	*SLC14A1*	Solute carrier family 14 (urea transporter), member1 (Kidd blood group)	−15.86	7.46	3.47	
204607_at	*HMGCS2*	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	−15.36	8.08	4.14	
207430_s_at	*MSMB*	Microseminoprotein, β-	−13.47	6.99	3.24	
239929_at	*PM20D1*	Peptidase M20 domain containing 1	−13.28	10.05	6.32	
214240_at	*GAL*	Galanin prepropeptide	−12.5	7.35	3.71	TNF-α(−), IFN-γ(−)
1559097_at	*C14orf64*	Chromosome 14 open reading frame 64	−11.89	6.35	2.78	
230197_s_at	*TPPP*	Tubulin polymerization promoting protein	−11.11	9.72	6.24	
205325_at	*PHYHIP*	Phytanoyl-CoA 2-hydroxylase interacting protein	−10.28	8.44	5.08	
234980_at	*TMEM56*	Transmembrane protein 56	−10.27	6.22	2.86	
235278_at	*MACROD2*	MACRO domain containing 2	−9.55	6.34	3.08	
205029_s_at	*FABP7*	Fatty acid–binding protein 7, brain	−9.42	9.31	6.07	
220801_s_at	*HAO2*	Hydroxyacid oxidase 2 (long chain)	−9.39	5.75	2.52	
205030_at	*FABP7*	Fatty acid–binding protein 7, brain	−9.29	10.33	7.12	
213920_at	*CUX2*	Cut-like homeobox 2	−9.26	5.93	2.72	
1555318_at	*HIF3A*	Hypoxia-inducible factor 3, α-subunit	−9.25	10.45	7.24	
221795_at	*NTRK2*	Neurotrophic tyrosine kinase, receptor, type 2	−9.22	8.41	5.20	
234513_at	*ELOVL3*	Elongation of very long-chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	−9.21	5.84	2.64	
201596_x_at	*KRT18*	Keratin 18	−9.17	8.60	5.40	
227803_at	*ENPP5*	Ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)	−8.93	6.45	3.29	
208331_at	*BPY2*	Basic charge, Y-linked, 2	−8.8	6.03	2.89	
222102_at	*GSTA3*	Glutathione *S*-transferase A3	−8.75	10.04	6.91	
223836_at	*FGFBP2*	Fibroblast growth factor–binding protein 2	−8.65	9.48	6.37	
213661_at	*DKFZP586H2123*	Regeneration-associated muscle protease	−8.64	10.15	7.04	
232602_at	*WFDC3*	WAP four-disulfide core domain 3	−8.62	8.27	5.16	

Table 2b continued in the following page

Table 2b. Continued

Probe set ID	Gene symbol	Gene title	Fold change (lesional vs. non-lesional)	Mean expression in skin samples		Cytokine regulation[1]
				Non-lesional	Lesional	
201650_at	*KRT19*	Keratin 19	−8.35	10.67	7.61	TNF-α, IFN-γ
239246_at	*FARP1*	FERM, RhoGEF (ARHGEF), and pleckstrin domain protein 1 (chondrocyte-derived)	−8.26	8.59	5.55	TNF-α
231859_at	*C14orf132*	Chromosome 14 open reading frame 132	−8.18	7.33	4.30	
207955_at	*CCL27*	Chemokine (C–C motif) ligand 27	−8.14	12.55	9.52	TNF-α
1564786_at	*LOC338667*	Hypothetical protein LOC338667	−7.82	7.36	4.40	
1557474_at	*LOC284578*	Hypothetical protein LOC284578	−7.81	5.66	2.69	
214598_at	*CLDN8*	Claudin 8	−7.67	10.82	7.88	
204416_x_at	*APOC1*	Apolipoprotein C-I	−7.62	10.28	7.35	
231535_x_at	*ROPN1*	Ropporin, rhophilin-associated protein 1	−7.58	6.59	3.66	
239304_at	*MFSD4*	Major facilitator superfamily domain containing 4	−7.43	7.42	4.53	
204032_at	*BCAR3*	Breast cancer anti-estrogen resistance 3	−7.37	8.00	5.11	
220425_x_at	*ROPN1/ROPN1B*	Ropporin, rhophilin-associated protein 1/ ropporin, rhophilin associated protein 1B	−7.28	7.82	4.96	
224646_x_at	*H19*	H19, imprinted maternally expressed transcript	−7.19	11.01	8.16	
205529_s_at	*RUNX1T1*	Runt-related transcription factor 1; translocated to, 1 (cyclin D-related)	−7.1	8.10	5.27	
228943_at	*MAP6*	Microtubule-associated protein 6	−7.08	5.61	2.79	
1560741_at	*SNRPN*	Small nuclear ribonucleoprotein polypeptide N	−7.06	6.85	4.03	

Abbreviations: FCH, fold change; FDR, false discovery rate; TNF, tumor necrosis factor.
[1]Genes that are induced by IFN-γ or TNF-α on keratinocytes (Nograles *et al.*, 2008).

identified association with *non-insulin-dependent diabetes* (185 genes, $P<10^{-8}$), as well as a broader 272-gene subcategory associated with *diabetes* ($P<10^{-8}$). The *cardiovascular disease* category includes a subcategory of 139 genes, including renin associated with *hypertension* ($P<10^{-7}$) and *atherosclerosis* (170 genes, $P<10^{-7}$). Surprisingly, the metabolic and cardiovascular associations were more highly significant than the cell cycle and inflammatory disease categories, although these were also significant in IPA.

An IPA of the whole psoriasis transcriptome identified in this study yielded *dermatological conditions* (379 genes, $P<10^{-70}$), *genetic disorder* (361 genes, $P<10^{-74}$), cancer (968 genes, $P<10^{-64}$), and *gastrointestinal disease* (893 genes, $P<10^{-26}$) as the four top associations, the subcategories of which contain the links to *non-insulin-dependent diabetes, coronary artery disease*, and *inflammatory bowel disease* previously discussed. Within the *inflammatory, immune response* and *cardiovascular disorders* categories, interesting previously unreported psoriasis genes included cytotoxic T-lymphocyte antigen (CTLA)-4, Toll-like receptor (TLR)-3, and renin. We confirmed via immunohistochemistry that protein products of these genes were expressed at high levels in psoriasis plaques (Figure 2). CTLA4 staining was observed on keratinocytes and dermal cells, whereas TLR3 was expressed mostly on keratinocytes. In contrast, renin was expressed at very high levels by scattered cells in the papillary and upper reticular dermis. Among the canonical pathways collection (Supplementary Figure S1d online), IPA-identified metabolism involved pathways such as *atherosclerosis signaling, PPARα activation, RAR activation, renin–angiotensin signaling, and leptin signaling* ($P<10^{-2}$ all cases). The enrichment of *PPARα/RAR* as well as *IL-1-mediated inhibition of RXR* agrees with data presented by Romanowska and colleagues (2010). Additionally, 3 of the top 10 pathways were macrophage-related pathways and included *Fcγ receptor–mediated phagocytosis in macrophages and monocytes*, and *iNOS production in macrophages*. *Wnt* and *FGF signaling* pathways were not significant in our analysis. IPA also identified transcription factors activated or in our transcriptome. Of relevance, downstream genes regulated by STAT1, 2, and 3 were found to be activated. Interestingly, the transcription factor NROB2 is implicitly activated, and its function of downregulating targeting genes could possibly explain suppressed PPARα and RAR network alterations.

Table 3. Confirmation of genes by RT-PCR testing and comparison with gene microarray results

	Real-time reverse transcriptase PCR				Gene microarray		
	Gene symbol	Fold change (lesional vs. non-lesional)	Log2 (fold change)	*P*-value	Fold change (lesional vs. non-lesional)	Log2 (fold change)	False discovery rate
Differentially expressed genes							
1	*IL19*	1359.74	10.41	3.43×10^{-17}	18.29	4.19	$<10^{-15}$
2	*DEFB4*	385.00	8.59	3.07×10^{-19}	15.68	3.97	$<10^{-15}$
3	*TNIP3*	341.29	8.41	1.73×10^{-9}	23.18	4.53	$<10^{-15}$
4	*SERPINB3*	121.92	6.93	9.00×10^{-19}	45.81	5.52	$<10^{-15}$
5	*SPRR2C*	33.16	5.05	7.55×10^{-14}	243.3	7.93	$<10^{-15}$
6	*HPSE*	32.35	5.02	8.79×10^{-12}	63.46	5.99	$<10^{-15}$
7	*OASL*	29.68	4.89	1.39×10^{-13}	238.28	7.90	$<10^{-15}$
8	*IVL*	13.95	3.80	1.29×10^{-7}	4.63	2.21	$<10^{-15}$
9	*OAS2*	12.95	3.70	7.54×10^{-10}	22.19	4.03	$<10^{-15}$
10	*PMCH*	12.45	3.64	1.19×10^{-5}	2.84	1.51	$<10^{-15}$
11	*CTLA4*	11.21	3.49	6.05×10^{-5}	16.31	4.03	$<10^{-15}$
12	*HERC6*	10.67	3.42	3.40×10^{-6}	16.13	4.01	$<10^{-15}$
13	*CXCL10*	9.42	3.24	7.79×10^{-7}	24.66	4.62	$<10^{-15}$
14	*CXCL11*	9.40	3.23	1.18×10^{-5}	2.06	1.04	4.17×10^{-6}
15	*IL1B*	8.80	3.14	7.61×10^{-7}	7.04	2.82	$<10^{-15}$
16	*MX1*	8.44	3.08	2.19×10^{-8}	8.71	3.12	$<10^{-15}$
17	*NAMPT*	5.93	2.57	7.75×10^{-8}	20.59	4.36	$<10^{-15}$
18	*STAT1*	5.81	2.54	4.37×10^{-7}	11.79	3.56	$<10^{-15}$
19	*CARHSP1*	5.78	2.53	3.73×10^{-9}	7.58	2.92	$<10^{-15}$
20	*MMP9*	5.56	2.47	8.38×10^{-4}	2.54	1.34	4.56×10^{-9}
21	*CCNE1*	5.54	2.47	5.33×10^{-5}	5.71	2.51	$<10^{-15}$
22	*CXCL9*	5.39	2.43	1.42×10^{-5}	15.68	3.97	$<10^{-15}$
23	*CCL18*	4.67	2.22	4.64×10^{-5}	4.77	2.25	1.77×10^{-9}
24	*STEAP4*	4.05	2.02	3.10×10^{-5}	8.33	3.06	$<10^{-15}$
25	*CCNB1*	4.00	2.00	7.16×10^{-6}	8.74	3.13	$<10^{-15}$
26	*S100P*	3.63	1.86	5.24×10^{-4}	3.2	1.68	7.60×10^{-14}
27	*CCNA2*	3.62	1.85	2.34×10^{-4}	14.28	3.84	$<10^{-15}$
28	*CEBPD*	2.03	1.02	1.34×10^{-2}	7.43	2.89	$<10^{-15}$
29	*ADAM10*	1.58	0.66	7.64×10^{-2}	4.41	2.14	$<10^{-15}$
30	*SNCA*	−1.27	−0.35	4.99×10^{-1}	−3.71	−1.89	$<10^{-15}$
31	*NR1H3*	−1.61	−0.69	1.74×10^{-2}	−2.69	−1.43	$<10^{-15}$
32	*NTRK2*	−1.68	−0.75	5.43×10^{-2}	−9.22	−3.20	$<10^{-15}$
33	*KRT33A*	−1.84	−0.88	4.28×10^{-1}	−2.19	−1.13	6.00×10^{-4}
34	*CD207*	−2.09	−1.06	1.75×10^{-1}	−2.96	−1.57	2.04×10^{-11}
35	*KRT73*	−2.14	−1.10	8.42×10^{-3}	−2.17	−1.12	6.86×10^{-5}
36	*KRT18*	−2.17	−1.12	3.87×10^{-4}	−9.17	−3.20	$<10^{-15}$
37	*LPL*	−2.21	−1.14	8.42×10^{-3}	−6.25	−2.64	7.02×10^{-13}
38	*KRT19*	−2.45	−1.29	1.28×10^{-3}	−8.35	−3.06	$<10^{-15}$
39	*ACTA2*	−2.75	−1.46	7.41×10^{-4}	−3.69	−1.88	$<10^{-15}$
40	*APOC1*	−3.12	−1.64	5.55×10^{-3}	−7.62	−2.93	$<10^{-15}$
41	*MUC1*	−3.55	−1.83	1.51×10^{-3}	−6.10	−2.61	2.15×10^{-11}
42	*FADS2*	−5.83	−2.54	2.02×10^{-2}	−6.43	−2.68	1.29×10^{-11}

Table 3 continued in the following page

Table 3. Continued

		Real-time reverse transcriptase PCR			Gene microarray		
	Gene symbol	Fold change (lesional vs. non-lesional)	Log2 (fold change)	*P*-value	Fold change (lesional vs. non-lesional)	Log2 (fold change)	False discovery rate
43	*FADS1*	−5.96	−2.58	6.69×10^{-4}	−15.95	−4.00	2.27×10^{-15}
Borderline genes							
44	*IL22*	168.03	7.39	5.67×10^{-14}	1.19	0.25	2.56×10^{-2}
45	*S100A7*	44.94	5.49	2.01×10^{-12}	1.62	0.70	$<10^{-15}$
46	*CD69*	2.43	1.28	1.82×10^{-2}	1.71	0.77	1.62×10^{-6}
47	*CD209*	1.49	0.57	3.52×10^{-1}	1.58	0.66	2.40×10^{-3}
48	*JAK2*	1.46	0.55	1.42×10^{-1}	1.47	0.56	2.21×10^{-7}
49	*ITGAM*	−1.15	−0.20	7.92×10^{-1}	1.63	0.70	5.00×10^{-4}
50	*ITGAX*	−1.25	−0.32	6.63×10^{-1}	1.69	0.76	1.49×10^{-8}

Abbreviations: DEGs, differentially expressed genes; RT-PCR, real-time reverse transcriptase PCR.
Genes 1–43 were chosen among those DEGs by microarray analysis, with the exception of gene 29 (*ADAM10*), which was borderline. Additional borderline genes (44–50) were also confirmed.

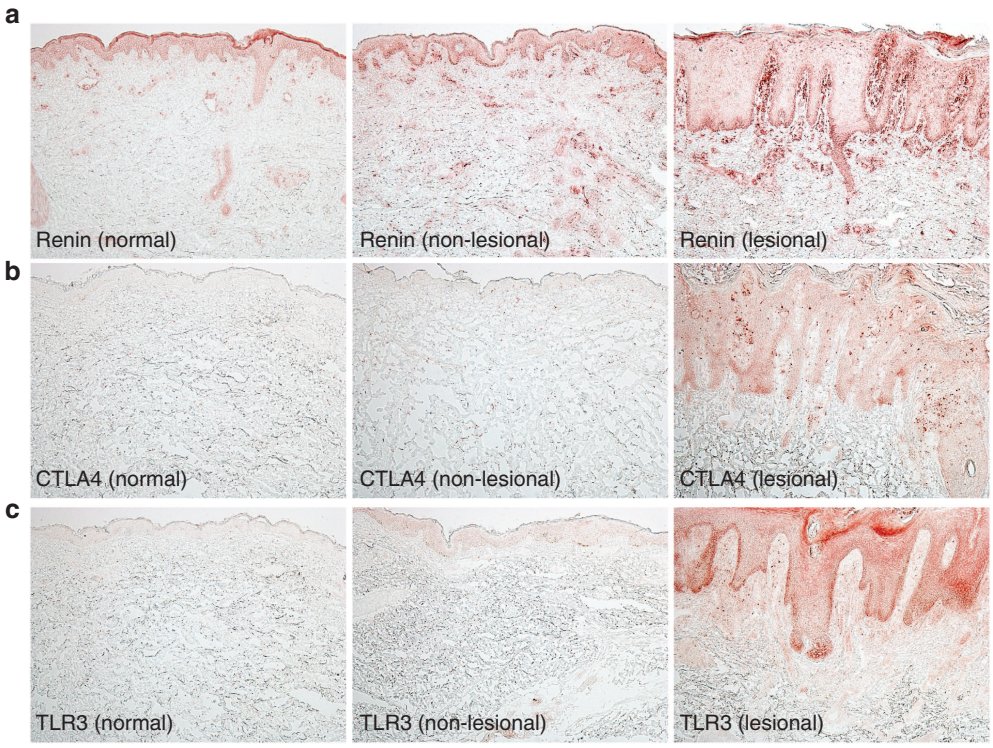

Figure 2. Protein expression of previously unreported genes detected in this transcriptome. Representative immunohistochemistry staining in normal, non-lesional, and lesional psoriasis skin ($n = 5$). (**a**) Renin was highly expressed by scattered cells in the papillary and upper reticular dermis mostly in lesional skin compared with non-lesional and normal skin. (**b**) Cytotoxic T-lymphocyte antigen (CTLA4) was expressed on keratinocytes and some dermal cells in lesional skin compared with very little expression on non-lesional skin and none on normal skin. (**c**) Toll-like receptor (TLR3) was strongly expressed on keratinocytes of lesional skin compared with a faint expression on normal and non-lesional skin. Scale bar = 100 μm.

Parallel increases in inflammatory elements in skin and blood

The overexpression of renin, as well as other cardiovascular, metabolic, and inflammatory markers in the skin, raised the question of whether these pathways are also dysregulated in the circulation of psoriatic patients. When profiling the expression of a 92-protein panel (Supplementary Table S6 online) in this psoriatic population, we detected increased expression of 12 proteins in the serum of psoriasis patients versus a control population of healthy individuals; increases ranged from 1.25-fold to >3.5-fold (*P*-values of 10^{-5}–10^{-50};

Table 4). Most of these products are inflammatory cytokines/chemokines or proteins responsive to cytokines. Each protein is associated with corresponding increases in mRNA levels in psoriasis lesions and *P*-values <0.05 for the mRNA increase (Table 4). An imbalance in race distribution was observed in the psoriasis versus healthy control populations; however, results of analyses conducted with race added as a covariate indicated no significant effect of race on the expression of these 12 proteins. Body mass index (BMI) as a covariate did not significantly affect gene expression for these products, but within overall serum measures, increased or decreased levels of some proteins, e.g, leptin and insulin, were affected by BMI (Supplementary Table S6 online). Also using a more sensitive assay for IL-17A, we found increased expression in the blood of psoriasis patients compared with healthy controls (Supplementary Figure S2 online).

DISCUSSION

Our study findings represent the largest set of DEGs in psoriasis to date, to our knowledge, and provide the most comprehensive molecular definition of moderate-to-severe disease based on lesional skin. This was accomplished using the same analytical criteria as employed in previously published studies with smaller sample sizes. We also expanded the disease definition by profiling serum samples for differentially regulated proteins and assessing their dysregulation in lesional skin. Results of gene expression profiling conducted via gene-set enrichment analysis demonstrated similarities between this study and previously reports (Oestreicher *et al.*, 2001; Zhou *et al.*, 2003; Yao *et al.*, 2008). Also, upregulated genes such as S100A12, SERPINB4/SERPINB3, and IL-8, and downregulated genes such as BTC, WIF1, THRSP, and WDR72 are among the top 15 within both our list and that reported by Gudjonsson *et al.* (2010). We have also confirmed many upregulated genes involved in signaling pathways believed to be central in psoriasis pathogenesis, including the IFN-γ, TNF, and IL-17 signaling pathways. Some of the specific upregulated genes include *OASL*, *CXCL1*, *STAT-1*, and *Mx-1* belonging to the IFN-γ signaling pathway; AKR1B10, IL1F9, and CXCL9 belonging to the TNF signaling pathway; and CCL20 and CXCL8 (IL-8) belonging to the IL-17 signaling pathway (Haider *et al.*, 2008; Nograles *et al.*, 2008).

In addition, we identified several genes biologically significant for psoriasis that were previously unreported, including *renin*, *CTLA4*, and *TLR3*. *Renin* is a gene involved in the renin–angiotensin signaling pathway that ultimately leads to aldosterone release, vasoconstriction, and an increase in blood pressure, and psoriatic patients have enhanced plasma renin activity and increased urinary aldosterone excretion (Ena *et al.*, 1985). The *CTLA4* gene, is one of the genes listed under metabolic disorder and diabetes in the metabolic disease functional pathway as is involved in negative regulation of T-cell proliferation as well as regulatory T-cell differentiation and immune response. In psoriasis-like murine skin, the induction of T-regulatory cells

Table 4. Increased expression of 12 proteins, as detected by serum and gene microarray assessments, in psoriasis patients (*n*=146) versus a control population of healthy individuals (*n*=162)

			Serum measurements					Gene array measurements				
Symbol	Name	*P*-value[1]	Fold change (psoriasis vs. healthy controls)	Healthy controls (*n*=162)	Psoriasis patients (*n*=146)	Psoriasis patients with BMI[2] <30 (*n*=75)	Psoriasis patients with BMI[2] ⩾30 (*n*=73)	Fold change (lesional vs. non-lesional)	*P*-value	False discovery rate	Non-lesional skin	Lesional skin
S100A12	ENRAGE	4.06×10^{-26}	2.30	25.46	58.55	55.94	60.22	889.1	$<10^{-20}$	$<10^{-20}$	2.72	12.52
ACPP	Prostatic acid phosphatase	1.17×10^{-35}	2.50	0.20	0.49	0.46	0.51	8.3	$<10^{-20}$	$<10^{-20}$	8.87	11.92
CCL22	MDC	$<10^{-50}$	3.03	409.31	1240.06	1227.13	1211.61	3.91	1.11×10^{-16}	1.18×10^{-15}	8.93	10.90
IL1RN	IL1RA	5.67×10^{-32}	2.65	68.97	182.84	138.65	223.63	3.03	2.86×10^{-11}	1.65×10^{-10}	3.93	5.53
TNPO1	MIP1β	1.52×10^{-39}	2.34	131.53	307.87	299.36	314.43	2.82	$<10^{-20}$	$<10^{-20}$	8.51	10.01
CCL2	MCP1	$<10^{-40}$	3.55	141.20	501.78	491.29	509.71	2.47	$<10^{-20}$	$<10^{-20}$	10.39	11.70
VEGFA	VEGF	1.74×10^{-29}	1.83	449.19	821.76	859.10	794.82	1.99	9.76×10^{-13}	6.61×10^{-12}	8.86	9.85
ICAM1	ICAM1	1.67×10^{-05}	1.26	134.07	169.41	153.51	184.43	1.84	7.74×10^{-08}	3.17×10^{-07}	6.68	7.56
IL15	IL-15	8.80×10^{-06}	1.25	0.65	0.81	0.80	0.81	1.84	1.85×10^{-09}	8.86×10^{-09}	6.69	7.57
TNFRSF1B	TNF-RII	7.32×10^{18}	1.40	3.50	4.91	4.60	5.21	1.53	5.45×10^{-08}	2.26×10^{-07}	8.94	9.55
TNF	TNFα	$<10^{-50}$	2.50	3.54	8.85	8.37	9.31	1.45[3]	1.25×10^{-08}	5.51×10^{-08}	3.11	3.65
CXCL5	ENA78	1.40×10^{-45}	3.04	0.75	2.29	2.08	2.48	1.14[4]	3.25×10^{-02}	6.28×10^{-02}	2.25	2.44

Abbreviations: BMI, body mass index; FDR, false discovery rate; RT-PCR, real-time reverse transcriptase PCR.
[1]*P*-values were adjusted to control the FDR and all resulted in FDR <0.05.
[2]BMI defined as baseline BMI <30 includes normal and overweight patients; BMI defined as baseline BMI $\geqslant30$ includes obese patients.
[3]TNF has previously been reported to be increased in psoriatic lesional skin when assessed via by RT-PCR (Suárez Fariñas *et al.*, 2010).
[4]CXCL5/ENA78 has previously been reported to be increased in psoriatic lesional skin (Zhou *et al.*, 2003).

involving CTLA4 signaling is one of the mechanisms for the therapeutic action of psoralen plus long-wave UV, a well-established psoriasis treatment (Singh *et al.*, 2010). TLR3 has a fundamental role in pathogen recognition and activation of innate immunity and is expressed in the keratinocytes of normal, non-lesional and psoriatic skin, and on monocyte-derived dendritic cells (Baker *et al.*, 2003). The interaction of TLR3 with its ligand can yield keratinocyte activation and the release of proinflammatory cytokines TNF and IL-8 (Begon *et al.*, 2007), and TLR3 signaling generally activates numerous IFN- and TNF-stimulated gene products that are also upregulated in psoriasis. However, because of the extensive overlap in regulated genes, activation of signaling by TLR3 cannot be definitively determined.

Given that psoriasis is increasingly recognized to be associated with comorbid conditions of obesity, diabetes, metabolic dysregulation, and cardiovascular diseases that may be related to inflammation in skin (Davidovici *et al*, 2010), it is of interest that associated functional pathways were also identified through IPA, including *metabolic disease* and *cardiovascular disease* (Supplementary Figure S1b online), leading to two potential mechanisms for increased association between psoriasis and metabolic and cardiovascular comorbidities. First, a product made in psoriatic plaques could produce diffusible hormone-like proteins that influence the biology of distant cells/tissues (e.g., renin, vascular endothelial growth factor and monocyte chemoattractant 9 protein-1 (CCL2); Table 4). Second, the IPA findings show dysregulated gene expression in psoriasis lesions in metabolic pathways associated with atherosclerosis, *PPARα and RAR activation, renin–angiotensin signaling, leptin signaling*, and others that may be important in comorbid conditions (Supplementary Figure S1d online). These networks imply activated transcription factors underlying observed changes with the interesting suggestion that activation of NROB2 could relate to suppressed PPARγ and RAR network alterations. Another example is the cytochrome *C* oxidase family (COX5A, COX7A1, and COX15), which is associated with mitochondrial dysfunction and in turn is regulated by nitric oxide (NO) signaling. Given the genetic association of psoriasis with inducible NO synthase (Stuart *et al.*, 2010) and increased expression of these NO-forming enzymes in this disease (Lowes *et al.*, 2005), the gene expression pattern seen in psoriatic skin may reflect cellular dysfunction at other sites that could be governed by common regulatory pathways such as the NO response. Endothelial cell function is both intermediately related to NO metabolism and dysfunctional in psoriasis patients (Karadag *et al.*, 2010). Although resting vascular tone is largely regulated by endothelial NO synthase (Naber *et al.*, 2001), we note that alterations in vascular NO metabolism have been previously associated with inflammation. Thus, one might speculate that alterations in constitutive versus inducible NO synthesis in tissues outside the skin could potentially alter endothelial/vascular function and lead to cardiovascular disease (Grassi *et al.*, 2011). The skin changes may offer a window into systemic cellular metabolism that cannot be directly assessed. Given that we studied patients across a range of BMIs, some gene expression associated with higher BMI ($\geqslant$30) might have been present. However, analyses by BMI subgroups did not detect any significant difference, and inclusion of BMI as a covariate in the analysis neither yielded significant findings nor altered analysis outcomes.

In conclusion, this evaluation of the psoriasis transcriptome, the largest study to date, to our knowledge, to assess global gene expression and serum protein profiling in a relatively homogenous group of patients with moderate-to-severe psoriasis not receiving systemic psoriasis therapy provides additional support for inflammation-related pathogenic mechanisms in psoriasis. This study also provides previously unreported insights into the biological changes that occur in lesional skin of patients with moderate-to-severe psoriasis as they relate to systemic psoriatic manifestations and comorbidities (i.e., cardiovascular disease and metabolic syndrome). Evaluating patients with uniformly severe disease appears to have enabled detection of previously unidentified metabolic/cardiovascular risk pathways, particularly because cardiovascular risk is more highly associated with extensive disease (Gelfand *et al.*, 2006; Mehta *et al.*, 2010). Ultimately, risk pathways must be reconciled with specific genes involved to derive meaningful associations that can serve as tools for future therapeutic targets and, hence, may eventually lead to prevention of psoriasis comorbidities. The utilization of large patient cohorts to develop disease profiles from multiple matrices will continue to significantly contribute to the understanding of the pathophysiology of disease and associated comorbidities.

MATERIALS AND METHODS

Further details are provided in the Supplementary Materials and Methods online.

Patients and tissue/serum samples

Skin punch biopsy samples were obtained from 89 patients with histologically confirmed chronic psoriasis vulgaris who were enrolled into an IRB-approved Phase 3, multicenter, randomized trial protocol (ACCEPT trial; Griffiths *et al.*, 2010). Patients entering this trial were similar to those in other Phase 3 psoriasis studies in that they were candidates for systemic treatment, had at least 10% of body surface area affected by plaque psoriasis, and some may have been treated previously with systemic agents. To enter the study, patients could not have used topical agents for 2 weeks before treatment, nor could they have used systemic agents within 4 weeks of the first treatment. In addition, they may not have used any biological agent within 3 months of the first administration of study agent or within five times the half-life of the biological agent before the first administration of study agent, whichever was longer. For each patient, baseline skin biopsies included both lesional and macroscopically normal non-lesional skin samples were collected. Lesional skin samples were isolated from a representative psoriatic target lesion ($\geqslant$3 cm). Serum samples were obtained from 162 healthy volunteers (Bioreclimation, Hicksville, NY) with approved written informed consent and a subset of 149 patients, of which 62 were also a part of the biopsy substudy, with psoriasis from the ACCEPT trial (Griffiths *et al.*, 2010). This

study was conducted in compliance with the Declaration of Helsinki Principles.

RNA processing and microarray hybridization

Skin biopsies were snap-frozen in liquid nitrogen and stored at −80°C until used. RNA was extracted using the Qiagen RNeasy Fibrous Tissue Mini Kit (QIAGEN, Valencia, CA) and later hybridized to GeneChip HG U133 Plus 2.0 (Affymetrix, Santa Clara, CA). Raw data have been deposited in NCBI's Gene Expression Omnibus and are accessible through accession number GSE 30999.

Statistical analysis

Succinctly, 3 samples were excluded from analysis because of low quality control measures. Images were scrutinized for spatial artifacts using Harslight (Suárez Fariñas *et al*. 2005b). Expression measures were obtained using GCRMA algorithm (Wu *et al*., 2004) and changes in lesional versus non-lesional skin were assessed using general linear model (Mardia *et al*., 1979; Freund *et al*., 1986). *P*-values were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). Transcripts with low expression were excluded. Genes showing FCH > 2 and FDR < 0.05 were considered to be part of the disease profile.

RT-PCR testing

Applied Biosystems (Foster City, CA) Taqman 16-gene and 48-gene low-density array cards were used for RT-PCR analysis. The 16-gene cards were tested on 13 healthy/normal and 20 pairs of psoriasis non-lesional and lesional skin biopsy samples. The 48-gene cards were tested on 12 healthy/normal and 43 pairs of psoriasis skin biopsy samples. Probe set identifications are provided in Supplementary Table S6 online. The resulting data were normalized to human acidic ribosomal protein expression (Gene Symbol: RPLPO).

Serum protein profiling

A 92-protein vendor-defined multiplex Luminex-based panel (Human Map 1.6 plus IL-17 and IL-23; Rules Based Medicine, Austin, Texas) was used to profile differential serum protein expression from healthy volunteers ($n = 162$) and patients with psoriasis ($n = 149$). The complete list of analytes in the Human Map 1.6 can be found at http://rulesbasedmedicine.com/products-services/humanmap-services/human-discoverymap.

Immunohistochemistry

Frozen tissue sections from normal, psoriatic lesional, and non-lesional skin ($n = 5$) were stained with mouse monoclonal antibodies for renin (AbD Serotec, Planegg, Germany (10 μg)), CTLA4 (Abcam, Cambridge, MA (1:100)) and TLR3 (Abcam(1:10)). Standard procedures were employed as previously described (Fuentes-Duculan *et al*., 2010).

CONFLICTS OF INTEREST

MS-F and JF-D have no conflicts of interest. JGK has consulted for/received honoraria from Janssen. CB, KH, and KL are employees of Janssen Research & Development LLC, a Johnson & Johnson pharmaceutical company.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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