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Authors

Kim, Jaehwan
Oh, Chil-Hwan
Jeon, Jiehyun
[et al.](#)

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Molecular Phenotyping Small (Asian) versus Large (Western) Plaque Psoriasis Shows Common Activation of IL-17 Pathway Genes, but Different Regulatory Gene Sets

Jaehwan Kim, MD, PhD¹, Chil-Hwan Oh, MD, PhD², Jiehyun Jeon, MD, PhD², Yoosang Baek, MD², Jaewoo Ahn, MD², Dong Joo Kim, BS^{1,3}, Hyun-Soo Lee, MD¹, Joel Correa da Rosa, PhD⁴, Mayte Suárez-Fariñas, PhD^{4,5}, Michelle A. Lowes, MD, PhD^{1,6}, and James G. Krueger, MD, PhD^{1,*}

¹Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY, USA

²Department of Dermatology, Korea University College of Medicine, Seoul, South Korea

³School of Medicine, Stony Brook University, Stony Brook, NY, USA

⁴The Center for Clinical and Translational Science, The Rockefeller University, New York, NY, USA

⁵Icahn School of Medicine at Mount Sinai, New York, New York, United States of America

⁶Division of Dermatology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA

Abstract

Psoriasis is present in all racial groups, but in varying frequencies and severity. Considering that small plaque psoriasis is specific to the Asian population and severe psoriasis is more predominant in the Western population, we defined Asian *small* and *intermediate* plaque psoriasis as psoriasis subtypes, and compared their molecular signatures with classic subtype of Western *large* plaque psoriasis. Two different characteristics of psoriatic spreading—vertical growth and radial expansion—were contrasted between subtypes, and genomic data were correlated to histologic and clinical measurements. Compared to Western *large* plaque psoriasis, Asian *small* plaque psoriasis revealed limited psoriasis spreading, but IL-17A and IL-17-regulated pro-inflammatory cytokines were highly expressed. Paradoxically, IL-17A and IL-17-regulated pro-inflammatory cytokines were lower in Western *large* plaque psoriasis, while T cells and dendritic cells in total psoriatic skin area were exponentially increased. Negative immune regulators, such as CD69 and

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Corresponding author: James G. Krueger, MD., PhD, D. Martin Carter Professor in Clinical Investigation, Co-Director of the Center for Clinical and Translational Science, Head of Laboratory for Investigative Dermatology, The Rockefeller University, New York, 1230 York Avenue, New York, NY 10065, Phone: (212) 327-8091, Fax: (212) 327-8232, kruegej@rockefeller.edu.

Availability of supporting data: Raw microarray data have been deposited in NCBI's Gene Expression Omnibus (GSE67853) and are accessible with the following link: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=srinkyqcnpubpbcf&acc=GSE67853>

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FAS, were decreased in both Western *large* plaque psoriasis and psoriasis with accompanying arthritis or obesity, and their expression was correlated with psoriasis severity index. Based on the disease subtype comparisons, we propose that dysregulation of T cell expansion enabled by downregulation of immune negative regulators is the main mechanism for development of large plaque psoriasis subtypes.

Keywords

Psoriasis; Subtype; Genomic comparison; Molecular phenotyping

INTRODUCTION

Psoriasis is a common skin disease affecting 2 to 3 percent of the world population (Koo, 1996a; Stern *et al.*, 2004; van de Kerkhof, 2008). It begins as a pinhead-sized papule on the skin that vertically grows to become raised, red, scaly patch, and radially expands to peripheral body surface area, progressing eventually to severe cutaneous disease in some individuals. Severe psoriasis is frequently associated with systemic inflammation and comorbidities, such as psoriatic arthritis, cardiovascular disease, diabetes, and depression (Boehncke *et al.*, 2011; Cohen *et al.*, 2008b; Devrimci-Ozguven *et al.*, 2000; Gladman *et al.*, 2005; Koo, 1996b; Neimann *et al.*, 2006; Schon and Boehncke, 2005). Although high throughput technologies have enabled us to perform comprehensive molecular characterization of psoriasis, the molecular determinants that are proportional to disease severity and extent are not yet understood. This is, in part, because psoriasis is a heterogeneous disease caused by complex interactions between genetic, environmental, and immunological factors, and none of the preclinical murine models fully represent the cellular changes and molecular features of psoriasis (Christophers, 2008; Danilenko, 2008; Guttman-Yassky *et al.*, 2011; Lowes *et al.*, 2014; Suarez-Farinas *et al.*, 2013; Swindell *et al.*, 2011). To overcome this translational gap and explore potential mechanisms of disease progression in humans, we identified subtypes of psoriasis in which disease progression is limited, and contrasted their cellular and molecular signatures with a more severe form of psoriasis.

Psoriasis is present in all racial groups, but in varying frequencies and severity. The prevalence is 2.5% in Caucasians, 1.3% in African Americans, but significantly lower in Asians (0.8% in India, 0.4% in China, and 0.3% in Japan) (Campalani and Barker, 2005; Gelfand *et al.*, 2005; Prashant, 2010). In Asians, less severe psoriasis is more predominant and a distinct phenotype has been particularly reported, known as “small plaque psoriasis” (Lew *et al.*, 2004; Youn, 2012). Small plaque psoriasis is the typical form of chronic plaque psoriasis in adults living in Korea and other Asian countries. This form of psoriasis has some resemblance to guttate psoriasis, but differs in that it is a chronic, stable form of psoriasis in adults across many years of disease persistence and overall skin surface involvement is usually <10% body surface area (Supplementary Table S1 online).

Considering that small plaque psoriasis is specific to the Asian population and severe psoriasis is more predominant in the Western population, we compared plaque psoriasis subtypes between Asian and Western populations to explore possible disease progression

mechanisms. We first subtyped Asian small and intermediate psoriasis based on the individual plaque size and PASI. Then, we confirmed Asian isoforms as true psoriasis subtypes by identifying a similar transcriptome and histology compared to moderate-to-severe Western large psoriasis. We next explored models of disease progression by contrasting different cellular and molecular signatures of the subtypes. The working models represented bi-dimensional features of disease progression by separating two different phases of psoriasis progression: vertical growth (epidermal hyperplasia) and radial expansion (the extension of overall psoriasis area and severity). By correlating the genomic data to the clinical and histologic measurements of disease progression, we propose that, rather than the increase in driver cytokines, dysregulation of T cell expansion enabled by downregulation of negative regulators is the main mechanism of disease progression to large plaque psoriasis subtypes and overall disease severity.

RESULTS

Clinical stratification of small and intermediate psoriasis in the Asian population

We studied 51 biopsy-confirmed psoriasis patients in Seoul, Korea, where small plaque psoriasis has been previously reported (Lew *et al.*, 2004). Patients were clinically stratified into Asian small versus Asian intermediate psoriasis by considering individual plaque size and disease severity measured by PASI. Compared to Western large psoriasis, which has large individual plaque size and typical PASI > 12 (Feldman, 2004; Schmitt and Wozel, 2005), we defined Asian small and intermediate psoriasis as psoriasis subtypes with limited disease progression or “mild” disease (Supplementary Figure S1 online). 43% (22/51) of patients had Asian small psoriasis, in which individual plaque size (< 2 cm) and the extent of psoriasis area and severity (mean PASI 6.5 ± 0.64) were both limited (Table S1 online) (Lew *et al.*, 2004). Smaller plaque size was consistent with thinner epidermal thickness, representing less epidermal hyperplasia in disease progression (Figure 1a). Asian small psoriasis also revealed low PASI (Figure 1b), even when psoriatic plaques were widely distributed on the trunk and/or extremities (Supplementary Figure S2 online).

Asian intermediate psoriasis comprised 57% (29/51), in which individual plaque size (> 5 cm) was larger than Asian small psoriasis (< 2 cm) but the extent of psoriasis area and severity (mean PASI 7.0 ± 0.93) was less than large psoriasis (PASI > 12). Psoriatic plaques were localized to the knees, elbows, scalp, and lower backs/buttocks. Otherwise, the plaques were distributed to limited areas of the trunk or extremities. Overall, the clinical features of Asian intermediate psoriasis were similar to mild cases of psoriasis in the Western population. Clinical photos and immunohistochemical images of 10 representative Asian plaque psoriasis patients are presented in Supplementary Figure S3 online.

Genomic and histologic confirmation of Asian small and intermediate psoriasis as true psoriasis subtypes

We obtained gene expression profiles with the Affymetrix Human Genome U133 Plus 2.0 Array, and established the psoriatic transcriptomes of Asian small, Asian intermediate, and Western large psoriasis by defining differentially expressed genes (DEGs) between lesional versus non-lesional skin within each subtype (> 2 fold change (FCH), < 0.05 false discovery

rate (FDR)). Among 9637 DEGs, 2674 (27.7%) were common to the three subtypes (Figure 1c). When the gene sets were narrowed down to a meta-analysis-derived transcriptome (MAD3) of Western large psoriasis for the identical array (Tian *et al.*, 2012), there was 66.9% commonality in DEGs (Figure 1d).

We next validated if the dysregulation of established psoriasis gene sets (Bowcock *et al.*, 2001; Gudjonsson *et al.*, 2009; Jabbari *et al.*, 2011; Suarez-Farinas *et al.*, 2012; Suarez-Farinas *et al.*, 2010; Tian *et al.*, 2012; Yao *et al.*, 2008) was equivalently observed in Asian small and intermediate psoriasis in comparison to Western large psoriasis. We performed Gene Set Variation Analysis (GSVA) with the combined z-score method and generated pathway enrichment scores from the observed gene expression levels. The analysis revealed that the enrichment scores of Asian small and intermediate psoriasis were not different from, or were even higher than the scores of Western large psoriasis ($p < 0.01$ and $FDR < 0.01$; Supplementary Figure S4 online).

Histological findings in Asian small and intermediate psoriasis also revealed hallmarks of histological findings in Western large psoriasis. In the psoriatic lesional skin of both Asian small and intermediate psoriasis, the epidermis revealed hyperplasia with focal parakeratosis (Supplementary Figure S3 online, immunohistochemical images). Key cellular subsets of psoriasis immunopathogenesis, CD3⁺ T cells and CD11c⁺ myeloid dendritic cells, accumulated in both subtypes. Numbers of CD3⁺ T cells and CD11c⁺ dendritic cells in Asian small psoriasis were not different from Western large psoriasis in slide sections of lesional skin (Supplementary Figure S5 online). Number of CD3⁺ T cells in Asian intermediate psoriasis was also not different from Western large psoriasis, while CD11c⁺ dendritic cells were more abundant in Asian intermediate psoriasis compared to Western large psoriasis.

Taken together, Asian small and intermediate psoriasis phenotypes were validated as psoriasis variants, sharing a common psoriasis transcriptome and histologic findings with Western large psoriasis (psoriasis vulgaris).

Models of disease progression emerge from subtype comparisons

We next explored models of disease progression by correlating two different phases of disease progression: vertical growth (epidermal hyperplasia) measured by epidermal thickness of lesional skin and radial expansion (the extension of overall psoriasis area and severity) measured by PASI (Figure 2). Since Asian small psoriasis was limited in both epidermal thickness and PASI, we considered it as a model of the initial stage of disease progression.

To explore mechanisms of vertical growth, we compared Asian small and intermediate psoriasis, since epidermal thickness was significantly different between the two subtypes, without a difference in PASI (Figure 2a). In this model, CD3⁺ T cell and CD11c⁺ dendritic cell infiltrates within the epidermis and dermal papillary area were significantly different (Supplementary Figure S6 online). In addition, CD3⁺ T cells and CD11c⁺ dendritic cells within the epidermis and dermal papillary area were linearly correlated with the epidermal thickness (Figure 2b and 2d; Supplementary Table S2 online).

To explore mechanisms of radial expansion, we compared Asian intermediate and Western large psoriasis, since PASI was significantly different between the two subtypes, without a difference in epidermal thickness (Figure 2a). In this model, the accumulated T cell and dendritic cell numbers in total psoriasis body surface area of Western large psoriasis ($CD3^+$ T cells: $6.24 \times 10^9 \pm 4.68 \times 10^9$, $CD11c^+$ dendritic cells: $5.13 \times 10^9 \pm 4.74 \times 10^9$) were exponentially higher than the numbers for Asian intermediate psoriasis ($CD3^+$ T cells: $1.18 \times 10^9 \pm 9.76 \times 10^8$, $CD11c^+$ dendritic cells: $1.45 \times 10^9 \pm 1.43 \times 10^9$) (Supplementary Figure S5 online). In addition, $CD3^+$ T cells and $CD11c^+$ dendritic cells in total psoriasis body surface area were highly correlated to PASI (Figure 2c and 2d; Supplementary Table S2 online).

Genomic exploration of disease progression models

To explore molecular correlates of disease progression, we simultaneously measured expression levels of 35 genes in both lesional and non-lesional skin of Asian small (N=16), Asian intermediate (N=21), and Western large (N=20) psoriasis by RT-PCR (Figure 3 and Supplementary Figure S7 online). In the model of the initial stage of disease progression, IL-17A and IL-17-regulated pro-inflammatory cytokines (IL-1B and IL-8) were highly expressed even before vertical growth and radial expansion. The expression levels of IL-17A, IL-1B, and IL-8 in RT-PCR were highest in lesional skin of Asian small psoriasis, and were significantly higher than in Western large psoriasis (Figure 3a).

In our disease progression model of the vertical growth phase phase (Asian small versus Asian intermediate psoriasis), epidermal hyperplasia was confirmed by high lesional expression of K16 in RT-PCR (Figure 3b) and Ki67 in microarray data (FCH 3.9, $p < 0.05$ and $FDR < 0.05$). IL-20, a key molecule for epidermal hyperplasia response, was expressed higher in Asian intermediate psoriasis in non-lesional skin (Figure 3b). In the multiple linear regression model predicting epidermal thickness from the RT-PCR expression levels of 35 psoriatic genes in lesional and non-lesional skin (expressions of Asian small and intermediate psoriasis in Figure 3 and Supplementary Figure S7 online), epidermal thickness of lesional skin increased in accordance with IL-17A expression in non-lesional skin ($\text{Epidermal thickness}_{\text{Lesional}} = 230.6 + 34.8 \times \text{DEFB103}_{\text{Lesional}} + 12.4 \times \text{IL-17A}_{\text{Non-lesional}} + 11.7 \times \text{IFN-}\alpha_{\text{Lesional}} - 35.9 \times \text{Fas}_{\text{Lesional}}$, $R^2 = 0.47$, adjusted $R^2 = 0.40$).

In our disease progression model of the radial expansion phase (Asian intermediate versus Western large psoriasis), we observed an increase in $CD3^+$ T cells (Figure 2c) and a paradoxical decrease in driver cytokines (IL-17A, IL-1B, IL-8, and IL-20) (Figure 3a and 3b) in psoriatic lesional skin. We hypothesized that rather than the increase in driver cytokines, dysregulation of T cell expansion is the main mechanism of the radial expansion phase. To explore this hypothesis, we first identified significant downregulation of negative regulator signaling in Western large psoriasis in comparison to Asian intermediate psoriasis (Figure 4a). Among the immune molecules whose function as a negative regulator have been described (Abrams *et al.*, 1999; Bovenschen *et al.*, 2011; Chen *et al.*, 2008; Cortes *et al.*, 2014; Gorbachev and Fairchild, 2010; Kagen *et al.*, 2006; Sancho *et al.*, 2005; Stranges *et al.*, 2007; Sugiyama *et al.*, 2005), downregulation of CD69, Fas, CTLA4, PD-L1, and FoxP3 was confirmed by RT-PCR in lesional skin of Western large psoriasis in comparison

to Asian small or intermediate psoriasis (Figure 3c). In addition, the expressions of CD69 (lesional and non-lesional), Fas (lesional and non-lesional), and CTLA4 (lesional) were negatively correlated with PASI (Figure 5 and Supplementary Table S2 online). Using a multiple regression model to predict PASI from the RT-PCR expression levels of 35 psoriatic genes in lesional and non-lesional skin (expressions of Asian intermediate and Western large psoriasis in Figure 3 and Supplementary Figure S7 online), PASI increased in accordance with the reduction of CD69 expression in non-lesional skin ($\text{PASI} = -18.2 + 5.5 \times \text{TNF-}\alpha_{\text{Non-lesional}} + 3.3 \times \text{DEFB103}_{\text{Non-lesional}} - 4.1 \times \text{CD69}_{\text{Non-lesional}} - 1.2 \times \text{IL-9}_{\text{Lesional}}$, $R^2 = .67$, adjusted $R^2 = .63$).

Disease progression beyond cutaneous lesions

When comparing comorbid diseases across the psoriasis variants of Asian small, Asian intermediate and Western large psoriasis, there was much more frequent detection of comorbid conditions with Western large psoriasis. The difference was statistically significant for Body Mass Index (BMI), psoriatic arthritis, obesity, hypertension, and depression ($p < 0.05$, Supplementary Table S3 online). In a subsequent pairwise comparison with Bonferroni correction, Western large psoriasis had a higher prevalence of psoriatic arthritis and obesity than both Asian small and intermediate psoriasis ($p < 0.017$). To understand the significant differences in comorbidities between Asian small or intermediate psoriasis versus Western large psoriasis, we tested for a difference in the activation of comorbidity-associated pathways between the subtypes (Figure 4b). As conditions comorbid with psoriasis are often associated with atherosclerosis (Cohen *et al.*, 2008a; Gladman *et al.*, 2005; Gonzalez-Juanatey *et al.*, 2007; Neimann *et al.*, 2006; Pyorala *et al.*, 1987), we selected gene sets of psoriatic arthritis, cardiovascular disease, and atherosclerosis for comparison. The pathway analysis revealed that psoriasis comorbidity-related gene sets were enriched only in Western large psoriasis, while there was no significant enrichment of the same gene sets in Asian small or intermediate psoriasis ($p < 0.001$ and $\text{FDR} < 0.001$) (Lee *et al.*, 2008).

Next, we confirmed clinically the downregulation of negative regulators contrary to the upregulation of driver cytokines in the skin of psoriasis patients with accompanying comorbidities (Figure 6). Compared to psoriasis patients without psoriatic arthritis, patients with psoriatic arthritis revealed lower expression levels of negative regulators (CD69 in lesional skin, FAS in both lesional and non-lesional skin) in contrast to higher expression levels of driver cytokines (TNF- α in non-lesional skin, IL-1B in both lesional and non-lesional skin) (Figure 6a). Similarly, obese psoriasis patients revealed lower expression levels of negative regulators (CD69 in lesional skin, FAS in both lesional and non-lesional skin, and FoxP3 in lesional skin) and a higher expression level of driver cytokine (IL-1B in non-lesional skin) (Figure 6b).

DISCUSSION

Recent trans-ethnic genome-wide meta-analysis of Asian and Western psoriasis populations observed the population-specific effects of the AA positions 114 and 144 of HLA-A as well as another 10 non-MHC psoriasis susceptibility loci (Yin *et al.*, 2015). These population-

specific risk factors might lead to ethnically different expression patterns of psoriasis-associated transcriptomes, resulting in different psoriasis prevalence and clinical phenotypes. In this study, we compared psoriasis transcriptomes of clinically different Asian and Western psoriasis cohorts by two independent measures of clinical phenotyping – epidermal thickness (vertical growth) and PASI (radial expansion). To understand functional disease status by molecular characterization, quantitative expression analysis of RT-PCR (Figure 3 and Supplementary Figure S7 online) and pathway analysis of microarray (Figure 4) were combined to compare against the mRNA transcriptome that has been established in previous studies (Jabbari *et al.*, 2011; Loscalzo, 2007; Perera *et al.*, 2014; Suarez-Farinas *et al.*, 2012; Suarez-Farinas *et al.*, 2010). Serving as a reference list of genes for the core pathogenesis of psoriasis, we utilized a meta-analysis-derived transcriptome built upon the same microarray platform (Figure 1d) (Tian *et al.*, 2012). This approach revealed that IL-17A and IL-17-regulated pro-inflammatory cytokines drive disease progression from the early stage of psoriatic plaque development, particularly as IL-17 expression was highest in Asian small psoriasis (Figure 3a). Currently, IL-17-targeted therapies that neutralize IL-17 (i.e., secukinumab and ixekizumab) have been studied in Phase I to III clinical trials and receive FDA approval only for “moderate-to-severe” psoriasis (typical PASI > 12) (Chiricozzi and Krueger, 2013). However, Asian forms of psoriasis, which are classified as “mild” disease (mean PASI < 7) in spite of significant disease extent, might also benefit from anti-IL-17 agents.

Our study also revealed that non-lesional skin around the psoriatic plaques is not normal but actively reflects disease progression status: the epidermal thickness and PASI increase in accordance with IL-17 and TNF- α in non-lesional skin, respectively. Also, there was a strong and significant correlation between PASI and negative immune regulatory gene expressions (CD69 and FAS) in non-lesional skin (Figure 5 and Supplementary Table S2 online). Given that excessive immune activation may be present in non-lesional skin, the expression of negative immune regulatory pathways may be important in restraining activation to below-thresholds of producing clinically detectable disease.

The function of negative regulators within lesional and non-lesional skin is likely important in preventing the disease progression of psoriasis. In accordance with PASI, CD3⁺ T cells were increased in psoriasis body surface area (Figure 2c and Supplementary Table S2 online), while the expression of negative regulators decreased proportionally (Figure 5 and Supplementary Table S2 online). Therefore, we hypothesized that the lack of negative regulator signals may allow for extensive expansion of disease-related T cell clones. In our study, the expression of FoxP3 in non-lesional skin increased as PASI increased, but the correlation was not linear (Figure 5 and Supplementary Table S2 online). However, the expression of CTLA4, the inhibitory co-receptor of Tregs, revealed significant linear correlation with PASI in lesional skin. The expression of the Fas receptor, which is a component of the Fas-FasL interactions employed by CD4⁺ CD25⁺ Tregs to restrict CD8⁺ T cell-mediated immune response in the skin (Gorbachev and Fairchild, 2010; Stranges *et al.*, 2007), also revealed significant correlation with PASI in both lesional and non-lesional skin. Fas is an important regulator of migratory dendritic cell function and was recently identified as central to human skin dendritic cell programming (Anandasabapathy *et al.*, 2014; Baratin *et*

et al., 2015). Potentially, negative immune regulators such as CTLA4 and Fas could be more important than classical Tregs in regulating T cell expression in psoriasis.

C-type lectin receptor, CD69, exerts a proinflammatory function *in vitro*, but recent *in vivo* studies revealed its function as an immunoregulatory molecule induced following activation (Sancho *et al.*, 2005). Since CD69 induction can block sphingosine 1-phosphate receptor-1-mediated T cell egress from lymphoid tissues through interaction with membrane helix 4, CD69 is demonstrated to be a critical determinant of prolonged T cell retention (Bankovich *et al.*, 2010; Mackay *et al.*, 2015; Shioh *et al.*, 2006). In this context, downregulation of CD69 in Western large psoriasis despite an exponentially higher number of CD3⁺ T cells in total psoriasis body surface area may represent how T cell expansion is dysregulated in psoriasis. Also, it is feasible that CD69 could be persistently expressed by a subset of regulatory T cells (Sancho *et al.*, 2005). Although CD69⁺ and CD69⁻ FoxP3⁺ Tregs exist in homeostasis, only CD69-expressing Tregs express high levels of CTLA4 and have potent suppressor activity secreting high amounts of TGF- β (Cortes *et al.*, 2014). Considering that TGF participates in the differentiation of both Tregs and T_H17 cells, and that Tregs in psoriasis patients can easily differentiate into IL-17A-producing cells, CD69 might be the key determinant of disease progression in psoriasis (Bettelli *et al.*, 2006; Bovenschen *et al.*, 2011; Cortes *et al.*, 2014; Veldhoen *et al.*, 2006). In our study, the expression of CD69 in both lesional and non-lesional skin was highly correlated with PASI in a negative direction (Figure 5 and Supplementary Table S2 online).

Taken together, we showed the utility of disease subtype comparisons with detailed cellular and molecular signatures to advance new hypothesis generation and future testing of concepts in clinical trials (Figure 7). A limitation of study is comparing subtypes between distinct ethnic populations under the assumption that psoriasis transcriptome profiles represent functional disease status regardless of ethnicity. Ethnicity may confound the correlation between RNA expression of genes involved in psoriasis pathogenesis and clinical phenotypes that might be influenced by other factors, such as genetic/environmental differences. To overcome this limitation, the concepts should be further tested in different subtyping strategies, and ultimately in clinical trials blocking driver cytokines and/or boosting negative regulators in an attempt to prevent disease progression.

MATERIALS & METHODS

A detailed Materials and Methods description is available in Supplementary Materials and Methods online.

Study design

This study was designed to conduct *ex vivo* human observation with human skin biopsy tissues for the purpose of subtype comparisons (Supplementary Figure S8 online). Psoriasis lesional and non-lesional skin biopsy tissues were obtained in accordance with the Helsinki Declaration and approved by the Institutional Review Board of Korea University Guro Hospital, Seoul, South Korea and the Rockefeller University, New York, NY, USA. Written informed consent was obtained from all patients. 59 psoriasis patients were enrolled at Korea University Guro Hospital, Seoul, South Korea and 21 patients were enrolled at the

Rockefeller University Hospital, New York, NY, USA (ClinicalTrials.gov; NCT01920906). 8 patients enrolled in Korea were excluded because the pathology report from independent dermatopathologists did not support the diagnosis of psoriasis. Asian small psoriasis was diagnosed according to the criteria described in Supplementary Table S1 online (Lew *et al.*, 2004). As widely used in randomized clinical trials, Western large psoriasis was defined as moderate-to-severe psoriasis vulgaris with PASI greater than 12 (Feldman, 2004; Schmitt and Wozel, 2005).

Three different subtypes (Asian small psoriasis, Asian intermediate psoriasis, and Western large psoriasis) were compared by clinical data (demographics and comorbidities), immunohistochemical analysis, gene expression profiling, and quantitative analysis of gene expression. For immunohistochemical analysis and gene expression profiling of Western large psoriasis, we utilized de-identified repository data from established cohorts: 1) Immunohistochemistry repository data from tissue bank in the Laboratory for Investigative Dermatology at the Rockefeller University, New York (N=69), 2) NCBI GEO repository data (GSE30999, N=65).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CTLA4	Cytotoxic T-Lymphocyte-Associated Protein 4
DEFB103	Defensin, Beta 103B
FasL	Fas ligand
FOXP3	forkhead box P3
IFN	Interferon
K16	Keratin 16
PASI	Psoriasis Area and Severity Index
PD-L1	Programmed death-ligand 1

TGF	Transforming growth factor
T_H17	T helper 17
TNF	Tumor necrosis factor
Treg	Regulator T cell

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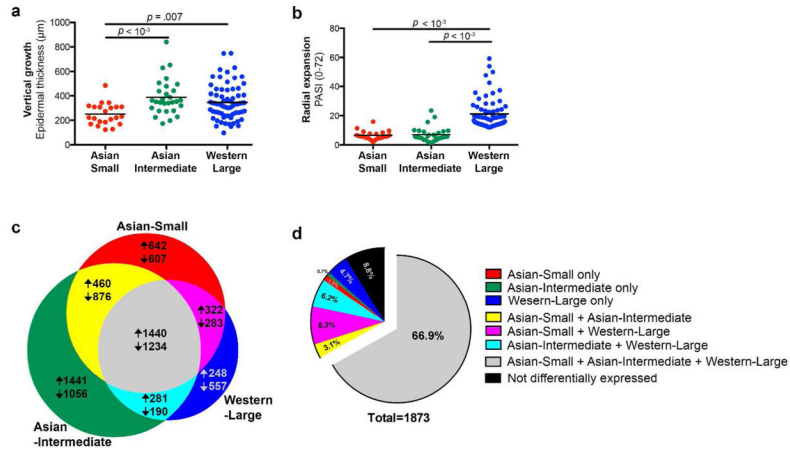


Figure 1. Comparison of epidermal thickness, PASI, differentially expressed genes (DEGs), and meta-analysis-derived psoriasis transcriptome between Asian small, Asian intermediate, and Western large psoriasis

(a) Asian small psoriasis with limited epidermal thickness in comparison to Asian intermediate or Western large psoriasis. (b) Western large psoriasis with higher PASI in comparison to Asian small or intermediate psoriasis. (c) Area-proportional Venn diagram comparing DEGs. 27.7% of all DEGs were shared by the three comparison subtypes. (d) DEGs among meta-analysis-derived psoriasis transcriptome for Affymetrix Human Genome U133 Plus 2.0 Array (MAD3)(Suarez-Farinas *et al.*, 2012). 66.9% of the meta-analysis-derived psoriasis transcriptome was differentially expressed in all subtypes (> 2 FCH and < 0.05 FDR, PASI: Psoriasis Area and Severity Index, FCH: fold change, FDR: false discovery rate)

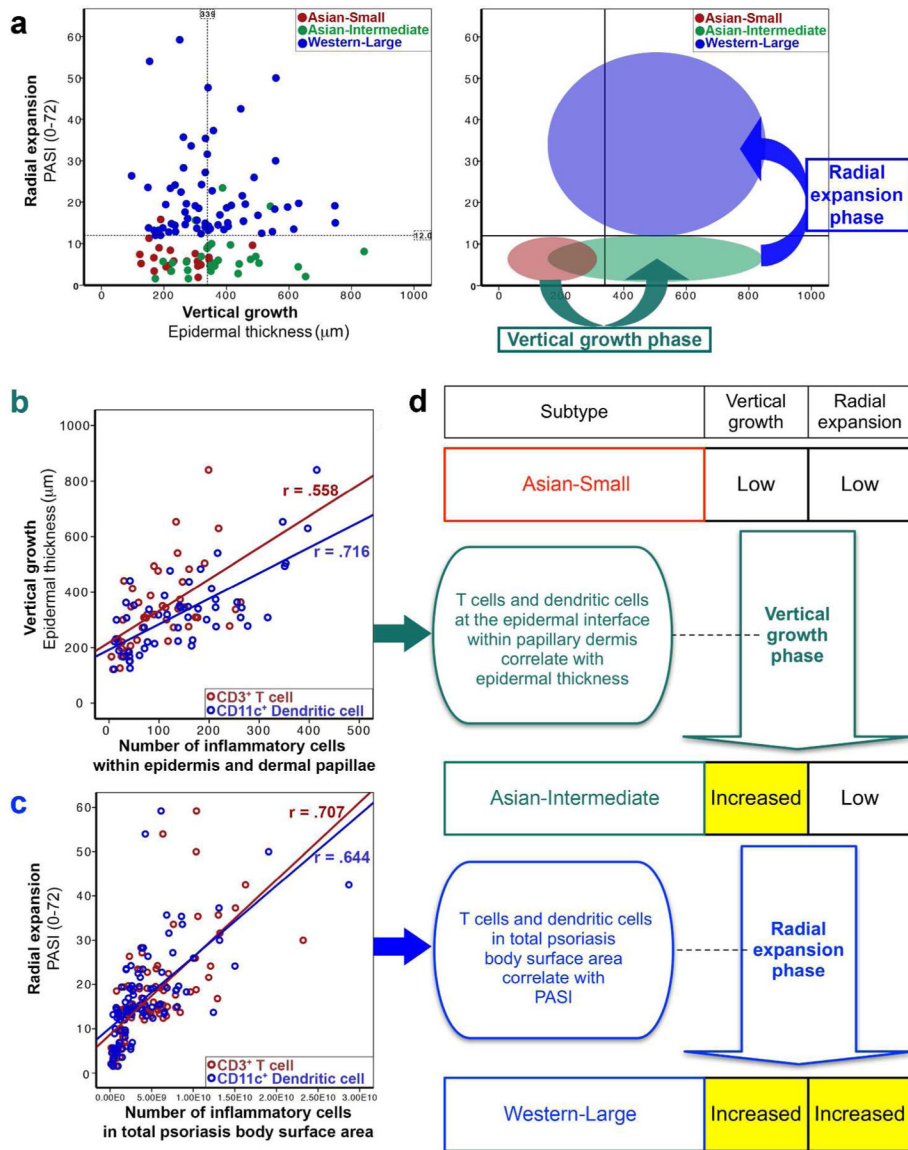


Figure 2. Exploratory models of disease progression

(a) Vertical growth and radial expansion models emerged from contrasting epidermal thickness and PASI between Asian small, Asian intermediate, and Western large psoriasis. (b) In the model of vertical growth phase, epidermal thickness was correlated with the numbers of CD3⁺ T cells and CD11c⁺ dendritic cells within the epidermis and dermal papillae (method of counting cells in epidermis and dermal papillae: Supplementary Figure S6 online). (c) In the model of radial expansion phase, PASI was correlated with CD3⁺ T cells and CD11c⁺ dendritic cells in total psoriasis body surface area (d) Summary of exploratory models (r = Pearson correlation, p < 0.0001, PASI: Psoriasis Area and Severity Index, Number of inflammatory cells in total psoriasis body surface area = cell count in the slide section × body surface area × proportion of psoriasis involvement).

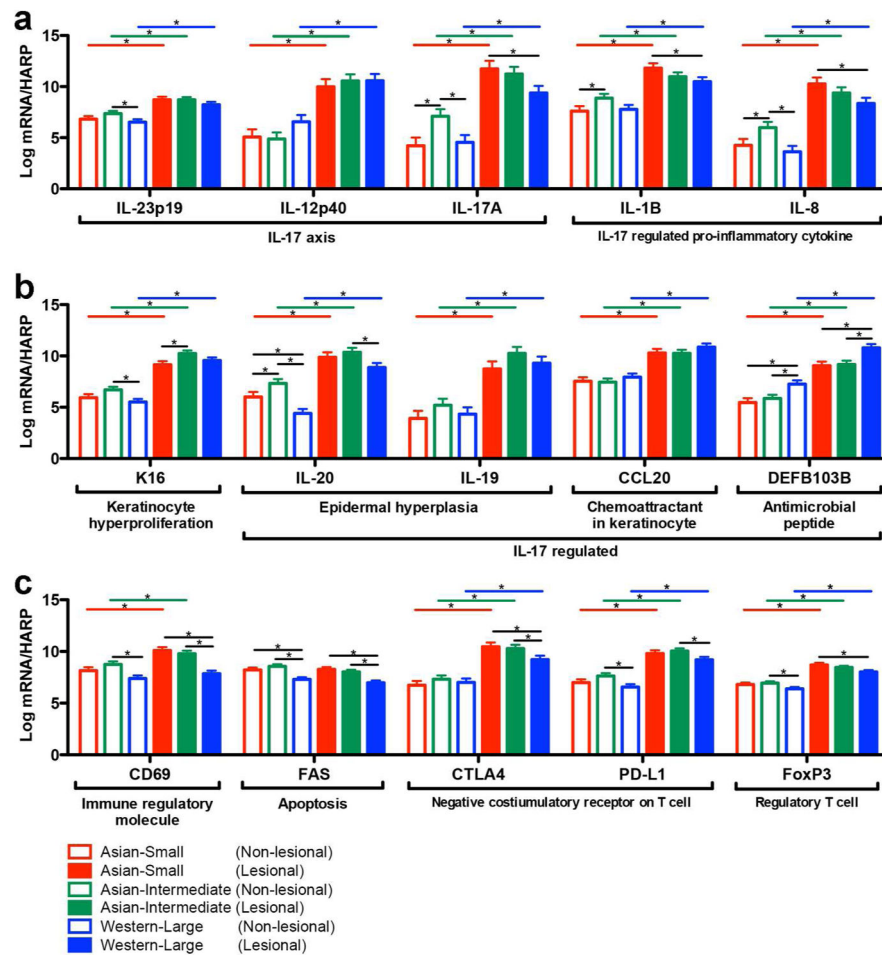


Figure 3. Quantitative comparison of gene expression in psoriatic lesional and non-lesional skin between Asian small (N=16), Asian intermediate (N=21), and Western large (N=20) psoriasis Expression levels of genes involved in psoriasis disease progression were quantified by RT-PCR (Gene expression: Log₂ conversion of mRNA expression normalized to human acidic ribosomal protein (HARP), **p* < 0.05).

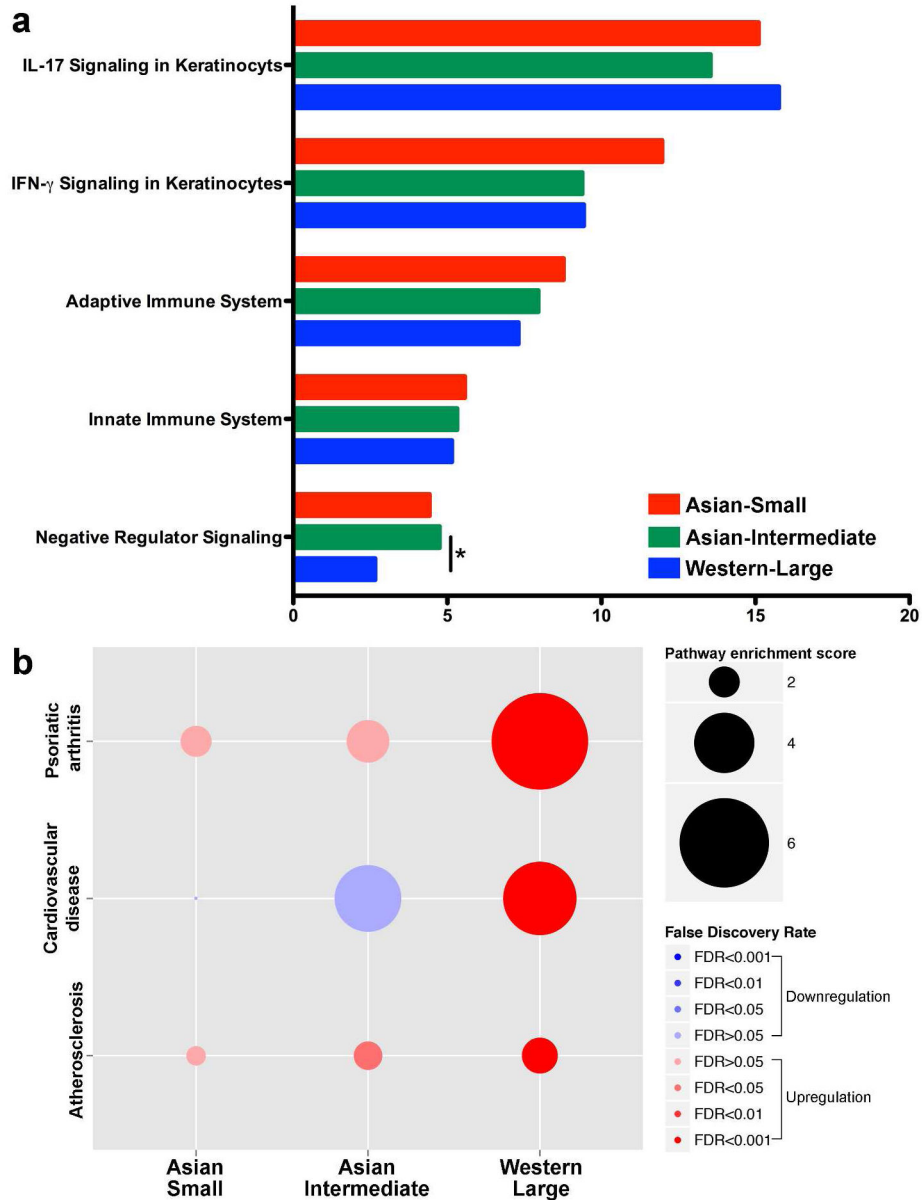


Figure 4. Psoriasis pathway enrichment scores

(a) The enrichment of pivotal immune pathways of IL-17 and IFN- γ signaling was not different between Asian small, Asian intermediate, and Western large psoriasis. Negative regulator signaling in Western large psoriasis was downregulated compared to Asian intermediate psoriasis. (* $p < 0.01$ and FDR < 0.01) (b) Pathways involved in psoriasis comorbidities (psoriatic arthritis, cardiovascular disease, and atherosclerosis) were significantly enriched only in Western large psoriasis ($p < 0.001$ and FDR < 0.001). The scores were generated by GSEA with the combined z-score method. Gene sets for pathway analysis were curated from published papers (Belasco *et al.*, 2014; Chiricozzi *et al.*, 2014), Molecular signatures database (<http://www.broadinstitute.org/gsea/msigdb>), and Gene ontology consortium (<http://geneontology.org>).

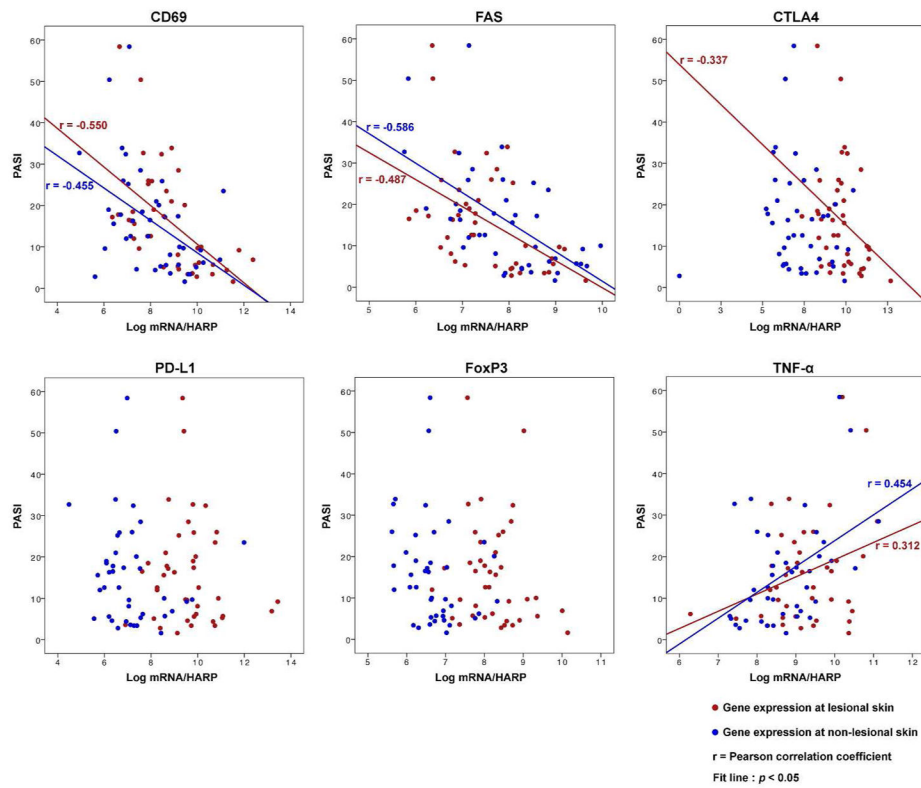


Figure 5. Correlation between PASI and expression levels of negative regulators in lesional and non-lesional skin

Overlay scatter plot to display the correlation between PASI and expression levels of negative regulators (CD69, FAS, CTLA4, PD-L1, and FoxP3) in lesional (red) and non-lesional (blue) skin. The expression of TNF- α in lesional and non-lesional skin is presented as a control (Gene expression: Log₂ conversion of mRNA expression normalized to human acidic ribosomal protein (HARP), PASI: Psoriasis Area and Severity Index).

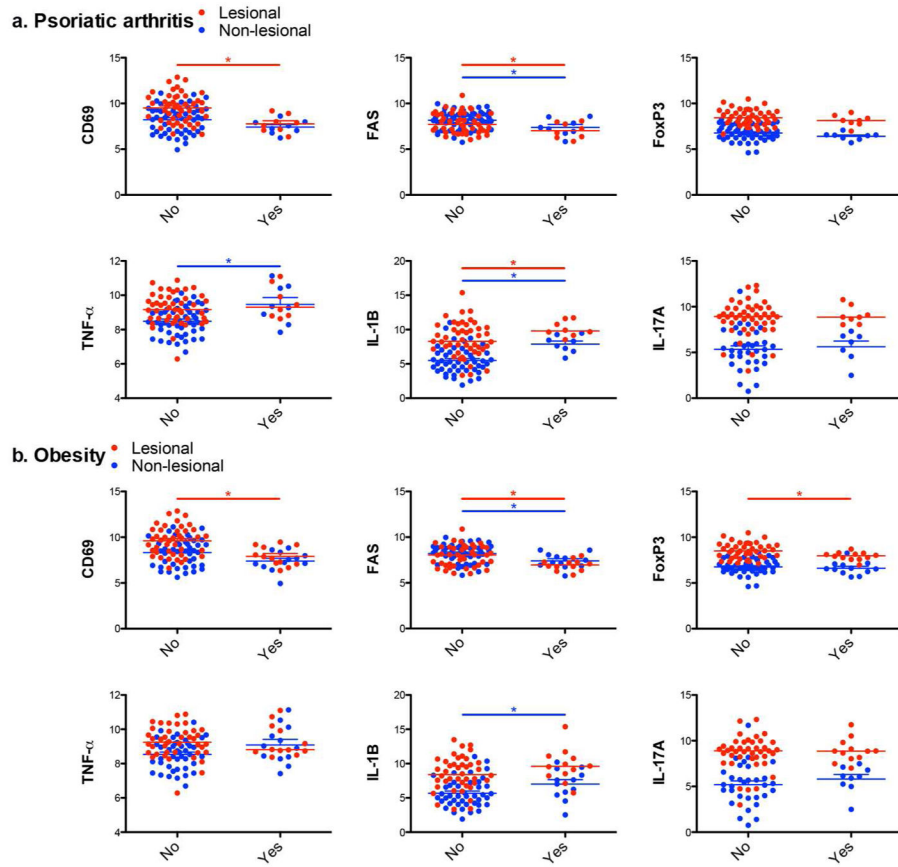


Figure 6. Downregulation of negative regulators and upregulation of driver cytokines in the skin of psoriasis patients with psoriatic arthritis/obesity

Overlay scatter plot to display different expression levels of negative regulators (CD69, FAS, and FoxP3) and driver cytokines (TNF- α , IL-1B, and IL-17A) in lesional (red) and non-lesional (blue) skin between psoriasis without (No) and with (Yes) comorbidities (Psoriatic arthritis (a), Obesity (b)). The data include total study population of Asian small, Asian intermediate, and Western large psoriasis. Gene expression: Log₂ conversion of mRNA expression normalized to human acidic ribosomal protein.

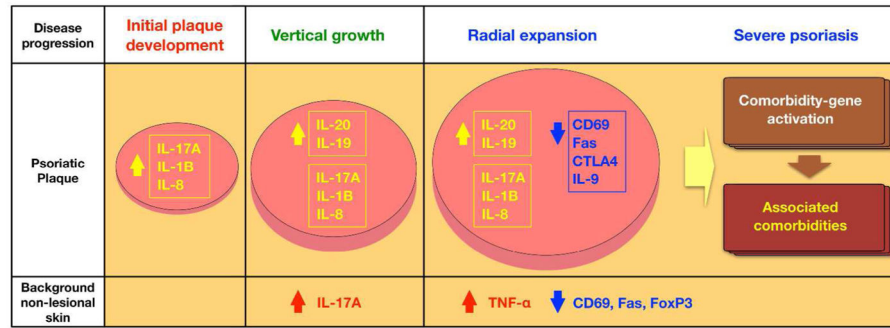


Figure 7. Molecular determinants of disease progression in psoriasis

Potential molecular determinants of disease progression at different stages were explored by correlating cellular and molecular signatures to histologic and clinical measurements of disease progression.