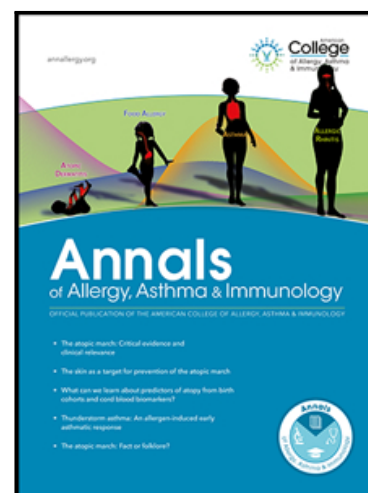


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**Atopic dermatitis in African American patients is T_H2/T_H22-skewed with T_H1/T_H17
attenuation**

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Abbreviations:

AA: African American

AD: Atopic dermatitis

CLDN: Claudin

EA: European American

ELOVL: Elongation of very long chain fatty acids protein

FLG: Filaggrin

GWAS: Genome Wide Association Study

GSVA: Gene Set Variation Analysis

KRT16: Keratin 16

LOR: Loricrin

MMP: Matrix metalloproteinase

SCORAD: Scoring of Atopic Dermatitis

STAT: Signal transducer and activator of transcription

TLDA: TaqMan® Low Density Array

TSLP: Thymic stromal lymphopoietin

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Contributions of Authors:

- **EGY and JGK:** Study design and conceptualization, patient recruitment, sample collection, manuscript revision
- **RDS:** Drafting of manuscript and figures, data analysis and presentation, immunohistochemistry
- **ABP and JG:** Statistical analysis
- **TCC, XZ, NZ, YE:** Contributions to RNA-Seq and RT-PCR
- **XP, JFD:** Immunohistochemistry

- **IC:** Cell counting
- **AFA:** Manuscript editing

INTRODUCTION

Atopic dermatitis/(AD) is a chronic inflammatory skin disease affecting all ethnic populations with varying rates of prevalence associated with disproportionate degrees of impact on healthcare.^{1, 2} The AD disease phenotype is characterized by T-cell and dendritic cell/(DC) infiltrates,^{3, 4} upregulation of inflammatory mediators,⁴ epidermal hyperplasia,⁵ and epidermal barrier defects with downregulation of differentiation proteins, lipids, and claudins.⁶⁻⁸ AD is currently considered to be a T_H2/T_H22 -centric disease, largely based on molecular profiling in European American/(EA) adults.^{4, 9} However, AD is increasingly recognized as a heterogeneous disease with significant variation in molecular phenotypes across different populations.^{10, 11} For instance, Asian and pediatric AD show increased T_H17 activation in addition to T_H2 skewing.¹⁰⁻¹³ This heterogeneity has important implications for future development and selection of targeted therapeutics, which thus far has been based primarily on pathogenic insights derived from the EA AD adult phenotype.^{4, 7, 8}

African Americans/(AA) are disproportionately impacted by AD with prevalence of 19.3% versus 16.1% in EA in the United States,^{14, 15} comparable with AD prevalence rates reported in African countries of up to 23%.¹⁶ Additionally, a U.S.-based study comparing health care utilization for atopic dermatitis in different ethnic groups found that although white patients had a greater number of per capita visits for all medical conditions and skin conditions, black patients had higher per capita numbers of visits for atopic dermatitis, and were 3 times more likely than whites to have an office visit at which atopic dermatitis was diagnosed.² This disproportionate impact is exacerbated by unique treatment challenges in this population, such as

higher required doses of cyclosporine A¹⁷ and narrow-band UVB,¹⁸ increased incidence of corticosteroid induced hypopigmentation,¹⁹ and greater risk of dryness due to increased transepidermal water loss/(TEWL)²⁰ and decreased ceramide-to-cholesterol ratio.²¹ The paucity of safe and effective treatments for moderate-to-severe AD in AA further highlights the need for biomarker research and identification of therapeutic targets in this population, thus allowing for accurate prediction of phenotype-specific responses to available targeted therapeutics, in addition to development of novel treatment strategies.

Still, molecular mechanisms of AD are profoundly understudied in this population.^{22, 23} Prior research in AA is limited to genome-wide association studies/(GWAS) which have detected some polymorphisms in risk loci within AD-associated genes, including *IL-7R*,²⁴ *TSLP*,^{24, 25} *IL-18R1*,²⁶ *FLG*,²⁶⁻²⁹ and *FLG2*²⁸ without molecular phenotypic correlation. We thus set to conduct the first global molecular profiling study of AA AD using RNA-seq. Our study identified significant variations in immune polarization and expression of epidermal barrier markers in AA AD as compared with a EA AD cohort.

METHODS

Sample collection

Biopsy specimens were collected from lesional and non-lesional (>10cm from active lesions) skin in 30 patients with chronic AD since childhood (15 AA, 15 EA), and from healthy skin in 16 control subjects (9 AA, 7 EA), under an institutional review board-approved protocol. Of the 12 AA patients studied, a heterozygous *FLG* mutation in the 2282del41 allele was found in 1 patient, and no mutations were found in R501X, R2447X, and S3247X. No heterozygous *FLG* mutations (R501X and 2282del4 allele) were found in the 4 EA patients studied. For several patients, *FLG* mutation analyses could not be completed due to lack of sample. Patients

avoided use of systemic immune-modulators and phototherapy for four weeks, and topical steroids or calcineurin inhibitors two weeks prior to sampling. Race and ethnicity data were patient reported and investigator confirmed. Patient characteristics are detailed in Table 1.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on frozen skin sections, using purified mouse anti-human monoclonal antibodies listed in eTable 1, as previously described.⁷ Epidermal thickness and cell counts were quantified using ImageJ V1.42 software (National Institutes of Health, Bethesda, MD).

RNA-Seq

RNA-Seq was performed with the Illumina HiSeq2500 (Illumina, San Diego, CA; 100 cycles, single-read sequencing, 8 samples per lane), as previously described.⁹ Sample quality was assessed using FastQC. Samples were aligned to human reference genome using STAR (open source aligner).³⁰ Mapped sequencing reads were assigned to genomic features using the featureCounts function from Rsubread library. Following standard transformation of counts to log-scale by voom transform,³¹ expression values were modeled using mixed-effect models with ethnic group and tissue type as fixed factors and a random effect for each patient. Fold-changes/(FCHs) for comparisons of interest were estimated and hypothesis testing was conducted using contrasts under the general framework for linear models in *limma* package. P-values from the moderated t-test were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure, which controls False Discovery Rate/(FDR). Differentially expressed genes/(DEGs) were defined by FCH>2.0 and FDR<0.05.

Quantitative real-time PCR (qRT-PCR)

RNA was extracted for real-time PCR/(RT-PCR) with the miRNAeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription to cDNA from RNA was carried out using the High Capacity cDNA Reverse Transcription Kit, and cDNA was amplified with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA). TaqMan Low Density Array/(TLDA) cards were used for qRT-PCR. Eukaryotic 18S rRNA was used as an endogenous control. Expression values (threshold cycle [Ct]) were normalized to *Rplp0*. All primers are listed in eTable 2.

Statistical analyses were performed with statistical language R (www.R-project.org). RT-PCR values were normalized to *Rplp0* by negatively transforming Ct values to $-dCt$. Undetected $-dCt$ values were estimated for each gene as 20% of the minimum across all samples. Expression values and cell counts were modeled in log₂-scale by linear mixed-effect models. Means of each group were estimated using *lsmeans* and comparisons of interest were tested using contrast. Summaries of mean expression for all markers are represented with a heatmap. Unsupervised clustering of lesional, non-lesional, and normal means were performed using Euclidean distance and average agglomeration criteria.

RESULTS

This study included 15 AA and 15 EA patients with moderate-to-severe AD (AA ages 18-59 [mean 40.6], SCORAD 38-94 [mean 56.9]; EA ages 23-69 [mean 47.8], SCORAD 44-77 [mean 58]), as well as 9 AA and 7 EA healthy control subjects (AA ages 23-56 [mean 41.3]; EA ages 31-56 [mean 46.4]). All AA patients had Fitzpatrick skin types V or VI. There were no significant differences in demographic parameters, including age and gender distribution, between AA and EA cohorts, or between AD cohorts and respective controls (Table 1). Clinical disease severity, as measured by SCORAD, was also not significantly different between AA and EA AD. Mean IgE was significantly higher in AA versus EA patients ($p < 0.0003$) as previously reported,³² although well above reference range (0-114kU/L) in both groups. Eosinophil counts were not significantly different between AD cohorts.

Clinical features of AD in AA and EA patients are depicted in Figure 1. While both AA and EA AD presented with lichenified patches, the lesions of EA patients appear erythematous, while those of AA patients are darker and more difficult to distinguish clinically from non-lesional skin. Additionally, AA patients were more likely to have extensor involvement (Figure 1B) and less frequent flexural involvement compared with EA patients, as previously observed.^{22,}

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AA AD tissues are characterized by epidermal hyperplasia and loricrin downregulation

Immunohistochemistry was performed to detect markers of epidermal hyperplasia, epidermal differentiation proteins, and immune cell infiltrates (Figures 2-3). Measures of epidermal hyperplasia included epidermal thickness, keratin 16/(KRT16) mRNA/protein expression, and number of Ki67⁺ cells. Epidermal thickness was significantly increased in

lesional skin of both EA and AA compared to respective controls ($p < 0.001$, Figure 2A,F). In both groups, all lesional samples stained positively for KRT16, with variable staining in non-lesional and lack of staining in control skin, corresponding with significantly upregulated *KRT16* mRNA in lesional versus normal and non-lesional skin in both AA and EA AD ($p < 0.001$, Figure 2B,G). Ki67⁺ cells were increased in lesional skin of both AD cohorts (Figure 2C,H).

Loricrin, a key differentiation protein, exhibited a less consistent distribution of protein expression in AA versus EA AD lesions, and *LOR* mRNA was significantly downregulated in EA and AA lesions ($p < 0.05$, Figure 2D,I). In contrast, the differentiation protein filaggrin showed more extensive interruptions in protein expression in EA AD lesional and non-lesional skin as compared with AA (Figure 2E). *FLG* mRNA mirrored this pattern, with downregulation in EA AD (approaching significance in lesional skin, $p < 0.1$) and lack of modulation in AA AD (Figure 2E,J).

Infiltration of FcεRI⁺ cells is greater in AA versus EA AD lesions

Significantly increased infiltration of CD3⁺T-cells and CD11c⁺myeloid DCs characterized both AA and EA AD lesional and non-lesional skin (Figure 3A-B,F-G). DCs marked by the high-affinity IgE receptor (FcεRI⁺), which also marks inflammatory DCs/(IDECs), were significantly increased in AA compared with EA lesional skin ($p < 0.05$, Figure 3C,H). Langerin⁺ langerhans cells were increased only in AA lesions, while neutrophil elastase⁺ neutrophils were significantly increased in lesions of both groups ($p < 0.05$ for neutrophils, Figure 3D-E,I-J).

RNA-Seq molecular profiling in AA AD shows attenuation of the T_H1-axis compared with EA AD

For global molecular profiling of the AD transcriptome in AA and EA patients, we performed RNA-Seq using Illumina HiSeq2500. Differentially expressed genes/(DEGs) in AD were defined by criteria of fold-change/(FCHs)>2.0 and false discovery rate/(FDR)<0.05 when comparing lesional or non-lesional AD versus respective normal skin, or lesional versus non-lesional skin of each group (eTable 3). Venn diagrams by upregulated and downregulated DEGs in lesional and non-lesional AD versus normal skin are displayed in Figure 4A. The AA AD lesional versus normal comparison included 1,812 DEGs (639 upregulated, 1,173 downregulated), of which 46% (828) were unique to AA and 54% (984) were shared between AA and EA. In the non-lesional AA AD versus normal skin comparison, 177 DEGs were identified (75 upregulated, 102 downregulated), of which 91% (161) were unique to AA and only 9% (16) were shared among AA and EA. Of note, when comparing AA and EA healthy control cohorts, only 2 named DEGs were identified by RNA-Seq, a non-coding RNA gene (*SNHG8*) and a mitochondrial protein encoding gene (*C21orf33*).

The top 50 commonly upregulated DEGs in AA and EA lesional skin included key genes previously associated with active AD lesions,^{9, 33} including markers of general inflammation (*MMP12*), hyperplasia (*KRT16*), T_H2 (*CCL17/18/26*) and T_H22 (*S100A7/8/9/12*). The top commonly downregulated DEGs were lipid metabolism genes (*ELOVL3* [fatty acid elongase 3], *FABP7* [fatty acid binding protein 7], *FADS1/2* [fatty acid desaturase 1 and 2]), terminal differentiation genes (*LOR*, *FLG2*), and claudins (*CLDN8/16/23*). However, several genes previously associated with the EA adult AD transcriptome, such as the T_H1-regulated *IL-12RB2*,

CXCL10, and *CXCL11* were exclusively upregulated in the EA AD but not the AA AD transcriptome.

To perform pathway-level comparisons between the EA and AA AD molecular profiles, we conducted Gene Set Variation Analysis/(GSVA) using previously published gene sets (Figure 4B).³⁴⁻³⁶ Innate and T_H1-related pathways including TNF, IFN- γ , and IL-12-mediated signaling pathways showed significantly greater upregulation in EA versus AA AD. T_H2-related genes, however, were similarly upregulated in both groups (Figure 4B,a-e). T_H17-regulated genes were significantly increased in lesional EA versus AA AD, while the T_H22/IL-22 pathway was not differentially expressed between AD cohorts (Figure 4B,f-g). Epidermal differentiation complex/(EDC) including cornified envelope/(CE) genes, as well as lipid genes, were downregulated in both groups (Figure 4B,h-i). A summary of these comparisons is provided as a bubble plot in eFigure 1.

To detect more specific immune phenotypic variations among AA and EA AD, we used a previously defined immune genes list^{5, 12, 37} shown as a heatmap depicting differences in FCH-expression among AD cohorts, normalized to gene expression of respective AA and EA healthy controls (Figure 5). The most significant differences in immune gene expression between groups were in the T_H1/IFN- γ pathway; *IL-12RB2*, *CCL4*, *CXCL10*, *CXCL11*, *MX1*, and *STAT1* were upregulated exclusively in lesional EA AD, and not in AA AD. Innate immune mediators *IL-1 β* and *IL-8* as well as T_H17-pathway genes (*IL-12B/p40*, *CCR6*, *CCR10*, *CXCL3*, *CAMP*, *S100P*) were also exclusively upregulated in EA. In contrast, T_H2 markers showed fewer differences between groups. Most T_H2 markers were either strongly upregulated in lesional and non-lesional skin of both groups (*IL-4R*, *IL-13*, *IL-7R*, *CCL17/18/22/26*, $p < 0.05$), or not upregulated in either group (*IL-4*, *IL-5*, *IL-9*, *IL-31*). However, *IL-10*, *CCL13*, and *TSLP* were upregulated exclusively

in AA AD. Of note, *HLA-DQA2*, a gene associated with total IgE in previous GWAS,³⁸ was also exclusively upregulated in AA. T_H22 genes (*IL-22*, *S100A8/9/12*) were upregulated to a similar extent in AA and EA AD. There were no significant differences in expression of these immune genes among AA and EA healthy control cohorts by RNA-Seq.

Epidermal barrier dysfunction, a hallmark of the AD disease phenotype, is characterized by downregulation of genes associated with lipid metabolism,^{33, 39} the EDC and cornified envelope,^{7, 40} and tight junctions (i.e. claudins [*CLDNs*]).^{41, 42} Differences in expression of a predefined barrier gene subset^{33, 40, 43} among AD cohorts normalized to respective healthy controls is displayed in a heatmap in eFigure 2. Differentially expressed terminal differentiation genes among AD cohorts included *FLG* and *OVOL1*, a gene involved in keratinocyte formation and barrier function that has been recently associated with AD susceptibility by GWAS.⁴⁴ Both of these genes were exclusively downregulated in EA AD. However, other epidermal differentiation genes such as *LOR* and *FLG2* were similarly downregulated in both AD cohorts. Most lipid metabolism genes and claudins were similarly downregulated in AA and EA AD, although some were either more downregulated (*FABP7*, *ELOVL3*, *CLDN8*) or exclusively downregulated (*CLDN3*, *CLDN10*) in AA. There were no significant differences in expression of barrier genes among AA and EA healthy control cohorts.

RT-PCR Validation

To validate RNA-seq findings, and to detect key genes in AD that have been shown to fall below detection limits in RNA-seq,⁹ we performed RT-PCR on a large panel of 66 inflammatory and barrier genes including those previously determined to play significant roles in the AD molecular phenotype.

Markers of general inflammation (*MMP12*) and T-cell migration (*CCR7*, *CCL19*, *CCL21*) showed significantly increased mRNA expression in both AA and EA AD lesions versus non-lesional and control skin ($p < 0.05$, Figure 6A, eFigure 3D-F). Innate immune markers *IL-1 β* and *IL-8* were significantly more upregulated (from respective controls) in EA versus AA AD lesions ($p < 0.01$), confirming RNA-seq findings, and *TNF* was significantly upregulated from control skin exclusively in EA AD lesions (Figures 6B-D). *IFN- γ* and the *IFN- γ* -induced *CXCL10* and *MX1* showed significantly higher upregulation in EA versus AA lesions ($p < 0.05$), as did T_H1 -related products *IL-12RB1* and *IL-27p28* ($p < 0.01$, Figures 6E-H, eFigure 3L). *CXCL11* was not induced at all in AA AD (eFigure 3K). In contrast, most T_H2 cytokines and chemokines were either upregulated to a similar extent in EA and AA AD lesions versus normal (*IL-5*, *IL-10*, *IL-13*, *CCL17/18/22/26/27*), or were significantly higher in AA versus EA AD (*CCL13*, $p < 0.05$, Figure 6I-M, eFigure 3O-P,T-W). T_H9 /IL-9 was also upregulated in both AD cohorts. While some T_H17 -related cytokines (*IL-36G*, *IL-23p19*) and antimicrobial peptides (*CXCL1*, *CAMP/LL37*, *LCN2*) showed significantly greater upregulation in EA versus AA AD lesions ($p < 0.05$, Figure 6O-Q, eFigure 3KK-LL), others were similarly induced in lesional skin of both groups (*IL-17A*, *IL-19*, *IL-20*, *IL-21*, *PI3*, *DEFB4*, *HBD3*, Figure 6N, eFigure 3BB-EE,JJ-MM). IL-22 and T_H17 / T_H22 -induced S100s (*S100A8/S100A9/S10012*) were highly upregulated in both EA and AA AD, with no significant differences between groups (Figure 6R-S, eFigure

3FF-GG). The T-reg marker *FOXP3* was also significantly upregulated in lesional skin of both groups (Figure 6T). A summary of expression of all markers measured by RT-PCR is presented as a heatmap in eFigure 4. A summary of overall key differences in immune and barrier gene expression among AA and EA AD is presented in Table 2.

To account for differences in serum IgE among our AA and EA AD cohorts, we performed an extrinsic AD-subset analysis of all markers measured by RT-PCR, including only those patients with serum IgE > 200 U/mL (n=13 AA AD, n=6 EA AD). While some comparisons lost significance due to smaller sample size, overall expression patterns remained consistent with the full cohort analysis; innate immune (TNF, IL-1 β), T_H1 (*IFN- γ* , *IL-12RB1*, and *IL-27p28*) and T_H17 markers (*IL-23R*, *IL-23p19*) were significantly more upregulated in EA versus AA AD lesions, while T_H2 and T_H22 markers showed similar expression among AD cohorts (p<0.05, eFigure 5).

T_H2, T_H9 and T_H22 markers and serum IgE correlate with disease severity in AA AD

In AA AD lesional skin, disease severity (SCORAD) was significantly associated with mRNA expressions of *IL-13* (T_H2), *IL-9* (T_H9), *IL-22* (T_H22), and the T_H17/T_H22-induced S100 genes (*S100A8/9/12*) (p<0.05, Figure 7A). *IL-9*, *S100A8*, and *S100A9* expressions maintained significant correlations with SCORAD in non-lesional AA AD skin. *LOR* expression was significantly negatively correlated with SCORAD in non-lesional AA AD, but not in EA AD, while *FLG* expression was not significantly correlated with SCORAD in either group. In EA AD, the strongest correlations with SCORAD were observed among T_H17 cytokines (*IL-17A*, *IL-17F*) in lesional skin (p<0.05, Figure 7B). Serum IgE was significantly correlated with SCORAD in lesional AA AD, but not in EA AD (Figure 7C).

DISCUSSION

This is the first global molecular profiling study of AD skin in African American patients, characterizing phenotypic variations among African American and European American AD cohorts. Despite similar severity and chronicity of AD in both cohorts (longstanding disease since childhood in all patients, lesions present >72 hours before biopsy, and no significant differences in epidermal acanthosis between groups), our data revealed significant variations in cellular infiltration as well as expression of immune and barrier genes between AA and EA AD. In this study, all gene expression results reported in AA and EA AD were normalized to expression in respective AA and EA healthy control samples, and there were no significant differences in gene expression among healthy control cohorts by RNA-Seq. Thus, the differences in AA and EA AD detected in this study cannot be attributed to variations among healthy control groups.

Our study showed that AA AD lesions had greater infiltration of atopic DCs marked by the high-affinity IgE receptor/ $\text{Fc}\epsilon\text{R1}^+$, but similarly increased infiltration of other immune cell populations as compared with EA AD. Ethnic variations have been described in $\text{Fc}\epsilon\text{R1}\alpha$ and $\text{Fc}\epsilon\text{R1}\beta$ subunit genes (*FCER1A*, *FCER1B*), both of which are associated with IgE and allergic disorders^{49, 50}; two mutations in *FCER1A* are frequent in AA and rare in EA,⁵¹ while a mutation in *FCER1B* associated with bronchial hyperresponsiveness is more prevalent in black populations, both regardless of asthma status.⁵² Although we did not find these genes to be significantly overexpressed in either AD population, we did identify other genes associated with total IgE by GWAS³⁸ as upregulated either exclusively in AA AD (*HLA-DQA2*) or in both AD cohorts (*IL-13*, *IL-4R*).

The increased infiltration of FcεR1+ DCs in AA corresponds with our finding of higher serum IgE in the AA versus EA cohort, as well as the significant correlation between serum IgE and SCORAD identified in AA but not EA AD. The observation of significantly higher serum IgE in AA versus EA individuals has been made numerous times in previous studies, not only in populations with allergic disease but also healthy, non-allergic study populations. For instance, studies of asthma consistently report higher total serum IgE in black versus white individuals, both with and without asthma.^{32, 45, 46} Additionally, African ancestry has been identified as a risk factor for high IgE in African admixed populations, in both asthmatic and normal healthy control cohorts.⁴⁷ Finally, studies that have assessed ethnic variations in serum IgE levels of healthy individuals from neonates to adults show significantly increased IgE levels in AA versus EA AD.^{45, 48} Therefore, it is more likely that our finding of higher serum IgE in AA is reflective of a common ethnic variation rather than a factor that influences differences in immune dysregulation among AA and EA AD. Accordingly, the results of our subset analysis of only those patients with high IgE (>200) were consistent with those of the full cohort analysis, indicating that the differences in T-helper axis polarization among AA and EA AD does not merely reflect differences in IgE status.

While recent molecular phenotyping studies in pediatric and Asian AD populations have shown significant variability in T_H17-axis involvement and epidermal barrier characteristics compared to EA adults, strong T_H2-axis upregulation has been consistently observed across AD populations.¹⁰⁻¹² Similar to this pattern, our results show robust T_H2-pathway upregulation in AA AD. In fact, some T_H2 markers were exclusively upregulated in AA AD (*TSLP*, *IL-10*, *CCL13*) by RNA-Seq, although most were similarly overexpressed in both groups. These findings are supported by one of the few GWAS performed in AA AD, which detected polymorphisms

unique to AA in *TSLP* and *IL-7R* that were associated with IgE and AD disease severity.²⁴ AA AD was also characterized by T_H2 -skewing, with significant upregulation of *IL-22*, a cytokine shown to promote hyperplasia and inhibit keratinocyte differentiation,^{53, 54} as well as the induced *S100* genes (*S100A8/9/12*), which are associated with epidermal proliferation and differentiation.^{55, 56} The contributions of the T_H2/T_H22 axes in AA AD are further demonstrated by the significant correlations of T_H2 (*IL-13*) and T_H22 (*IL-22*, *S100A8*, *S100A9*) markers with SCORAD.

A striking feature of the AA AD molecular profile is the lack of induction of T_H1 markers known to be upregulated in chronic EA AD, including *IFN- γ* , the *IFN- γ* -induced *CXCL10*, *CXCL11*, and *MX1*, as well as *IL-12RB1* and *IL-27p28*.⁵ *IL-12RB1* is an important component of *IL-12/IL-23* cytokine signaling,⁵⁷ while *IL-27p28* induces T_H1 -differentiation through a *STAT1* mechanism.^{58, 59} A similar pattern was observed in T_H17 -regulated markers, including those that were exclusively upregulated in EA AD by RNA-Seq (*IL-12p40*, *CAMP/LL37*) and those showing significantly lower expression in AA as compared to EA AD (*IL-36G*, *IL-23p19*, *CXCL1*, *LCN2*). Additionally, the significant correlations of T_H17 markers (*IL17-A/IL-17F*) with SCORAD observed only in EA AD indicate that the T_H17 axis may play a lesser role in AA AD.

The previous paradigm of AD as a biphasic disease with a T_H2 -to- T_H1 transition from acute to chronic (>72 hours) lesions⁶⁰ has since been replaced by the more nuanced phenomenon of progressive T_H2/T_H22 -upregulation from acute to chronic disease, with more significant T_H1 -upregulation and subtle T_H17 increases during this transition.⁴ Our current study shows a lack of T_H1 -upregulation in chronic AA AD, challenging the notion of T_H1 -skewing as a universal hallmark of chronic AD. Taken together with the lack of T_H1 -upregulation observed in chronic

AD lesions from early pediatric patients,¹² these data argue against a pathogenic role for the T_H1 axis in AD.

Prior studies have described mutually antagonistic relationships between the T_H1 and T_H2 axes, where high IFN- γ and IL-12 levels inhibit T_H2 cytokine expression via transcription factor suppression.^{61, 62} More recently, T_H2/T_H17 reciprocal regulation has been described where IL-13 was shown to repress expression of T_H17 genes, and IL-17A to similarly repress T_H2 genes.^{63, 64} Thus, our observations of T_H1/T_H17-attenuation and T_H2-skewing in AA AD are likely to be interrelated rather than isolated phenomena. In fact, the significantly higher mean IgE levels measured in the AA AD cohort may be at least partially attributed to the absence of IFN- γ expression in this phenotype, allowing T_H2 expression and therefore IgE production to go unopposed.

Given the strong T_H2-bias in AA AD, the lack of *FLG* downregulation in this group (as opposed to significant downregulation in EA) was surprising in context of data supporting a role for T_H2 cytokines (*IL-4*, *IL-13*) in suppressing *FLG* expression.⁶⁵ However, it is important to note that the four most common *FLG* loss-of-function mutations found in European AD populations, which are associated with diminished filaggrin protein expression, have been reported as absent in African populations^{66, 67} and present in only 3.2% of AA, a frequency that could be somewhat attributable to European admixture.^{27, 68} Recently, Margolis et al. reported the presence of two loss-of-function mutations in *FLG2*,⁶⁹ a gene encoding for FLG2 protein which is associated with barrier disruption, in AA children.⁷⁰ Consistently, our study identified *FLG2* as significantly downregulated in both AA and EA, while *FLG* was downregulated exclusively in EA, corresponding with differences in protein expression patterns of FLG between groups. In addition to *FLG2*, other barrier genes were notably downregulated in both AA and EA

AD, such as *ELOVL3*, which plays a role in elongation of long-chain fatty acids and prevention of transepidermal water loss,⁷¹ claudins (*CLDN8*), which are essential components of epidermal tight junctions,⁷² and finally *LOR*, whose expression in non-lesional skin significantly negatively correlated with SCORAD (while *FLG* was not correlated). These data indicate that genes other than *FLG* may contribute significantly to overall barrier disruption in AA AD.

Overall, our data showed that AA AD is T_H2/T_H22-biased, with T_H2 and T_H22 markers as well as IgE correlating significantly with disease severity, and characterized by attenuation of T_H1 and T_H17 axes relative to EA despite chronic, longstanding disease. We also identified barrier products other than FLG that may play a prominent role in the barrier dysfunction in AA AD. Limitations of this study included small sample size, limited data on *FLG* mutation status, and lack of ancestry informative marker/(AIM) analysis. Future studies in larger populations that implement AIM investigation would allow for appropriate analyses of more admixed populations. The results of this study provide rationale for stratified analyses of future AD targeted therapeutic clinical trials by ethnicity, in order to understand whether ethnic variations in AD molecular phenotypes translate to differential treatment responses to polar T-cell antagonism.

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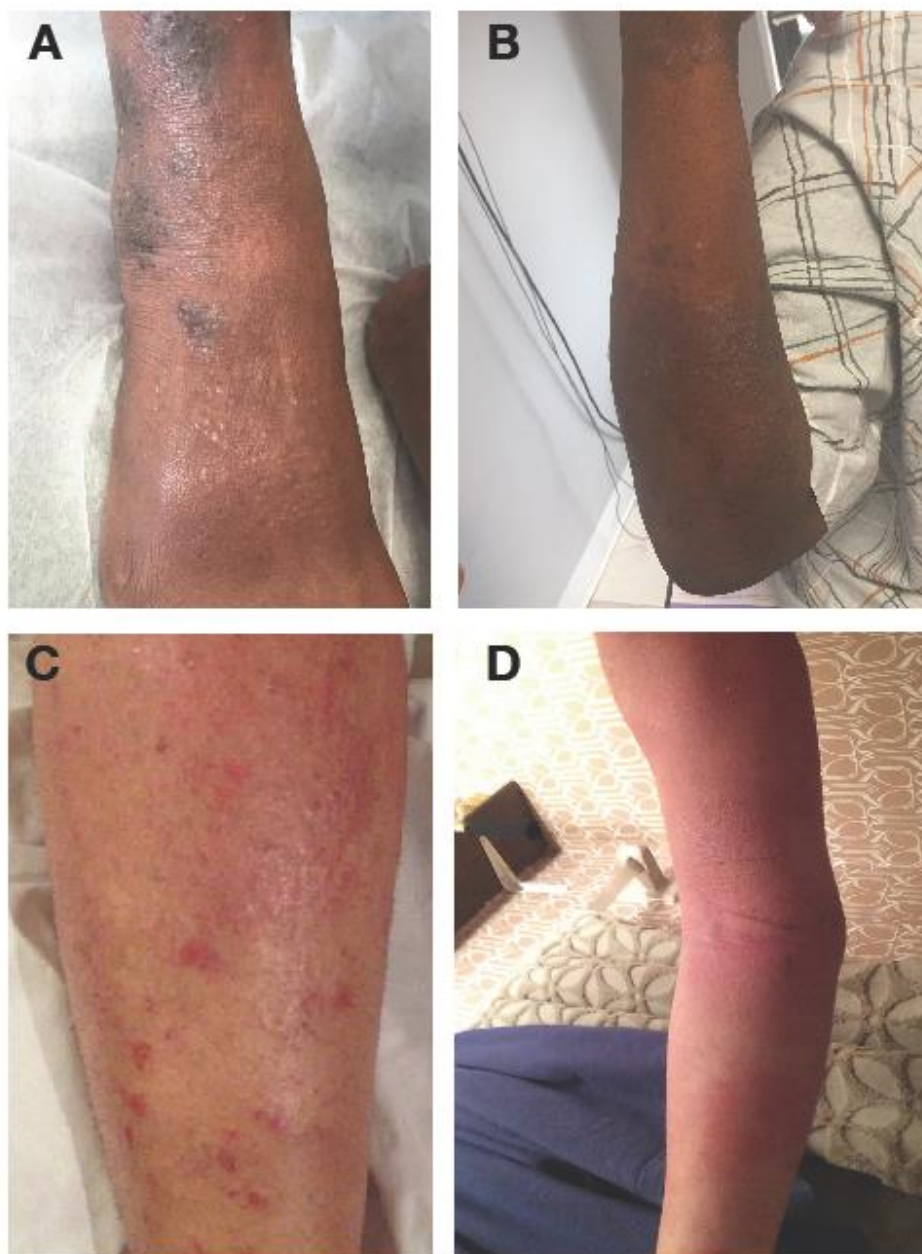
Figure Legend:

Figure 1. Representative clinical images of AA (A-B) and EA (C-D) patients with AD.

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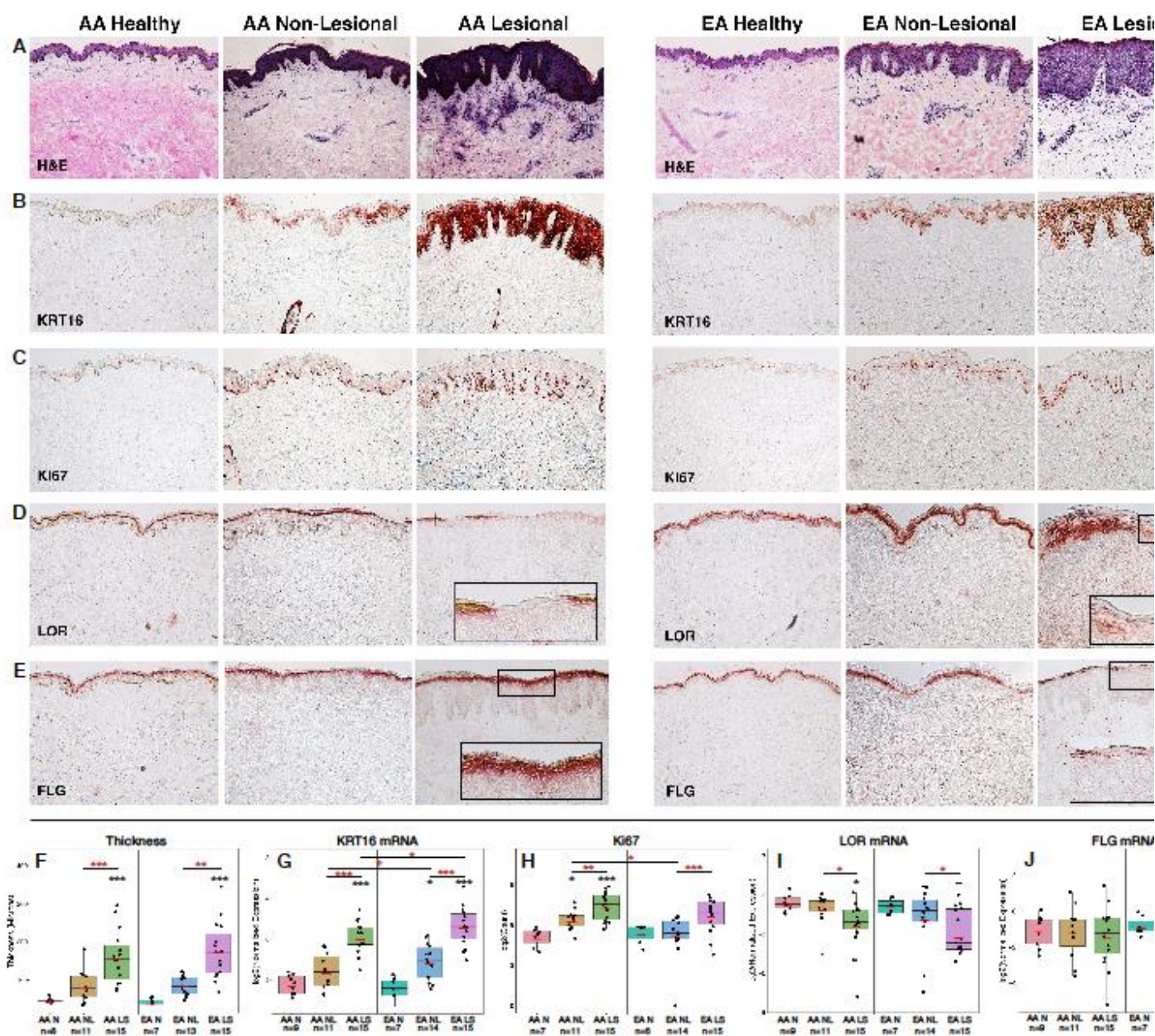


Figure 2. A-E, Representative staining in biopsies from AA and EA patients with AD and healthy controls. F-J, Quantification of epidermal thickness, Ki67+ keratinocytes, and mRNA expression of K16, LOR and FLG. Red bars represent means. Black stars denote comparisons to

control skin. *LS*, Lesional; *NL*, non-lesional; *N*, Normal. $+P<0.1$, $*P<0.05$, $**P<0.01$, $***P<0.001$.

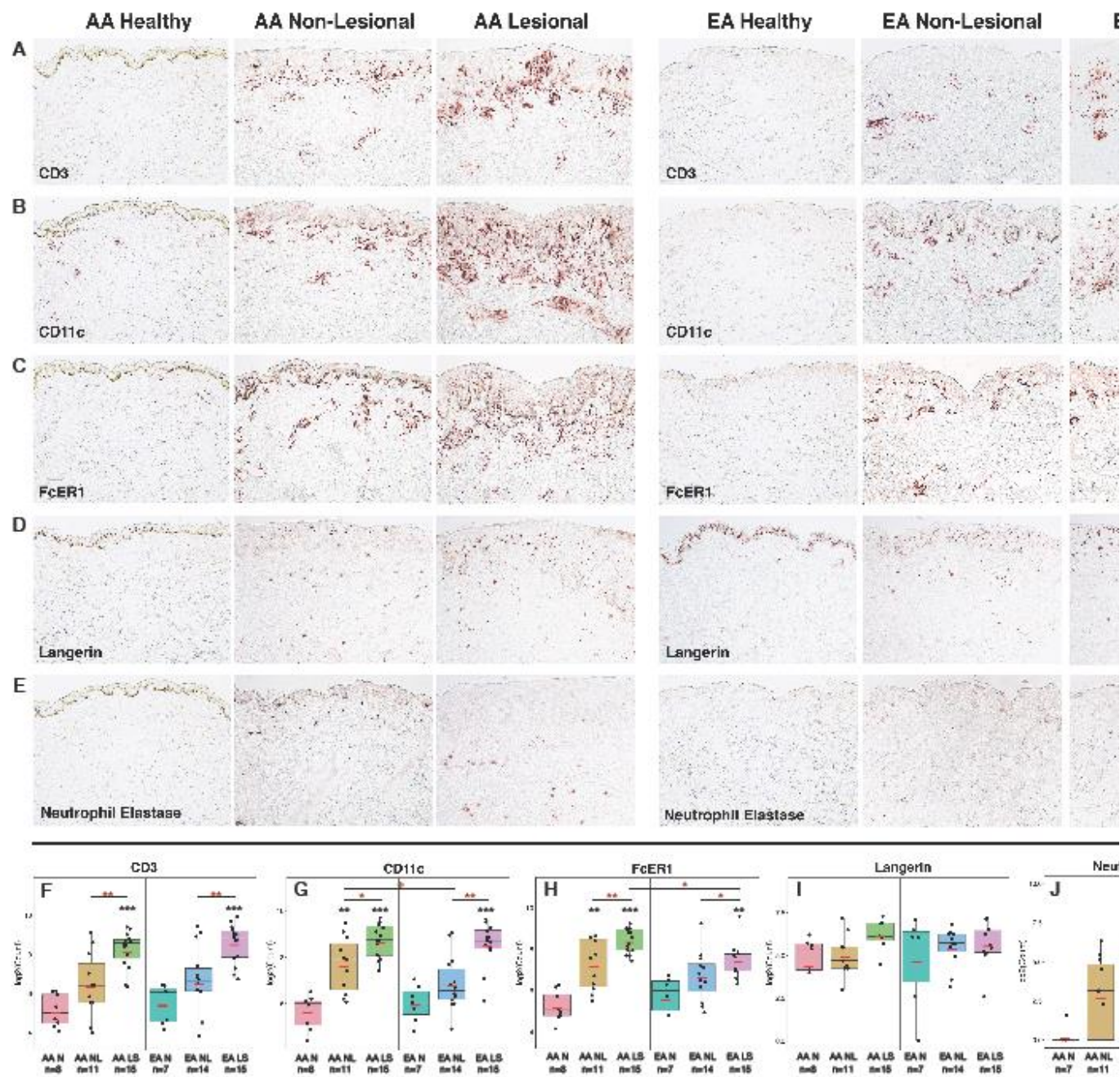


Figure 3. A-E, Representative immunohistochemistry staining in biopsies from AA and EA patients with AD and healthy controls. CD3⁺ T-cells, CD11c⁺ DCs, FcεR1⁺ DCs, Langerin⁺ langerhan's cells, and neutrophil elastase⁺ neutrophils staining and counts (F-J) are shown.

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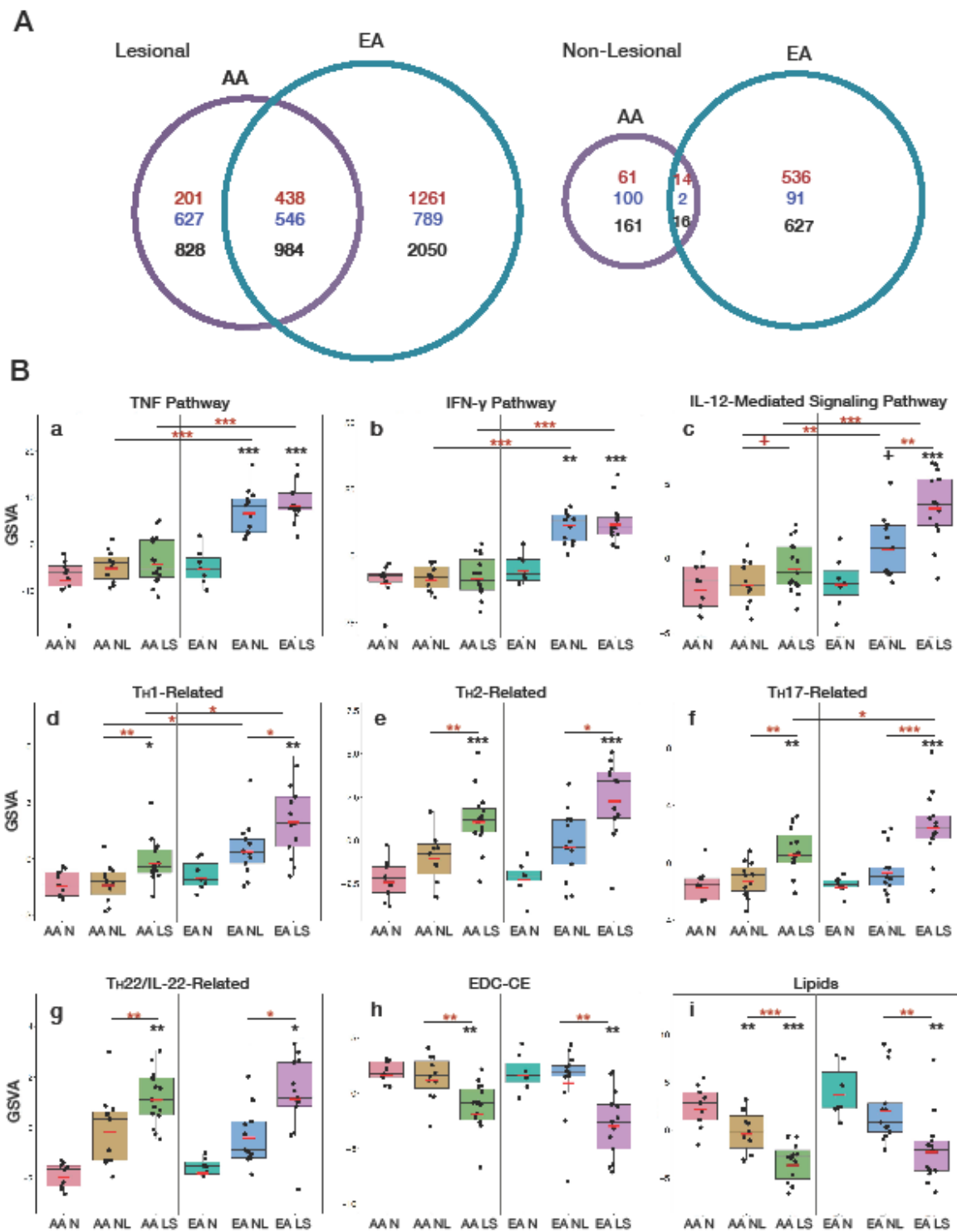
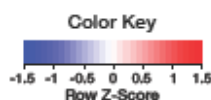


Figure 4. A, Venn diagrams of upregulated (*red*), downregulated (*blue*), and total (*black*) DEGs in lesional and non-lesional AA and EA AD versus respective healthy control skin. **B,** Gene set variation analyses (GSVA) for major immune pathways (**a-g**) and subsets of epidermal differentiation complex/cornified envelope (EDC-CE) genes (**h**) and lipid genes (**i**).



DEG	African American		European American		Fold-Change (FCH)							
					African American				European American			
	NL	LS	NL	LS	LS vs. N	NL vs. N	LS vs. NL		LS vs. N	NL vs. N	LS vs. NL	African American vs. European American
PI3					10.10**	2.56	3.98+		21.80***	2.12	10.36***	-2.17
IL22					3.97*	1.77	2.21		5.99**	1.70	3.52**	-1.51
IL12R2					1.89**	1.27	1.49+		2.28***	1.28	1.78***	-1.20
IL20					6.21**	2.96	2.10		11.00***	2.82	3.89**	-1.77
CCL1					7.99**	2.22	3.59*		12.00***	2.31	5.19***	-1.50
S100A9					48.60***	6.72	7.23*		159.00***	9.65*	16.56***	-3.26
CD83					2.37**	1.24	1.91*		2.35*	1.07	2.32***	-1.38
IL13					5.31**	2.21	2.41		12.30***	2.57	4.79***	-2.32
IL4R					2.28***	1.41	1.61**		3.28***	1.50+	2.19***	-1.44
CECR1					5.71**	3.98+	1.44		12.00***	2.74	4.37***	-2.10
UXCL10					4.36*	2.82	1.55		7.58*	2.38	3.19**	-1.74
CCL2					2.40*	1.48	1.62		3.45**	1.05	3.27***	-1.44
CCL7					1.87	1.33	1.49		2.35*	1.07	2.18*	-1.25
IL12B					1.99	1.30	1.53		2.81*	1.01	2.78*	-1.41
S100A2					39.20***	4.32	9.08**		59.00***	2.58	22.90***	-1.50
IL36A					12.00***	2.42	4.96**		17.00***	1.52	11.20***	-1.42
CD80					2.90*	1.19	2.44**		3.59**	-1.07	3.83***	-1.23
CCL17					14.90***	0.24	3.51*		17.40***	3.98	4.38***	-1.37
S100A12					16.00***	5.74	4.80*		24.40***	1.44	16.90***	-1.36
LCR2					2.93	1.26	2.33		2.88	-1.17	3.35*	-1.02
CTLA4					8.81***	2.34	3.77*		6.34**	2.25	2.81*	1.36
LCR					3.71***	1.83	2.02*		3.21**	1.76	1.82*	1.16
CCL19					2.72*	1.26	2.16+		2.26+	1.29	1.75*	1.21
CD18					10.10**	2.56	3.96*		6.81**	2.15	3.17*	1.49
ICG8					4.71**	1.61	2.92*		3.31*	1.49	2.22*	1.42
CCL22					9.71***	3.98+	2.87*		8.78***	1.08+	2.85**	1.11
OASL					9.46***	1.50	6.28***		7.70***	1.63	4.73***	1.23
CD28					2.85*	1.07	3.06**		2.30+	1.18	2.02*	1.20
CCL16					38.90***	8.23+	4.73**		45.20***	11.70**	3.86*	-1.16
CCR7					4.32***	1.22	3.53**		4.87***	1.80	2.71**	-1.13
IL32					2.44**	1.14	2.13*		2.71**	1.46	1.85*	-1.31
CECR4					2.63**	1.20	2.19*		2.88**	1.60	1.80*	-1.10
CD3E					2.64*	1.13	2.34*		3.10**	1.72	1.80+	-1.37
IL7R					2.41*	-1.13	2.73*		3.21**	1.43	2.25**	-1.33
CD3D					2.73*	-1.30	3.55**		2.89*	1.38	2.09*	-1.06
S100A7					53.50***	10.10*	5.28*		25.20***	2.46	10.20***	2.12
NRP12					49.10***	12.60*	3.81*		28.80***	3.66	7.87***	1.71
KCL2					1.37	1.07	1.78		1.07	-2.39	2.57*	1.27
CXCL1					10.60***	4.35	2.44		8.87***	-1.04	9.23***	1.20
CCR9					4.25*	2.51	1.69		3.60+	1.16	3.10*	1.18
CCL20					4.52*	2.72	1.66		3.45*	1.23	2.81*	1.31
CCL26					18.50***	7.07*	2.62+		12.70***	5.60**	2.26+	1.48
ITK					3.27*	1.62	2.01		2.54*	1.41	1.86*	1.15
CCR4					5.95**	1.60	3.72*		3.68*	1.40	3.43**	1.23
IL2RA					3.96**	1.68	2.37*		2.90*	1.18	2.47*	1.37
TNFRSF9					12.10***	2.95	4.09*		5.95**	1.64	3.63**	2.03
CCR1					2.71*	1.39	1.95*		2.01	1.11	1.81*	1.35
IL36G					4.88***	2.71+	1.80		4.98***	1.27	3.91***	-1.02
CD69					2.04	1.30	1.57		2.48	-1.79	4.43**	-1.23
IL36RN					2.32**	1.87	1.17		2.77*	1.05	2.64***	-1.44
CCL13					6.87***	2.10	3.27*		2.45	1.54	1.59	2.81
FASLG					3.47*	1.40	2.47		2.18	1.88	1.16	1.59
IL10					5.93**	3.00	1.98		2.05	1.01	2.02	2.90
TNLP					2.52*	1.89	1.33		-1.18	-2.00	1.69	2.96
IL15					2.44**	2.21+	1.18		1.45	1.21	1.45	1.58
CCL3					4.62**	2.47	1.88		20.10***	4.18*	4.81***	-4.45
CCR3					1.98	1.41	1.40		5.63**	1.97	2.86*	-2.84
IL6					1.68	1.14	1.47		10.20**	1.72	5.93***	-6.07
ILN					2.51	1.35	1.83		24.90***	3.20	7.79***	-9.92
IL19					1.81	1.15	1.58		6.91**	2.00	3.46**	-3.82
CANP					1.17	-1.43	1.67		4.83**	1.44	3.35**	-4.13
CXCL11					1.33	1.42	1.07		3.11*	1.05	3.11*	-3.95
CCL4					1.33	1.45	-1.09		5.45*	1.41	3.85**	-4.08
JAK3					2.88***	1.75	1.64		5.91***	2.20*	2.69***	-2.06
CCL11					1.76	-1.03	1.81		3.80*	1.31	2.91**	-2.15
TNFRSF4					1.82	1.08	1.68		4.35***	1.33	3.27***	-2.39
TGAI					2.26*	1.13	1.98*		5.97**	1.87	3.19***	-2.64
IRF1					1.77**	1.50	1.18		2.54***	1.59+	1.60**	-1.44
STAT1					1.39	1.17	1.19		2.09**	1.30	1.61**	-1.50
IL11					1.04	-1.21	1.25		2.36	-1.11	2.63*	-2.28
LAG3					2.35*	1.85	1.27		3.24**	1.87	1.74*	-1.38
IL18					2.16	1.12	1.93		5.09*	1.24	4.12**	-2.35
IL26					3.19*	1.21	2.65*		5.83**	2.58	2.26*	-1.83
ITGB2					1.51	-1.14	1.72*		2.18**	1.37	1.60*	-1.45
NR1					1.82*	1.00	1.82*		3.03***	1.66	1.82*	-1.66
CXCL2					2.25*	1.07	2.09		4.63**	1.77	2.62**	-2.06
CCR5					2.01*	-1.15	2.31*		3.88**	1.55	2.51***	-1.93
IL24					1.90	-1.13	2.14		6.91**	2.24	3.08*	-3.64
CCL8					1.40	-1.86	2.18		4.77*	1.52	3.13*	-3.41
CCR2					1.55	-1.08	1.68		2.74**	1.54	1.78*	-1.77
TGFB1					1.63**	1.12	1.46*		2.42***	1.75**	1.39*	-1.48
CCL23					3.07*	1.89	1.63		3.75*	2.92	1.28	-1.22
CD2					2.55*	-1.23	3.13**		4.83***	2.58*	1.88*	-1.90
CECR3					1.72	1.23	1.39		4.07***	2.32+	1.76*	-2.37
IL27					1.05	-1.26	1.33		1.83*	1.76	2.73**	-2.22
LAT					1.66*	1.15	1.45		3.19***	1.23**	1.43*	-1.92
CCR6					-1.10	-1.57	1.52		2.27*	1.72	1.32	-2.49
TBX21					-1.56	-2.59	1.66		3.32*	1.66	2.00+	-5.18
CCR12					1.84	1.75	1.05		4.58**	3.35*	1.37	-2.49
S100P					1.05	1.79	-1.70		3.10*	2.99+	1.04	-2.96
CCL27					1.66*	2.10*	-1.27		2.19**	1.65	1.33	-1.32
CCR2					1.12	1.24	-1.11		2.03*	-1.31	2.65***	-1.82
CCL16					-2.09	-1.32	-1.50		3.00*	-2.07	-1.45	-1.44
PDE8B					-1.39	-1.23	-1.11		-2.44***	-1.30	-1.87***	1.75
PDE11A					-2.11	-1.01	-2.08+		-3.65**	-1.24	-2.95**	1.73
PDE9A					-1.92*	-1.35	-1.42		-2.03*	1.01	-2.06***	1.06
IL17D					-2.40**	-1.74	-1.38		-2.10**	1.02	-2.15**	-1.34
IL34					-2.76***	-1.27	-2.02**		-2.08**	1.03	-2.08**	-1.37
ITF13					-2.63*	-1.67	-1.57		-2.58*	-1.69	-1.53	-1.02
IL37					-12.00***	-1.89	-6.33***		-9.12***	-1.94	-4.71***	-1.31

Figure 5. Heatmap of immune DEGs by RNA-seq in AA and EA AD, normalized to respective healthy control skin. *Red*, greater expression; *blue*, lower expression. *FCH*, fold-change; *FDR*, false discovery rate; +FDR-adjusted $P < 0.1$, *FDR-adjusted $P < 0.05$, **FDR-adjusted $P < 0.01$, ***FDR-adjusted $P < 0.001$.

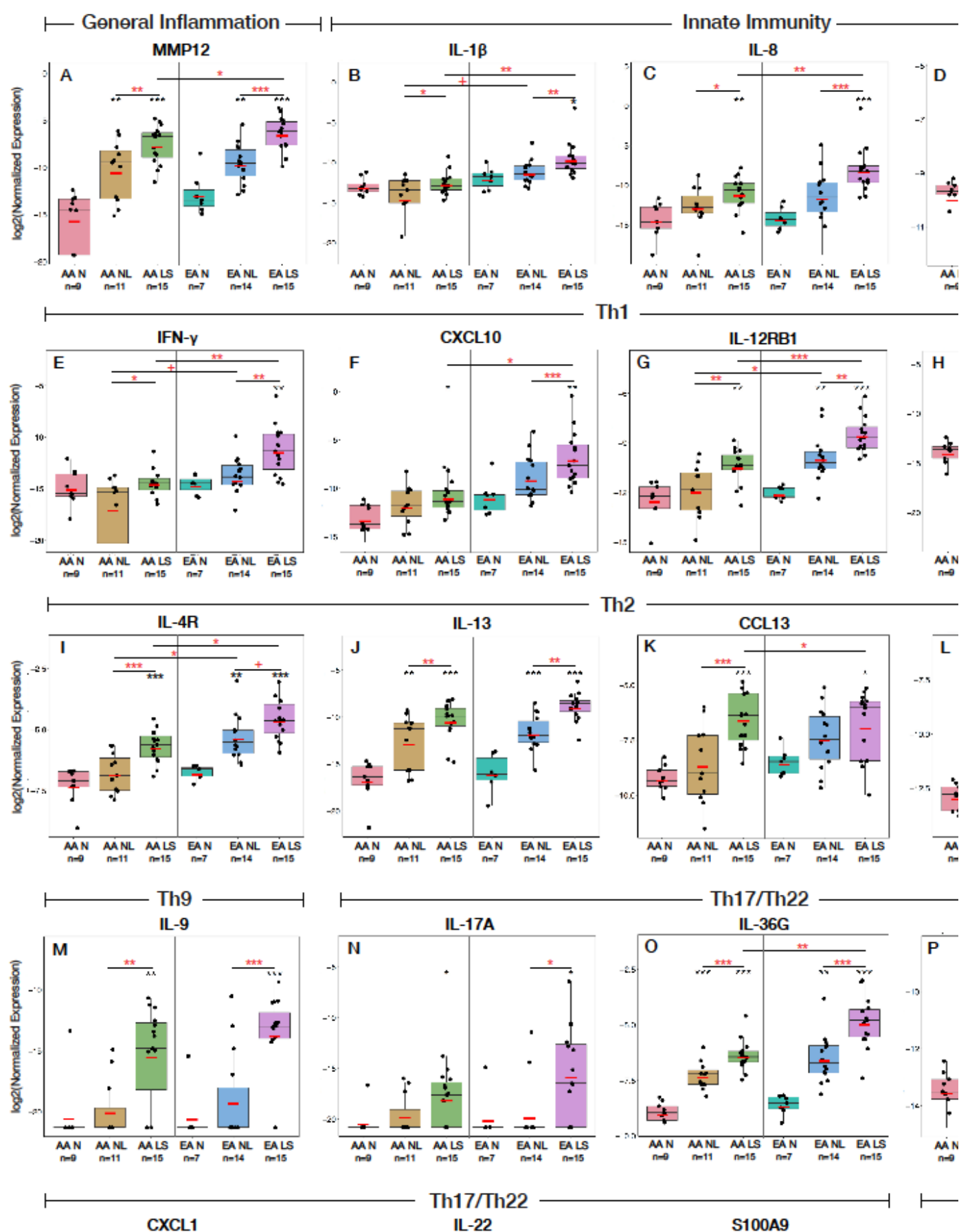
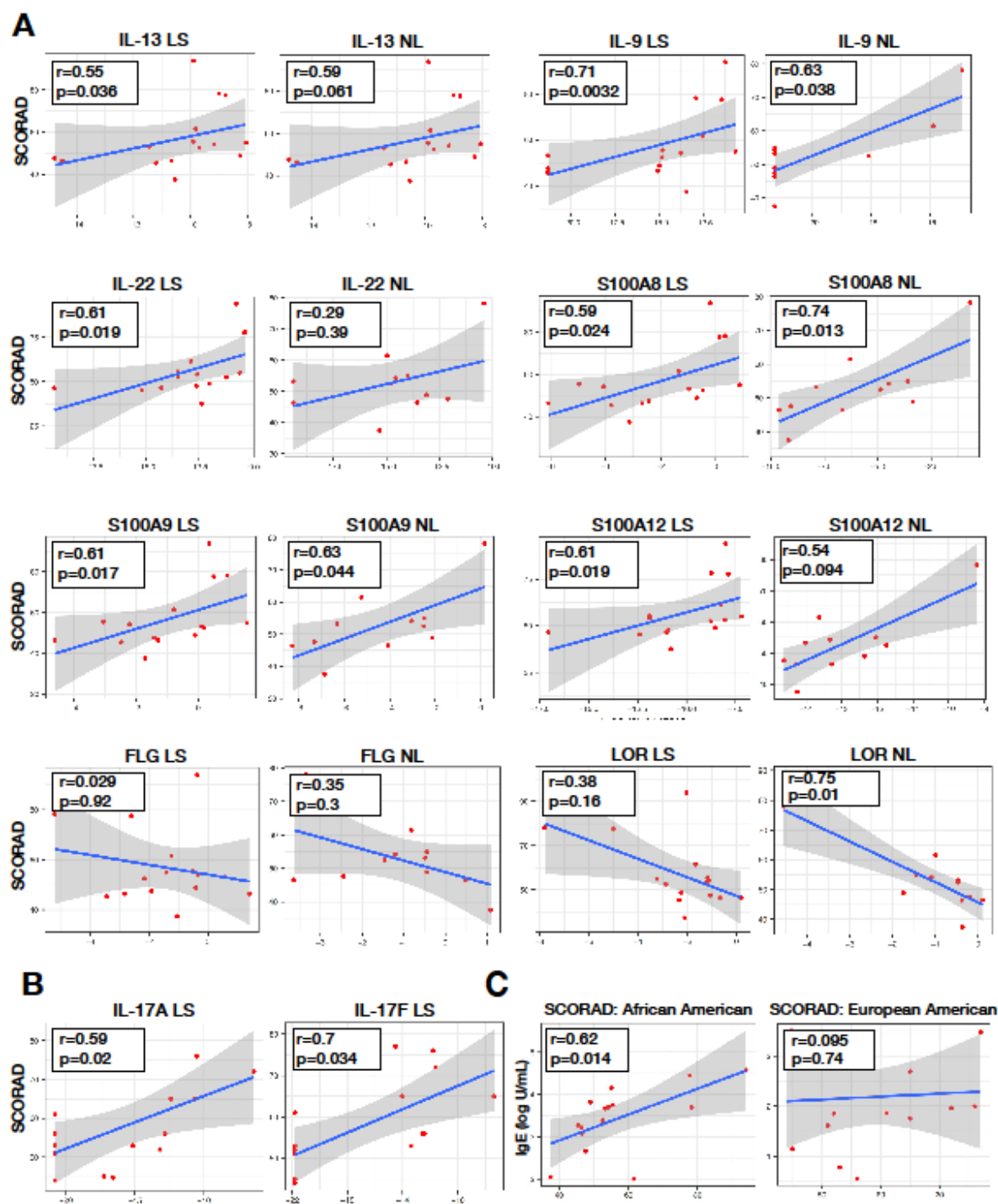


Figure 6. A-T, Quantitative real-time PCR comparisons of selected inflammatory and barrier markers are shown in boxplots. Red bars represent means. Black stars denote comparisons to healthy control skin. *LS*, Lesional; *NL*, non-lesional; *N*, Normal. $+P<0.1$, $*P<0.05$, $**P<0.01$, $***P<0.001$.



Characteristic	AA AD (n = 15)	EA AD (n = 15)	AA Healthy (n = 9)	EA Healthy (n = 7)	P value AA vs. EA AD	P value AA AD vs. Healthy	P value EA AD vs. Healthy
Age (y), mean (SE)	40.6 (3.5)	47.8 (3.6)	41.3 (4.3)	46.4 (4.0)	0.3499	0.9417	0.6924
Sex (male/female)	6/9	7/8	7/2	3/4	0.7125	0.1049	1.0000

Figure 7. A, Spearman correlation of AD clinical disease severity (SCORAD) with mRNA expression of T_H2, T_H9, and T_H22-related genes and barrier genes in AA lesional and non-lesional skin. **B,** Correlation of SCORAD with mRNA expression of T_H17-related genes in lesional skin of EA. **C,** Correlation of serum IgE with SCORAD in AA and EA AD. *LS*, Lesional; *NL*, non-lesional.

Table 1. Patient Characteristics

SCORAD index, mean (SE)	57.1 (3.9)	58.1 (2.8)	-	-	0.7047	-	-
IgE (kU/L) (SE)	7620 (2820)	769.1 (286.5)	-	-	0.0003	-	-
% eosinophils, mean (SE)	5.4 (0.9)	4.9 (0.9)	-	-	0.6095	-	-

SE=standard error, SCORAD= Scoring of AD (disease severity), AA=African American, EA=European American

Table 2. Key differences in expression of immune and barrier genes among AA and EA AD

	Upregulated Genes (Immune)	Downregulated Genes (Barrier)
Genes similarly expressed in AA and EA AD	T_H2: <i>IL-4R, IL-13, IL-7R, CCL17, CCL18, CCL26</i> T_H22: <i>IL-22, S100A7, S100A8, S100A9, S100A12</i>	<i>LOR, FLG2, FADS1, FADS2, CLDN16, CLDN23</i>
Genes showing increased or exclusive modulation in EA AD	Innate: <i>IL-1β, IL-8, TNF</i> T_H1: <i>IFN-γ, IL-12RB2, IL-27p28, CCL4, CXCL10, CXCL11, MX1, STAT1</i> T_H17: <i>IL-12B/p40, IL-36G, CXCL1, CXCL3, CAMP/LL37, LCN2, S100P</i>	<i>FLG, OVOL1</i>
Genes showing increased or exclusive modulation in AA AD	T_H2: <i>IL-10, CCL13, TSLP</i>	<i>FABP7, ELOVL3, CLDN8, CLDN3, CLDN10</i>