Microbe-host interplay in atopic dermatitis and psoriasis

Nanna Fyhrquist et al.

Despite recent advances in understanding microbial diversity in skin homeostasis, the relevance of microbial dysbiosis in inflammatory disease is poorly understood. Here we perform a comparative analysis of skin microbial communities coupled to global patterns of cutaneous gene expression in patients with atopic dermatitis or psoriasis. The skin microbiota is analysed by 16S amplicon or whole genome sequencing and the skin transcriptome by microarrays, followed by integration of the data layers. We find that atopic dermatitis and psoriasis can be classified by distinct microbes, which differ from healthy volunteers microbiome composition. Atopic dermatitis is dominated by a single microbe (Staphylococcus aureus), and associated with a disease relevant host transcriptomic signature enriched for skin barrier function, tryptophan metabolism and immune activation. In contrast, psoriasis is characterized by co-occurring communities of microbes with weak associations with disease related gene expression. Our work provides a basis for biomarker discovery and targeted therapies in skin dysbiosis.
Interactions between commensal or pathogenic microbes and the hosts they colonize are central to the maintenance of homeostasis and the initiation of disease. This rapidly advancing field is now starting to bear the fruits of interdisciplinary efforts but our understanding of microbe–host interactions is still limited.

Skin represents a primary tissue interface, where accessibility to both microbiome species and underlying tissue provides a unique opportunity for studies into host–microbiome interactions in the initiation and maintenance of atopic/allergic or autoimmune-type inflammation. Recent advances in analyzing microbial gene sequences in healthy skin has provided a comprehensive understanding of the classes of microbes and their diversity occupying distinct topographical niches. Pioneering studies have expanded into shotgun sequencing approaches to further probe the taxonomic diversity and biogeography of microbes in small cohorts of healthy individuals. Commensal skin microbe control adaptive skin immune homeostasis through interaction with specific subsets of antigen-presenting dendritic cells (DCs) and effector T-cell populations, supporting their own survival, and protecting against the overgrowth of pathogens. Insights into the association of distinct microbial classes with inflammatory skin disease and their impact on the host genome are just emerging. In atopic dermatitis (AD), as a model of atopic/allergic inflammatory disease, commensal skin microbes are associated with disease flares. AD-related dysbiosis is frequently characterized by the colonization by Staphylococcus aureus, and simultaneous loss of other, potentially beneficial species. S. aureus colonizes skin effectively, and expresses several virulence factors with proven roles in the pathogenesis of AD by studying effects in cell and animal model. S. aureus is associated with severity of the disease, and may be the result of a combination of detrimental effects from S. aureus on the one hand, and the loss of beneficial effects from other members of the skin microbiota on the other hand. Therefore, the use of antimicrobial therapeutics that target S. aureus may not be the optimal choice as they may also wipe out beneficial species or strains, and break mutualistic interactions between the skin and its microbiota. Psoriasis (PSO) appears to play a regulatory role, which is reduced in disease.

Microbiota-based classification of AD and PSO. We next asked whether the skin microbiome discriminates inflammatory skin pathologies. A supervised learning pipeline was employed to construct classifiers to explore the key sets of microbial taxa. We identified 26 microbes discriminating AD vs. HV cohorts with an area under the curve (AUC) of 0.94 (class errors HV = 0.03, AD = 0.27, Fig. 2a). The most discriminative taxa were of the genus Staphylococcus, including S. aureus (Z = 14.0), S. epidermidis (Z = 5.8), Staphylococcus spp. (Z = −6.9) and Burckholderia spp. (Z = −7.5). An NMDS analysis highlights the role of S. aureus in differentiating AD (red dots) from HV (blue dots; Supplementary Fig. 4a). We identified 24 microbes that differentiate PSO vs. HV with an AUC of 0.85 (class errors HV = 0.09, PSO = 0.33, Fig. 2b). The top discriminating microbes were C. simulans (Z = 15.5), Neisseriaceae g. spp. (Z = 6.9), C. kroppenstedtii (Z = 5.5), Lactobacillus spp. (Z = −8.4) and Lactobacillus iners (Z = −3.5). An NMDS analysis demonstrated a clear separation boundary between HV (green dots) and PSO (green dots; Supplementary Fig. 4b). We identified 15 microbes that differentiate between AD and PSO with AUC of 0.86 (class errors AD = 0.29, PSO = 0.13, Supplementary Fig. 4c). The top microbes were S. aureus (Z = 14.0), S. epidermidis (Z = −3.9), Finegoldia spp. (Z = −6.5), C. simulans (Z = −5.0), and C. kroppenstedtii (Z = −4.4). An NMDS analysis highlights the importance of S. aureus in AD (Supplementary Fig. 4d).

Results

The skin microbiotas in AD and PSO are highly distinct. Skin swabs were collected using a standardized protocol (Supplementary Fig. 1a–c). A total of 3.36 million 16S rRNA gene reads were analyzed using QIIME v1.8.0 resulting in the identification of 17,725 operational taxonomic units (OTUs) at 99.3% identity level. After removal of rare OTUs, 3,342 remained. Blasting sequences against the Greengenes 16S rRNA gene database revealed healthy skin microbiomes consistent with previous reports. An overall analysis indicated clear differences between AD and HV (Fig. 1a, b, Supplementary Fig. 2), confirmed by nonmetric multidimensional scaling (NMDS) analysis of the 95 most abundant OTUs (Supplementary Fig. 3a, Supplementary Table 1). AD also showed a reduction in diversity (Supplementary Fig. 3b). A total of 51 highly abundant OTUs showed significant differences between HV, AD and PSO (Fig. 1a).

We additionally carried out a careful analysis of the influence of confounding factors, including age, anatomical location, gender and clinical center (Supplementary Table 2, Supplementary Fig. 3c). After correction for the confounding effects, 8 out of 17 confounded OTUs from AD and 5 of 13 OTUs from PSO retained significance. For example, C. kroppenstedtii was associated with age, S. aureus with anatomical location and Lactobacillus sp. with gender, but they all remained significantly associated with disease after correction (p < 0.05). Associations were tested using the Kruskal–Wallis test for body site and institution, the Mann–Whitney U test for gender, and Spearman correlation for age. The remaining 11 most significant OTUs are shown in Fig. 1c. The most significant result is an increase in the abundance of S. aureus in AD, associated with a significantly lower abundance of OTUs representing strictly anaerobic bacteria in AD (Fig. 1d).

Communities of microbes associate with AD or PSO. To understand the interactions between communities of microbes under different disease states, we used network principles to express co-occurrence relationships. We found distinct
SparCC correlation between taxa resulted in 19 species with differences between the community structures of microbes associated with AD and PSO. For microbes associated with AD, SparCC\(^1\) correlation between taxa resulted in 19 species with a correlation above a threshold of 0.2 (\(p < 0.05\), Fig. 2c). \(S\). \(aureus\) negatively correlated with species including \(Corynebacterium\) spp., \(S\). \(epidermidis\), Tepidimonas spp. and \(Phylobacterium\) spp. Of the 24 microbes identified as important for PSO classification (Fig. 2b), 16 species showed significant SparCC correlation (SparCC > 0.2, \(p < 0.05\), Fig. 2d). The most discriminant taxa \(C\). \(kroppenstedtii\) and \(C\). \(kroppenstedtii\) displayed positive correlations...
**Fig. 1** Characterization of the skin microbiome in AD and PSO. The results show a typical range of skin microbiomes in HV (n = 115) and significant changes in AD (n = 82) and PSO (n = 119). **a** An evolutionary tree based on 16S rRNA gene sequences, abundance and statistical significance of the 95 most abundant OTUs. The blue color intensity in the heat map shows the relative abundance of each OTU. The three Staphylococcus OTUs (indicated by asterisks) were calculated using a wider scale, due to their relatively high abundance in certain samples. The length of the green vertical bars indicate nonparametric statistical score (Kruskal–Wallis test, FDR, p < 0.05). The color bars by each OTU number indicates bacterial phylum (green: Firmicutes; red: Proteobacteria; cyan: Bacteroidetes; blue: Cyanobacteria; orange: Actinobacteria). **b** The most abundant bacterial groups depicted for HV, AD and PSO. c Statistical analysis (Mann–Whitney U test (FDR, p < 0.05)) of the 11 OTUs showing the most significant changes in AD and/or PSO vs. HV after correction for confounding factors. The values on the x-axis are in number of reads, out of a total of 8495 reads/sample. The asterisks indicate statistically significant differences and correspond to p < 0.01 (**) and p < 0.001 (**). The center line in the boxplots corresponds to the median, the bounding box is the interquartile range (IQR) and the whiskers are defined as 1.5 times IQR. The color bars by each OTU number indicates bacterial phylum (green: Firmicutes; red: Proteobacteria; cyan: Bacteroidetes; blue: Cyanobacteria; orange: Actinobacteria). The source data files used to generate the present figure are available from the NCBI Sequence Read Archive under accession PRJNA554499.

**Fig. 2** Microbiota-based classification of AD and PSO. **a** Variable importance for the best set of discriminatory AD (n = 82) taxa identified through Random Forest feature selection and classification (RF) analysis. Bars are colored by selection frequency (Red = OTU selected in all folds, blue = OTU selected in one fold). **b** Variable importance for the best set of discriminatory PSO (n = 119) taxa identified by RF analysis. **c** Co-occurrence network of AD-associated OTUs selected by RF. **d** Correlation network of PSO-associated OTUs selected by RF. The source data files used to generate the present figure are available from the NCBI Sequence Read Archive under accession PRJNA554499.
with *Streptococcus* spp., *P. anaerobius* and, *Anaerococcus* spp., *Neisseriaceae* g. spp., and *Rothia dentocariosa* respectively. Comparison across microbial interactions in PSO indicate that rather than a single species dominating the microbial landscape (as in AD), we observe multiple species associated with this disease type.

**Overlaps and distinctions of AD and PSO transcriptomes.** The transcriptomes of full-thickness skin biopsies were defined based on a stringent $10^{-5}$ FDR level and a fold change (FCH) of 1.5, or greater. A principal component analysis (PCA) revealed a clear separation between HV, AD, and PSO (Fig. 3a). Differential gene expression analysis identified 1232 genes differing between AD
The AD and PSO skin transcriptomes. a Projection of AD (red, n = 82), PSO (orange, n = 119) and HV (green, n = 115) transcriptome profiles in the subspace spanned by the two first components of the principal component analysis (PCA) performed on the 1000 most variant genes. b Venn diagram of the differentially expressed genes in the AD vs. HV, PSO vs. HV and PSO vs. AD contrasts (identified using the Limma linear model and empirical Bayes method, cut-off: log2 FC $>$ 0.58 and FDR $p < 1 \times 10^{-5}$, corrected using the Benjamini–Hochberg method). c IPA canonic pathway analysis of significantly enriched functions in AD and PSO gene signatures and the PSO vs. AD contrast. d Statistical analysis (unpaired t test) of selected top up- and downregulated genes. The center line in the dot plots corresponds to the mean, and the error bars to the standard deviation. The source data files used to generate the present figure are available from EBI ArrayExpress under accession E-MTAB-8149.

and HV, and 2525 between PSO and HV, and 1051 genes shared between AD and PSO (Fig. 3b).

For functional insight into the disease-specific genes, we performed Ingenuity Pathway Analysis (IPA), revealing significant overrepresentation of $T_{H2}$ and $T_{H1}$ signaling, dendritic cell maturation and iCOS-iCOSL signaling in helper T cells in AD, and Interferon signaling, LPS-IL-1-mediated inhibition of RXR function, the Inflammosome pathway and Th17 signaling in PSO. The levels of Interferon signaling and p38 MAPK signaling distinguished PSO from AD (Fig. 3c).

$TNF$ and $IFNG$ were identified as upstream regulators in both AD and PSO, whereas $IL4$ and $IL13$ emerged as unique features in AD, underpinning the central role of $T_{H2}$-associated signaling in AD (Supplementary Fig. 5b). Gene Ontology (GO) enrichment analysis highlighted chemotaxis, inflammatory response, and extracellular matrix organization in AD, and leukocyte activation in both AD and PSO (Supplementary Fig. 6). Among top upregulated genes in AD and PSO we observed inflammatory mediators ($S100$ proteins, defensins, matrix metalloproteinases, IL-1 family cytokines), T helper-related genes ($CCL1$, $CCL18$, $IL17A$, $IL22$, PI3/Elafin), barrier genes ($KRT16$, $SERPINB4$, $KLK9$, $FLG2$, $LCE5A$, $CLDN8$), and genes involved in tryptophan (trp) metabolism ($KYNU$). Top downregulated genes included $IL34$, anti-inflammatory $IL37$, and $NOS2$ (Fig. 3d, Supplementary Fig. 7).

The $S. aureus$-induced host gene signature in AD. Taking advantage of our large microbiome and transcriptome datasets, we addressed the interplay between host and microbes. We stratified AD samples into “high” and “low” groups, based on microbial abundance, including top ($n = 27$) and bottom ($n = 25$) tertiles in the analysis. The low abundance samples were devoid of $S. aureus$, whereas high abundance samples exhibited in all cases a high abundance of $S. aureus$ (87–99%).

First, we explored the microbial gene repertoire of $S. aureus$ high and low groups. Using PICRUSt which predicts functional content based on the 16S rRNA gene marker, we observed the enrichment of bacterial toxins, the two-component system and glycolysis in the $S. aureus$ high group compared with the low group (Supplementary Fig. 8a–b). The majority of the functions were contributed by $S. aureus$ OTUs (Supplementary Fig. 8c). To validate OTU-based predictions, we performed whole genome sequencing (WGS) on a limited sample set, showing close agreement between the two independent sequencing methods (Supplementary Fig. 9). WGS data confirmed the enrichment of major bacterial toxins, hld and plc (alpha- and delta-toxin, respectively), and genes associated with galactose metabolism, the phosphotransferase system, the two-component system, and glycolysis and gluconeogenesis, in the $S. aureus$ high samples (Supplementary Fig. 8c).

Next, we investigated gene expression profiles in the underlying skin. Comparison of the transcriptomes between $S. aureus$ high and low samples revealed a set of 256 significant genes (FDR $< 0.05$, $FCH \geq 1.5$) (Supplementary Fig. 10a, Supplementary Table 3). To explore whether the $S. aureus$-regulated genes were relevant to global features of AD pathophysiology, we created a co-expression network based on pairwise Pearson correlation ($r > 0.7$), using all significant AD-associated genes. Network community detection identified ten distinct modules, enriched for disease-relevant functions (Fig. 4a, Supplementary Fig. 10c). Projecting $S. aureus$-regulated genes onto the AD network revealed significant enrichment in genes that mapped to modules M1 and M5 (hypergeometric test, FDR $< 0.05$), associated with keratinocyte differentiation, and extracellular matrix organization, respectively (Fig. 4b).

Functional analysis of the $S. aureus$-regulated genes using GO and IPA revealed the enrichment of keratinization and skin development (Fig. 4c), and $T_{H17}$ signaling and tryptophan (trp) degradation (Supplementary Fig. 11a), respectively. IPA predicted $IL1B$, $TNF$ and $IFNG$ as top upstream regulators (Fig. 4d) and leukocyte migration and development of epithelial tissue as downstream effects (Fig. 4e). While upregulated genes were enriched for inflammatory signaling (Supplementary Fig. 11b), downregulated genes were over-represented for mainly skin development (Supplementary Fig. 11c). Top genes included skin barrier and antimicrobial factors ($S100A7$, $DEFB4A/B$, $S100A9$, $MMP12$, $FLG2$, $CLDN8$, $ADAM12$), components of trp metabolism ($KYNU$, $TDO2$, $KMO$), immune activation ($IL1B$, $CCL2$, $CCL19$), and $T_{H12}$ signaling ($IL4R$, $IL5$, $IL13$, $PI3$, $TNFRSF4$, $CCR4$). Moreover, $HIF1A$ and its targets $HK2$ and $PFKP$ were among the significantly regulated genes (Fig. 4e, f, Supplementary Fig. 10b, d–e).

Since the kynurenine pathway was enriched in the $S. aureus$ “high” samples, we performed transcriptomic reconstruction of trp breakdown, indicating the accumulation of 3-hydroxyanthranilic acid (3-HAA) in the skin (Supplementary Fig. 12a). Further, we investigated to what extent AD-associated strains of $S. aureus$ may depend on trp, and isolated 32 $S. aureus$ strains from AD patients with moderate to severe manifestation of the disease. We found that 66% of the isolated strains grew independent of trp (Supplementary Fig. 12b, Supplementary Table 4), and this observation was further supported by our WGS data (73% trp biosynthesis-related genes in the $S. aureus$ high samples) (Supplementary Fig. 12c).

Finally, to explore the impact of $S. aureus$ on gene targets identified by this study, organotypic human epidermal equivalents were topically exposed to $10^6$ CFU $S. aureus$ for 24 h, followed by measurement of gene expression by qPCR (Supplementary Fig. 13a–c). $S. aureus$ significantly induced the expression of $DEFB4$, $PI3$, $IL4R$ and $S100A9$ (Supplementary Fig. 13d).

Integrative analysis of the PSO microbiome and transcriptome. Next, we combined the PSO microbiome with associated skin transcriptomes. Testing the top discriminating microbes in PSO in terms of their ability to partition the skin transcriptome did not yield significant results. Therefore, we constructed a co-expression network based on PSO differentially expressed genes, and partitioned it into 12 modules as described above (Supplementary Fig. 14a). We identified associations between the top 25 most differentially abundant taxa between PSO and HV, and module eigengenes derived from the PSO co-expression
network, using linear models implemented in the MaAsLin package. A linear model controlling for body site, institution, age and gender effects was fitted for each microbe—module eigen-gene pair. Overall, six associations were identified (FDR < 0.20) (Supplementary Fig. 14b). Negative associations were identified between Corynebacterium spp. and three co-expression modules; M2, M3 and M10. M3 was strongly associated with the cell cycle, whereas M2 and M10 were enriched for inflammatory pathways including interferon signaling, and TH1 and TH2 activation (Supplementary Fig. 14c). These results suggest that Corynebacterium spp. may play a regulatory role which is reduced in disease however. Overall, our results indicate that microbiome–host associations in PSO are considerably less well defined than observed in AD.

Microbiome associates with clinical severity in AD. To investigate the effect of microbiota and gene expression on clinical...
severity, we performed feature selection coupled with multivariate regression. Genes and microbes were ranked according to their correlation with SCORAD or PASI indices and then regression models were trained on the top $N$ features ($N = 5$–$50$ in increments of five), and the best performing model was selected. Prediction of SCORAD from microbial abundance was optimal with the top 35 species with Random Forest regression (Supplementary Fig. 15a). The accuracy was relatively modest (MAE = 12.35, correlation true vs. predicted = 0.55) suggesting that variability in microbial abundance and clinical severity are likely explained by other factors. Amongst the most highly correlated species were Tepidimonas spp., Propionibacterium acnes, and S. aureus (Supplementary Fig. 15b). Most of the selected species were negatively correlated with the exception of S. aureus, indicating that microbial diversity may be inversely associated with clinical severity in AD. Diversity correlation with SCORAD revealed a weak but significant correlation (cor = $-0.27$, $p = 0.01$).

Transcriptomic data outperformed microbial abundance for predicting SCORAD. The top model identified 15 genes using linear regression as the best set of predictive genes (Supplementary Fig. 15c), (MAE = 9.84, correlation true vs. predicted = 0.66). Amongst the top genes were SEMA3D, IGSF10, LGR5 and CASP10 (Supplementary Fig. 15d). No associations between clinical severity and microbial abundance in PSO were identified.

Finally, oozing and crusting in the AD lesions indicative of infection, correlated significantly with the abundance of S. aureus, supporting the link between colonization by S. aureus and clinical severity in AD (Supplementary Fig. 16).

Discussion
Microbe–host interactions may be central to skin homeostasis, and dysbiosis may drive disease$^{10,15}$. However, to what extent the skin microbiota may associate with the host skin phenotype and underlying mechanisms, remains elusive. To achieve a better understanding of the dialogue between the skin and the skin microbiome, we used skin as a model and AD and PSO as proxies for T$_{H2}$-associated atopic and T$_{H2}$-1-associated autoimmune inflammation, respectively, giving us the opportunity to compare two types of inflammation and the associated microbiomes and transcriptomes. Here we present the findings of a large cohort ($n = 316$) combining the analysis of skin microbiomes and associated transcriptomes, identifying unique gene profiles that characterize healthy vs. inflamed skin. We show that while AD is dominated by one single microbial species, multiple species associate with PSO, and the abundance of the dominating species in AD, S. aureus, correlates with disease relevant gene expression (overview of the main findings in Fig. 5).

Analysis of the two patient groups shows distinct differences between microbiomes. The most significant change, the increase in S. aureus abundance in AD, is not present in all samples. A number of AD lesions contain little or no S. aureus, representing potentially different endotypes of the disease which deserve further investigation. The changes associated with psoriasis are more complex than for AD and involve many different bacteria, including Corynebacteria and Finegoldia. Through exploring classification models as a way to discriminate disease state, several of the OTUs were also strong predictors of disease status. Lactobacillus appeared to be consistently depleted in both diseases, whereas disease-specific species such as S. aureus in AD and C. simulans and C. kroppenstedtii in PSO were important for prediction in disease. Moreover, we identified two distinct community patterns by means of co-occurrence analysis. In AD, it was clear that S. aureus dominated the microbial landscape and negatively correlated with several skin commensals, such as S. epidermidis and Corynebacterium spp, and thus may be associated with the depletion of potentially regulatory or protective microbes. Importantly, recent studies have shown that S. epidermidis may specifically limit the growth of S. aureus$^{22}$, and disease severity is inversely correlated with the abundance of S. epidermidis relative to S. aureus$^{14}$. In contrast to the dynamics revealed in AD, the PSO-associated interaction network suggested that multiple, co-occurring species, is a more representative model. Previous studies have shown that while single taxa are unable to discriminate between PSO and healthy skin, the combined relative abundances of selected genera (Corynebacterium, Streptococcus and Staphylococcus) attain significance across the groups$^{15}$. In our hands, the same genera display clear patterns of co-occurrence, increasing in relative abundance in PSO lesions compared with healthy skin.

We observed a remarkable loss of strictly anaerobic bacteria in AD, indicating a switch in the skin microbiome from anaerobic to aerobic metabolism. Healthy skin is normally O$_2$ deprived$^{23}$, but a dry flaky skin and an impaired epidermal barrier function—characteristics seen in AD$^{24}$, may increase oxygenation and select for the low abundance of strictly anaerobic bacteria such as Lactobacillus spp or Finegoldia spp. At anaerobic conditions bacteria are fermenting organic matter, e.g. in skin the amino acid serine originating from filagrin degradation, forming in particular lactic acid, propionic acid and other short chain fatty acids (SCFA). These metabolites lower the skin pH to pH < 5.5, maintaining a protective acidity of the skin. Potassium lactate is also one of the most relevant “natural moisturizing factors” of healthy skin. Furthermore, gram-positive anaerobe cocci such as Finegoldia, Anaerococcus, and Peptoniphilus, stimulate rapid induction of antimicrobial peptides response in human keratinocytes, which could be an important signaling mechanism to the keratinocytes when the skin is injured$^{25}$. In the complete or partial absence of these organisms, danger signaling in keratinocytes and other barrier functions could be impaired, potentially favoring colonization by S. aureus.

We identified AD and PSO transcriptomes which overlap substantially with previously published studies$^{26}$–$^{29}$. While
transcriptomic changes in PSO were dramatic, microbial changes were rather small. Yet, the opposite applied to AD lesions, suggesting a nonlinear relationship between the skin microbiota and transcriptome. Instead, the communication between host and microbiota might depend on defined subsets of genes, and the interaction is likely bi-directional, with the microbiota influencing host gene expression, which in turn forms a habitat for specific microbes.

Previous studies have identified colonization by *S. aureus* in AD; however, little is known about its potential mechanistic impact. We identified differentially regulated host genes between AD lesional skin samples containing high or low abundance of *S. aureus*, functionally enriched for mainly three activities: skin barrier function, immune activation and trp metabolism. The *S. aureus* high associated microbiome was overrepresented by bacterial toxins (alpha-toxin and delta-toxin), the two-component system and glucose metabolism, essentially contributed by *S. aureus* specific OTUs. The highly adaptable and potent pathogen *S. aureus* is known to express a multitude of virulence factors, including toxins that impact on the skin barrier and the immune system. The pore forming alpha-toxin, for instance, which was highly enriched in the *S. aureus* “high” samples, likely plays a key role in the disruption of the skin barrier in AD patients, and through being able to activate the samples, likely plays a key role in the disruption of the skin barrier in AD patients, and through being able to activate the skin.

The host responded to *S. aureus* through upregulating beta-defensins (e.g., DEFB4), and the expression of S100 protein family members (S100A8, S100A9, S100A7). Beta-defensins are potent antimicrobial peptides, contributing directly to local immune responses as chemoattractants for leukocytes, and through activating antigen-presenting cells (APCs). Psoriasis (S100A7) preferentially kills *Escherichia coli*, but has also bactericidal activity against *S. aureus*. Being upregulated by proinflammatory cytokines, psoriasin functions as a T cell and neutrophil chemotactic agent. S100A8 and binding partner S100A9 are, in turn, expressed and released by activated phagocytes, and have powerful antimicrobial activities through the sequestration of essential trace elements. The S100A8/A9 complex also protects the host from infection by triggering toll-like receptor 4 (TLR4) and receptor for advanced glycanation end-products (RAGE)-mediated inflammatory pathways, and through the recruitment of neutrophils. Thus, besides targeting microorganisms, the antimicrobial defense response promotes inflammation, possibly feeding additionally into AD pathology.

Further, samples abundantly colonized by *S. aureus*, displayed significant changes in the expression of barrier genes, such as CLDN8, a component of tight junctions (TJs). TJs are key to epithelial barriers, reside immediately under the stratum corneum, and regulate the passage of water, ions and solutes through the skin. TJ defects, including dysregulated CLDN8, are associated with AD. Moreover, the expression of metalloproteinases ADAM12 and MMP12, which are implicated in tissue remodeling, cytokine and growth factor shedding, cell migration and adhesion, was modified. Alpha-toxin is known to specifically interact ADAM10, leading to the cleavage of cadherins in epithelial cell tight junctions, and resulting in the destruction of cell-cell contacts, and consequently, the expression of ADAM10 was modified in *S. aureus* high samples.

Among proinflammatory factors that were influenced by *S. aureus*, we note dysregulation of IL1B, CCL2, CCL19 and members of the Th2 signaling pathway. IL1B is a central mediator of inflammation, affecting all cells of the innate immune system and playing a key role in the differentiation and function of adaptive lymphoid cells. The chemokine CCL2, which is expressed mainly in basal keratinocytes, acts through attracting monocytes, dendritic cells, Th1 and Th2 cells, and the chemokine CCL19 is involved in the colocalization of CCR7-positive dendritic cells with CCR7-positive T cells, facilitating antigen presentation and activation of T cells. Among Th2 associated genes, we observed the induction of chemokine receptor CCR4, cytokines IL5 and IL13, cytokine receptor IL4R, and TNFRSF4, all central mediators of allergic pathomechanisms. Finally, the AD-associated peptidase inhibitor 3 P13/elafin, a product of Th17 activation and implicated in Th2 differentiation, was significantly modulated in *S. aureus* high samples.

Colonization of the skin by *S. aureus* activates defense and tissue repair responses, which cause increased metabolic demands, requiring the tissue to switch to glycolysis. When densely present, *S. aureus* may generate localized hypoxia and
biofilms, triggering hypoxia inducible factor (HIF) transcription factors, which drive the metabolic changes and shape the immunological response. In the *S. aureus* high samples, we observed significant upregulation of HIF1A, and its target genes HK2, PPFK and IL1B. HK2 and PPFK are key regulatory enzymes in glucose metabolism. While glycolysis in the host is necessary to combat infection, a recent study shows that staphyloccocal glycolysis is equally necessary to cause infection. In line, we observed increased levels of glucose and galactose metabolism and glucose transport, in the *S. aureus* high associated microbiome, suggesting enhanced ability to compete with the skin for limited oxygen and glucose. Staphyloccoci also express hypoxia activated genes, some of them which are part of the two-component system, regulating metabolic activity in anaerobic conditions. Accordingly, the two-component system was significantly enriched in the *S. aureus* high samples, including genes which facilitate anaerobic respiration and biofilm formation.

The essential amino acid trp and its catabolites are emerging as important mediators of host–microbe interactions, with multiple effects on host physiology on the one hand, and consequences for the survival of the microorganisms on the other hand. Recent studies have shown attenuated trp metabolic pathways in the skin microbiota of AD patients, and protective functions of microbial trp catabolites in AD. In turn, the host may use trp “depletion”, or “starvation” as an antimicrobial strategy. We generated a transcriptomic reconstruction of trp breakdown in atopic inflammation (Supplementary Fig. 13a), and detailed analysis of the pathway indicated significant upregulation of TDO2, KMO and KYNU, while alternative endpoints of the pathway were not regulated. Hence, the result suggests accumulation of 3-hydroxyanthranilic acid (3-HAA), and the pathway being locked into the generation of this metabolite. Interestingly, 3-HAA is considered an inflammatory mediator, and has been shown to interact directly with neuronal cells. The depletion of key nutrients by the host is thought to be a mode of host defense during bacterial colonization, and certain microbes, including *staphylococci*, are sensitive to the depletion of trp by the host. While investigating whether such mechanisms are at play in AD during *S. aureus* colonization, we found that the majority of *S. aureus* strains in AD patients were independent of supplied trp.

Taken together, our findings in AD patients imply that *S. aureus* may actively modulate the skin barrier function, while triggering an inflammatory response, including Th2 type signaling, potentially interfering with the regulation of *S. aureus* colonization and perhaps contributing to the exacerbation of clinical symptoms. Moreover, dense colonization by *S. aureus* may cause local hypoxia, leading to metabolic reprogramming of the host and its microbiota, and amplification of the inflammatory response. Our findings also suggest that *S. aureus* promotes trp degradation and the accumulation of proinflammatory metabolites in the host, which may help the host to maintain a defensive immune barrier, but may also feed into AD pathology. Finally, AD patients may be preferentially colonized by *S. aureus* strains that are capable of trp biosynthesis, providing them with a colonization advantage in the atopic environment. However, the defense mechanism of limiting trp may also result in adverse effects, i.e, repressing colonization by benign commensal bacteria, creating more dysbiosis.

Unlike in AD, where one species, *S. aureus*, was identified as the dominant microbe, we observed that multiple species demonstrated increased abundance in PSO. To establish a relationship between host transcriptomic profiles and microbial abundances, we screened PSO-associated microbes against modules of co-expressed genes, but detected only weak associations between potential pathogens and the expression of host transcripts. Several modules were found to be associated with microbial abundance, four of which were associated with one particular taxon corresponding to *Corynebacterium* spp. This species was negatively associated with a module enriched for “interferon signaling”, suggesting a potential protective, or regulatory role of pathways relating to psoriatic inflammation. This is of particular importance, since interferon signaling represents a key event during the initiation of psoriatic inflammation. However, since the identified correlations with bacteria were weak, further studies are warranted for unraveling the potential role of fungi or viruses in psoriatic inflammation.

Finally, we applied regression algorithms to highlight potential links between transcriptomic and microbial biomarkers with disease severity. In AD a set of 35 microbes was identified and linked to clinical severity. Of these species, all, except *S. aureus*, were negatively correlated with disease severity. The association between the abundance of *S. aureus* and severity has been observed in previous studies, and specific strains of *S. aureus* contribute to a varying degree to severity, adding to the complexity of AD disease. We lack *S. aureus* strain information in this study, but identify genes which appear to be driven by *S. aureus*. More than half of these genes are relevant to AD pathophysiology, overlapping with the AD transcriptome, but only a handful of these genes are associated with disease severity, suggesting that the *S. aureus* signature is driven mainly by *S. aureus*, not by severity. Lastly, we conclude that the host transcriptome predicted patient SCORAD to greater accuracy than the microbiome, suggesting that gene expression is a better predictor of clinical severity.

This study represents a large cohort with a rich dataset giving an opportunity for further detailed analysis of specific microbe–host interaction. For additional insight, full genetic diversity of the microorganisms need to be explored, adding information concerning strains and the functional potential, as well as information related to yet unexplored microorganisms including fungi, viruses and archaea. Principles of microbe–host interactions will need to be added to the concept of homeostasis and disease, and only an integrated approach will be able to follow the complex ecology of human health, paving the way for medical interventions that aim at preserving health-associated homeostasis between humans and their microbiota.

**Methods**

subcutaneous recruitment and sampling. Adult patients (18–83 years) with moderate-to-severe chronic AD (SCORAD score > 25, n = 91) and plaque-type PSO (PASI score > 7, n = 134) as well as healthy volunteers (n = 126) were recruited for the study (Supplementary Fig. 1). Microbiome samples and skin biopsies were obtained from areas with active disease in the upper back or posterior thigh in AD patients, and from the upper and lower back in PSO patients. Healthy volunteers were sampled in corresponding skin areas.

Each subject underwent a physical examination by a dermatologist and the medical history was recorded. The diagnoses were made by a dermatologist based on clinical presentation, personal history, laboratory findings and the criteria of Hanifin and Rajka. The exclusion criteria included concomitant autoimmune diseases (rheumatoid arthritis, diabetes, alopecia areata, etc.), the use of systemic antibiotics within 2 weeks and systemic immunosuppressive therapy or phototherapy or systemic biologic agents within the previous 12 weeks prior to screening. Before skin sampling, the biopsy sites were left untreated for at least 2 weeks and cleansing with only the non-antibacterial Dove soap was allowed and washing was avoided for 24 h prior to sampling. The patients or healthy volunteers who did not match these clinical exclusion criteria were removed from the study. The following biological samples were then obtained and submitted to analysis: (1) microbiome samples from upper/lower back, posterior thigh or buttocks (PSO, AD, HV) with no prior cleaning or preparation of the skin surface using sterile gloves to prevent cross-contamination were obtained placing a sterile ring (2.5 cm diameter) onto the appropriate skin area, 1.5 ml phosphate-buffered saline (PBS) was supplemented into the ring and the area sampled scraping a glass rod in a circular motion ten times to the left and to the right. Subsequently, the microbiome-enriched PBS was harvested and stored. In addition, mock samples containing only PBS were collected at each sampling time in order to assess contamination. (2) 6 mm punch biopsies from skin at the “microbiome” sites were taken in local anesthesia. Subsequently, samples were stored in RNAlater (Sigma-Aldrich) and

**Subject recruitment and sampling.** Adult patients (18–83 years) with moderate-to-severe chronic AD (SCORAD score > 25, n = 91) and plaque-type PSO (PASI score > 7, n = 134) as well as healthy volunteers (n = 126) were recruited for the study (Supplementary Fig. 1). Microbiome samples and skin biopsies were obtained from areas with active disease in the upper back or posterior thigh in AD patients, and from the upper and lower back in PSO patients. Healthy volunteers were sampled in corresponding skin areas.

Each subject underwent a physical examination by a dermatologist and the medical history was recorded. The diagnoses were made by a dermatologist based on clinical presentation, personal history, laboratory findings and the criteria of Hanifin and Rajka. The exclusion criteria included concomitant autoimmune diseases (rheumatoid arthritis, diabetes, alopecia areata, etc.), the use of systemic antibiotics within 2 weeks and systemic immunosuppressive therapy or phototherapy or systemic biologic agents within the previous 12 weeks prior to screening. Before skin sampling, the biopsy sites were left untreated for at least 2 weeks and cleansing with only the non-antibacterial Dove soap was allowed and washing was avoided for 24 h prior to sampling. The patients or healthy volunteers who did not match these clinical exclusion criteria were removed from the study. The following biological samples were then obtained and submitted to analysis: (1) microbiome samples from upper/lower back, posterior thigh or buttocks (PSO, AD, HV) with no prior cleaning or preparation of the skin surface using sterile gloves to prevent cross-contamination were obtained placing a sterile ring (2.5 cm diameter) onto the appropriate skin area, 1.5 ml phosphate-buffered saline (PBS) was supplemented into the ring and the area sampled scraping a glass rod in a circular motion ten times to the left and to the right. Subsequently, the microbiome-enriched PBS was harvested and stored. In addition, mock samples containing only PBS were collected at each sampling time in order to assess contamination. (2) 6 mm punch biopsies from skin at the “microbiome” sites were taken in local anesthesia. Subsequently, samples were stored in RNAlater (Sigma-Aldrich) and...
subjected to further analyses (Supplementary Fig. S1a). The study was approved by the appropriate local Institutional Review Boards (University of Helsinki, Dnr 911/13/03/2011; Heinrich Heine University Düsseldorf, 3647/2011; King’s College London, 11/H0802/6) and all subjects provided written informed consent before participation.

Upper back and thigh posterior were chosen to represent skin areas with AD lesions, while upper back and lower back were chosen for PSO lesions, at the same time to avoid avoiding harm due to the surgical procedure of obtaining skin biopsies (Supplementary Fig. 1a). Only samples from active skin disease, i.e., lesional skin, were included in the study, and normal skin from HV was used as a control. In HV, samples were taken from sites corresponding to the sampling sites in AD and PSO. The QIAPamp UCP Pathogen Mini Kit (Cat.No: 19092) according to the manufacturer’s instructions. In brief, brief pellets were resuspended in 500 μl Buffer ATL and vortexed for 10 min at maximum speed using Pathogen Lysis Tubes containing glass beads. The samples were transferred to fresh Beckman tubes and incubated in 15.5 mg/ml lysozyme (Sigma) for 30 min at 37 °C. Fifty milliters microtubes protease K was added and the samples were then incubated for 10 min at 56 °C. Addition of 250 μl of Buffer APL2 was followed by incubation at 70 °C for 10 min. Ten milliters RNA-grade glycogen (20 mg/ml, Thermo Scientific) were added to maximize DNA recovery. Ethanol was added to a final concentration of 25%. DNA was extracted and washed using spin columns, and subsequently eluted in 50 μl of Buffer AVE.

For 16S rRNA amplification and preparation for sequencing, 2.5 μl template were amplified in RT–PCR gradeWater (Life technologies), 3% DMSO, with 1× PCR HF buffer using Phusion Hot start II DNA polymerase, 200 μM dNTPs (all Thermo Fisher Scien), and 500 nM custom primers (Eurofins MWG Operon). One universal forward primer (341F 5′- CACTACGGGNGGCWGCAG with adaptor B, Lib-L) was paired with one of 104 barcoded reverse primers (805r 5′-GACTACHVGGGTATCTAATCC with adaptor A, Lib-L) (Supplementary Table 5). Each barcode consisted of seven nucleotides, contained no homopolymers, and a pair of barcodes differed in at least two positions. Each PCR was run in triplicates and the PCR products from each sample were pooled. A negative control PCR reaction lacking template was included for all primer pairs in each run. The PCR was run for 30 cycles. The PCR products were purified from the reaction using Dynabeads MyOne Carboxylic Acid (Life Technologies, Cat.No: 35401) and TruSeq precipitation buffer (16% PEG-6000, 1.5 M NaCl) according to the manufacturer’s instructions. DNA concentrations were measured by real-time PCR (Bio-Rad CFX96 Touch 35401) and TruSeq precipitation buffer (16% PEG-6000, 1.5 M NaCl) on the manufacturer. The DNA samples were run in triplicates and the PCR products from each sample were pooled. A negative control PCR reaction lacking template was included for all primer pairs in each run. The PCR was run for 30 cycles. The PCR products were purified from the reaction using Dynabeads MyOne Carboxylic Acid (Life Technologies, Cat.No: 35401) and TruSeq precipitation buffer (16% PEG-6000, 1.5 M NaCl) on the manufacturer.

For 454 amplicon sequencing, emulsionPCR was performed on the amplicon library using a large volume emPCR (Lib-L, v2 reagent kit) according to the manufacturer’s amplicon protocols and pyrosequenced (one way read direction) on a Genome Sequencer FLX–Titanium instrument (Roche/454 Life Sciences) at Science For Life Laboratory (SciLifeLab), Stockholm. Each library was sequenced in both regions of a two region gasketed 70 × 75 mm Titanium PicoTiterPlate, and filter settings. Samples containing only water were sequenced in order to assess contamination step to avoid amplification of other OTUs as possible and to discover close to all relevant bacteria in the skin. The noninvasive sampling protocol was designed to specifically sample bacteria on the surface of the skin as the total amount of bacteria in the deeper skin layers is expected to be low. 16S rRNA gene PCR was carried out directly from the DNA preparation, without another amplification step. For 16S rRNA gene sequencing negative controls were carried out according to standard protocols and relevant controls and low-quality data was removed using AmpliconNoise, which is the gold standard in the field. The same, easily reproduced sampling protocol was used in all centers and the DNA preparation, PCRs, sequencing and analysis were carried out in one facility using standard methods.

Three samples of poor quality were removed from the OTU table (1 PSO and 2 HV, Supplementary Fig. 1b). Abundances were normalized using the Trimmed Mean of M-values method (TMM), implemented in the edger Bioconductor package. Good’s coverage and Shannon diversity index was calculated using QIMERE v1.8.0.

To preserve statistical power, only OTUs present in more than 25% of all samples were analyzed. Differentially abundant OTUs were identified from the filtered set by comparing the abundance distribution of each OTU across the three clinical groups (HV vs. PSO vs. AD) with the Kruskal–Wallis test (FDR, p < 0.05). Microbiome–disease-specific associations were detected testing for differences in the abundance distribution of each differentially abundant OTU between the diseased (PSO and AD) and HV groups with the Mann–Whitney U test (FDR, p < 0.05).

For analysis of confounding variables, associations between OTU abundance and nonclinical factors (age, gender, body site and institution) were tested for the 16S rRNA gene dataset using: (1) Mann–Whitney U–Wallis test for body site and institution; (2) Mann–Whitney U test for gender and (3) Spearman correlation for age. FDR correction was used to correct for multiple testing. Additionally, the same association tests were carried out within the PSO and AD groups to search for disease-factor interactions. Then, a linear model was fitted for each OTU to account for the discovered nonclinical factors associations in the microbiome–disease association test. The general linear model equation is: OTU_abundance = clinical_group + nonclinical_factors and it was fitted using the lm function of R. p values from the test for the disease were FDR-corrected for multiple hypothesis testing.

Bacterial aerobes vs. strict anaerobes were determined according to Berges’ Manual of Systematic Bacteriology. Anaerobe abundance comparison between AD and HV was performed using a Mann–Whitney U test. Additionally, to account for the systematic increase of S. aureus in AD, the same test was repeated only with samples in which the aforementioned bacterium was not detected.

For visualization of results, the phylogenetic tree in Fig. 1a was calculated using ClustalW2 and it was visualized, along with associated data using interactive Tree of Life, ITEO and Adobe Illustrator. NMDs analysis was performed using phyloseq. Boxplots were generated in R version 3.1.0 with the ggplot2 package version 1.0.1, with whiskers extending to 1.5 IQR. Outliers were removed.
between S. aureus high and S. aureus low groups using Student’s t-test followed by Benjamini–Hochberg multiple testing correction. Visualization was carried out using STAMP software\(^60\) and R v.3.5.1.

**Microarray of genomic RNA transcriptional profiles.** The skin biopsy samples were stored in RNA later and total RNA was extracted from the tissue samples using the RNeasy Fibrous Tissue Mini kit (Qiagen). Tissue samples were homogenized using the FastPrep-24 instrument (Nordic Biolabs AB), and RNA was extracted according to the manufacturer’s instructions. The yield and purity of RNA in the samples were confirmed using a Nanodrop spectrophotometer and a fluorometer to verify absence of inhibitors (R260/280: 2.1; R260/230 nm: 1.3). RNA integrity was quantified by electrophoresis and performed using Agilent dedicated Lab-on-chip (RNA6000 Nano and Pico kits). RNA Integrity numbers and 28S/18S ratio averages were respectively 8.6 and 2.0.

One hundred nanograms of total RNA was amplified according to Affymetrix protocols (Affymetrix® GeneChip® Whole Transcript (WT) Expression Arrays). Based on expertise of Institut Curie genomic platform, MAQC A RNA samples (Universal RNA, Stratagene, P/N: 740000) were implemented to series of RNA samples were controlled using a Nanodrop spectrophotometer and a fluorometer to verify absence of inhibitors (R260/280: 2.1; R260/230 nm: 1.3). RNA Integrity numbers and 28S/18S ratio averages were respectively 8.6 and 2.0.

One hundred nanograms of total RNA was amplified according to Affymetrix protocols (Affymetrix® GeneChip® Whole Transcript (WT) Expression Arrays). Based on expertise of Institut Curie genomic platform, MAQC A RNA samples (Universal RNA, Stratagene, P/N: 740000) were implemented to series of RNA samples were controlled using a Nanodrop spectrophotometer and a fluorometer to verify absence of inhibitors (R260/280: 2.1; R260/230 nm: 1.3). RNA Integrity numbers and 28S/18S ratio averages were respectively 8.6 and 2.0.

**Dimensionality reduction of transcriptomics data.** Transcriptomes were de-duplicated using STAMP software\(^60\) and R v.3.5.1.

Rheinwald–Green protocol\(^14\) and stored in liquid nitrogen. For the generation of HEES, primary human keratinocytes were seeded onto 3.0 µm pore size transwell filters (Thincerts, Greiner Bio-One). Prior to seed, these transwells were incubated with 100 µg/ml rat-tail collagen (BD Biosciences) diluted in sterile cold PBS at 4 °C for 1 h. Afterwards, excessive coating was carefully aspirated and filters were washed once with cold, sterile PBS. First, the primary keratinocytes were cultured in a submerged manner in proliferation medium (CoT-prime, CellLincTec) for 2 days to form a homogenously distributed monolayer. One day later, medium was changed to differentiation medium (60% 3D barrier, CellLincTec complemented with 40% Dulbecco’s modified Eagle medium, Sigma). On the third day the transwell inserts were lifted to the air–liquid interface to induce differentiation and stratification of the primary keratinocytes. HEES were cultured in differentiation medium until the end of the culture and they were refreshed with differentiation medium every other day.

On day 7 of air–liquid interface culture 10⁵ CFU S. aureus in PBS (cultured as described above) was applied to the HEES. For control HEES only PBS was applied. After 24 h HEES were harvested for qPCR analysis.

RNA isolation, cDNA synthesis and qPCR analysis was performed as described earlier\(^81\). All primers were designed and used as described previously\(^84\) (Supplementary Table 5). Target gene expression was normalized to the expression of the house-keeping gene human acidic ribosomal phosphoprotein P0 (RPLP0). The ΔAC method was used to calculate relative mRNA expression levels\(^86\).

For morphological analysis, HEES were fixed in 4% formalin solution for 4 h and embedded in paraffin. 6 µm-slicemicroscopy sections were stained with hematoxylin and eosin (H&E, Sigma–Aldrich) or DAPI stain solution (DAPI Fluoromount-G, Southern Biotech) as described previously\(^66\).

**Tryptophan dependence assay.** The growth of S. aureus strains isolated from moderate-to-severe atopic dermatitis patients was analyzed in tryptophan-containing vs. tryptophan-depleted culture media. The tryptophan-depleted, conditioned medium was derived from IFN-γ-activated, IPO-positve human glioblastoma cells 86HG39 that efficiently inhibit the growth of tryptophan auxotrophic S. aureus strains\(^16\), 86HG9 cells were cultured in Iscove’s modified Dulbecco’s medium (IMDM) (Gibco, Grand Island, USA), supplemented with 5% fetal bovine serum and heat-inactivated calf serum (FCS) in culture flasks (Costar, Cambridge, USA) in a humidified incubator (37 °C, 10% CO₂). 1 × 10⁶ cells per cell flask were cultured with 1000U/ml IFN-γ (R&D Systems, Minneapolis, USA) for 72 h and the conditioned medium was harvested. As controls, medium from unstimulated 86HG9 cells (tryptophan-free RPMI 1640 Medium (Gibco, Grand Island, USA) (with or without tryptophan supplementation) were used. For the tryptophan dependence assay, a 24 h old colony was picked, resuspended in PBS (Gibco, Grand Island, USA) and serial diluted. The conditioned medium as well as control media (200 µl) were inoculated with 10 µl of the bacterial dilution containing 10–100 colony forming units (cfu). Colony forming units (cfu) counting was monitored by measuring the optical density of resuspended cultures at 620 nm. Tryptophan dependency was validated by growth restoration in tryptophan-containing cultures.

**Classification and feature selection.** In order to identify the best set of representative microbiota for disease class, we used a supervised learning approach with Random Forest classification models. The data were divided into training and testing models for three contrasts (Healthy vs. AD, Healthy vs. PSO and PSO vs. AD) with the aid of the classification methods that best discriminate disease groups. To identify the most stable predictors of disease, we first removed under-represented species by filtering out those that were present in less than 15 samples for each contrast. Next, to select the most discriminant OTU features, Random Forest feature selection implemented in the R package Boruta was used under a tenfold cross-validation framework with ten randomized repeats. Selected features were ranked by Boruta according to the variable importance score Z, which represents the average loss in accuracy after permutation of attribute values across samples. OTUs with a mean Z score greater than 0.2 were considered for further analysis. The fold change between healthy and disease patients was calculated to identify depletion or increased abundance in disease. After selection of features, the classification model was built within the R package randomForest. We evaluated the performance of the model using the R package ROCPR and report the mean AUC across all folds\(^88\). NMDs with Bray–Curtis distance using the selected feature sets with the function metaMDS from the R package Vegan was used for visualization purposes only. Selection frequency was calculated as the percentage of times a variable was selected by Boruta across all folds over all randomizations.

**Microbial co-occurrence networks.** We constructed interaction networks on all OTUs present in at least 5% of samples resulting in a core microbiome of 589 OTUs. We employed SparCC\(^39\) on the OTU abundance, which calculates a corrected correlation coefficient designed specifically for assessing the correlating relationships between taxa in microbiome studies. The statistical significance of correlations was evaluated against an empirical null distribution obtained with 100 bootstrap iterations (p < 0.05 and SparCC > 0.2). Network visualizations were generated in Cytoscape.

**Dimensionality reduction of transcriptomics data.** To select genes for integration, differential analysis between healthy and diseased samples was performed using the limma package reducing the total number of host transcripts under consideration from 32,633 to 16,716 AD and 22,433 PSO-associated transcripts.
Identification of host–microbial interactions. For each module identified through network community detection, feature reduction was performed by calculation of the module eigengene which is equivalent to the first principal component of module gene expression. To test for associations between microbial taxa and module eigengenes, we used the MaAsLin (https://huttenhower.sph.harvard.edu/maaslin) package which fits linear models between arcsine square root transformed OTU relative abundances and metadata after removal of statistical outliers. We assessed the relationship between the top 25 most significant disease-associated microbes and module eigengene pairs while correcting for potential confounding factors using the model: microbe + module + anatomical location + institution + age + gender. p values were corrected using the Benjamini–Hochberg method and microbe–module associations with FDR < 0.20 were considered as significant. In AD, the top most abundant microbe (S. aureus) was used for stratification of the patient cohort, and differential gene expression was compared between samples exhibiting the top and bottom tertiles of S. aureus abundance. Network modules enriched for S.aureus-associated genes were identified using hypergeometric tests followed by Benjamini–Hochberg multiple testing correction. For modules with significant microbial association, we evaluated the overrepresentation of pathways using Ingenuity pathway analysis.

Microbiome, transcriptome and disease severity associations. Regression algorithms were employed for the task of identifying gene and taxonomical markers that can predict the severity of disease indices. For transcriptomic data, one score is computed for each gene, quantifying its degree of correlation with severity. The genes are then ranked in descending order according to their scores and a series of regression models are trained onto the top N genes (N=5–50 in increments of five). The trained model can then be used to predict the severity of new samples. To ensure that the constructed regression model can generalize well to unseen samples, a leave-one-out cross-validation scheme is applied to identify performance. Briefly, one sample is made blind to the process of calculation of correlation and training of regression model, and is used to estimate the accuracy of the trained model. In leave-one-out cross-validation, each sample is held out exactly once and the prediction errors are averaged over all samples. The optimal number of genes is then chosen as the set that corresponds to the lowest mean absolute prediction error. The same procedure was applied to OTU abundance data to identify a signature that correlates with severity of disease. Six multivariate regression methods were applied including linear regression, SVM, S-NN, M5P, RF and Cubist and the model with the lowest mean absolute error was chosen.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data underlying Figs. 1–4, Supplementary Figs. 1–12, 14–16 and Supplementary Tables 1–3 are available from the NCBI Sequence Read Archive under accession PRJNA554499 and from EBI Array Express under accession E-MTAB-8149. Data underlying Supplementary Fig. 13 is provided as a Source Data file. All other data are available from the corresponding author upon reasonable requests.

Received: 17 May 2018 Accepted: 27 August 2019
Published online: 16 October 2019

References

1. Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449, 811–818 (2007).
2. Cho, I. & Blaser, M. J. The human microbiome: at the interface of health and disease. Nat. Rev. Genet. 13, 260–270 (2012).
3. Grice, E. A. & Segre, J. A. The skin microbiome. Nat. Rev. Microbiol. 9, 244–253 (2011).
4. Bieber, T. Atopic dermatitis. N. Engl. J. Med. 358, 1483–1494 (2008).
5. Nelse, F. O., Kaplan, D. H. & Barker, J. Psoriasis. N. Engl. J. Med. 361, 496–509 (2009).
6. Grice, E. A. et al. Topographical and temporal diversity of the human skin microbiome. Science 324, 1190–1192 (2009).
7. Oh, J. et al. Biogeography and individuality shape function in the human skin metageneome. Nature 514, 59–64 (2014).
8. Naik, S. et al. Commensal aerobic–aerobic cell-cell interaction specifies a unique protective skin immune signature. Nature 520, 104–108 (2015).
9. Linehan, J. L. et al. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. Cell 172, 784–796.e178 (2018).
10. Kong, H. H. et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 22, 850–859 (2012).
11. Geoghegan, J. A., Irvine, A. D. & Foster, T. J. Staphylococcus aureus and atopic dermatitis: a complex and evolving relationship. Trends Microbiol. 26, 484–497 (2018).
12. Vu, A. T. et al. Staphylococcus aureus membrane and disaccharide lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. J. Allergy Clin. Immunol. 126, 985–993 (2010). 993 e981-983.
13. Nakamura, Y. et al. Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. Nature 503, 397–401 (2013).
14. Byrd, A. L. et al. Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis. Sci. Transl. Med. 9, eaal6651 (2017).
15. Alekseyenko, A. V. et al. Community differentiation of the cutaneous microbiota in psoriasis. Microbiome 1, 31 (2013).
16. Gao, Z., Tseng, C. H., Strober, B., E. P., Z. & Blaser, J. M. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. PLoS ONE 3, e2719 (2008).
17. Statnikov, A. et al. Microbiomic signatures of psoriasis: feasibility and methodology comparison. Sci. Rep. 3, 2620 (2013).
18. Caporaso, J. G. et al. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 33–36 (2010).
19. Friedman, J. & Alm, E. Inferring correlation networks from genomic survey data. PLoS Comput. Biol. 8, e1002687 (2012).
20. Newman, M. E. Modularity and community structure in networks. Proc. Natl Acad. Sci. USA 103, 8577–8582 (2006).
21. Morgan, X. C. et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 13, R79 (2012).
22. Nakatsuji, T. et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci. Transl. Med. 9, eaal6480 (2017).
23. Tholen, S. et al. Skin barrier defects caused by keratinocyte-specific deletion of ADAM17 or EGFR are based on highly similar proteome and degradation alterations. J. Proteome Res. 15, 1402–1417 (2016).
24. Weidinger, S. & Novak, N. Atopic dermatitis. Lancet 387, 1109–1122 (2016).
25. Zeeuwen, P. L. et al. Gram-positive anacrococci are underrepresented in the microbiome of filaggrin-deficient human skin. J. Allergy Clin. Immunol. 139, 1368–1371 (2017).
26. Esaki, H. et al. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. J. Allergy Clin. Immunol. 135, 153–165 (2015).
27. Inkeles, M. S. et al. Comparison of molecular signatures from multiple skin diseases identifies mechanisms of immunopathogenesis. J. Invest. Dermatol. 135, 151–159 (2015).
28. Quanranta, M. et al. Intracellular genome expression analysis reveals a specific molecular signature of psoriasis and eczema. Sci. Transl. Med. 6, 242ra290 (2014).
29. Suarez-Farinaz, M. et al. Alopeica areata profililing shows TH1, TH2, and IL-23 cytokine activation without parallel TH17/TH22 skewing. J. Allergy Clin. Immunol. 136, 1277–1287 (2015).
30. Kobayashi, S. D., Malachowa, N. & DeLeo, F. R. Pathogenesis of Staphylococcus aureus abscesses. Am. J. Pathol. 183, 1518–1527 (2015).
31. Craven, R. R. et al. Staphylococcus aureus alpha-hemolysin activates the NLRP3 inflammasome in human and mouse monocytic cells. PLoS ONE 4, e7446 (2009).
32. Rohel, I., Yang, D., Oppenheim, J. I. & Hohlhains, T. Human beta-defensin 2 and 3 and their mouse orthologs induce chemotaxis through interaction with CCR2. J. Immunol. 184, 6688–6690 (2010).
33. Glaser, R. et al. The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption. J. Invest. Dermatol. 129, 641–649 (2009).
34. Abtin, A. et al. The antimicrobial hostodimer S100A8/S100A9 (calprotectin) is upregulated by bacterial flagellin in human epidermal keratinocytes. J. Invest. Dermatol. 130, 2423–2430 (2010).
35. Wang, S. et al. S100A8/A9 in Inflammation. Front. Immunol. 9, 1298 (2018).
36. De Benedetto, A. et al. Tight junction defects in patients with atopic dermatitis. J. Allergy Clin. Immunol. 127, 773–786 (2011). e771-777.
37. Mangosyi, Y. & Gutturay-Susky, E. Immune pathways in atopic dermatitis, and definition of biomarkers through broad and targeted therapeutics. J. Clin. Med. 4, 858–873 (2015).
38. Mukhopadhyay, S. et al. Matrix metalloproteinase-12 is a therapeutic target for asthma in children and young adults. *J. Allergy Clin. Immunol.* 126, 70–76 (2010). e16.

39. von Hoven, G. et al. Dissecting the role of ADAM10 as a mediator of Staphylococcus aureus alpha-toxin action. *Biochem. J.* 473, 1929–1940 (2016).

40. Garlanda, C., Dinarello, C. A. & Mantovani, A. The interleukin-1 family: back to the future. *Immunity* 39, 1003–1018 (2013).

41. Giustizieri, M. L. et al. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J. Allergy Clin. Immunol.* 107, 871–877 (2001).

42. Pedersen, M. B., Skov, L., Menne, T., Johansen, J. D. & Olsen, J. Gene expression time course in the human skin during elicitation of allergic contact dermatitis. *J. Invest. Dermatol.* 127, 2585–2595 (2007).

43. Brunner, P. M. et al. A mild topical steroid leads to progressive anti-inflammatory effects in the skin of patients with moderate-to-severe atopic dermatitis. *J. Allergy Clin. Immunol.* 138, 169–178 (2016).

44. Wickersham, M. et al. Metabolic stress drives keratinocyte defenses against Staphylococcus aureus infection. *Cell Rep.* 18, 2742–2751 (2017).

45. Lone, A. G. et al. Colonization of epidermal tissue by Staphylococcus aureus produces localized hypoxia and stimulates secretion of antioxidant and caspase-14 proteins. *Infect. Immun.* 83, 3026–3034 (2015).

46. Wilde, A. D. et al. Bacterial hypoxic responses revealed as critical determinants of the host-pathogen outcome by TnSeq analysis of Staphylococcus aureus invasive infection. *PLoS Pathog.* 11, e1005341 (2015).

47. Zollner, T. M. et al. Colonization with superantigen-producing *Staphylococcus aureus* for asthma in children and young adults. *J. Allergy Clin. Immunol.* 127, 1183–1189 (2011).

48. Yu, J. et al. A tryptophan metabolite of the skin microbiota attenuates psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J. Allergy Clin. Immunol.* 127, 2585–2595 (2007).

49. Yu, J. et al. A tryptophan metabolite of the skin microbiota attenuates psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J. Allergy Clin. Immunol.* 127, 2585–2595 (2007).

50. Xu, Y. et al. Indoleamine 2,3-dioxygenase in antimicrobial defence and immune-regulation: tryptophan depletion versus production of toxic kynurenines. *Curr. Drug Metab.* 8, 257–244 (2007).

51. Böh, A., Toldo, J., Fülop, F. & Vecsei, L. Changing the face of kynurenines and neurotoxicity: therapeutic considerations. *Int. J. Mol. Sci.* 16, 9772–9793 (2015).

52. Reizis, B., Colonna, M., Trinchieri, G., Barratt, F. & Gilliet, M. Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system? *Nat. Rev. Immunol.* 11, 558–565 (2011).

53. Zollner, T. M. et al. Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. *Clin. Exp. Allergy* 30, 994–1000 (2000).

54. Rothe, M. J. & Grant-Kels, J. M. Diagnostic criteria for atopic dermatitis. *Lancet* 348, 769–770 (1996).

55. McDonald, D. et al. An improved Greengene taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* 6, 610–618 (2012).

56. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461 (2010).

57. Ruan, J. [Berger’s Manual of Systematic Bacteriology (second edition) Volume 5 and the study of Actinomycetes systematic in China]. 521, 769–786 (2012).

58. Letunic, I. & Bork, P. Interactive Tree Of Life (iTOL): an online tool for interactive tree display and annotation. *Bioinformatics* 23, 127–128 (2007).

59. Langille, M. G. et al. Predictive functional profiling of microbial communities using 16S rRNA gene sequences. *Nat. Biotechnol.* 31, 814–821 (2013).

60. Parks, D. H., Tyson, G. W., Hugenholtz, P. & Beiko, R. G. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124 (2014).

61. Gauthier, L., Figueiredo, O., Lannan, A., Bao, F. & Figueiredo, O. The missing link between indoleamine 2,3-dioxygenase mediated immune suppression and antioxidant performance in R. Bioinformatics 21, 3940–3941 (2005).

62. Parks, D. H., Tyson, G. W., Hugenholtz, P. & Beiko, R. G. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124 (2014).

63. Gautier, L., Cope, L., Bolstad, B. M. & Irizarry, R. A. affy—an analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20, 307–315 (2004).

64. Heinrichsdorf, J., Schulte, H. & Schütt, K. Identification of a keratinizing epithelium in culture by a novel serum-free formulation of keratinocytes. *Cell Cult. Cell Biol.* 53, 521–530 (2013).

65. Berghofer, J. G. et al. Psoriasis risk genotypes of the late corni fi er. *Psoriasis* 7, 198–203 (2008).

66. Smits, J. P. H. et al. Immortalized N/TERT keratinocytes as an alternative cell source in 3D human epidermal models. *Sci. Rep.* 7, 11838 (2017).

67. Muller, A., Hessler, K., Schmidt, S. K., Speller, K., Mackenzie, C. R. & Daubener, W. The missing link between indoleamine 2,3-dioxygenase mediated antibacterial and immunoregulatory effects. *J. Cell Mol. Med.* 13, 1125–1135 (2009).

68. Singh, T., Sander, O., Beerenwinkel, N. & Lengauer, T. ROCr: visualizing classifier performance in R. *Bioinformatics* 21, 3940–3941 (2005).
Nanna Fyhrquist, Gareth Muirhead, Stefanie Prast-Nielsen, Marine Jeanmougin, Peter Olah, Tiina Skoog, Gerome Jules-Clement, Micha Feld, Mauricio Barrientos-Somarribas, Hanna Sinkko, Ellen H. van den Bogaard, Patrick L.J.M. Zeeuwen, Gijs Rikken, Joost Schalkwijk, Hanna Niehues, Walter Däubener, Silvia Kathrin Eller, Helen Alexander, Davide Pennino, Sari Suomela, Ioannis Tessas, Emilia Lybeck, Anna M. Baran, Hamid Darban, Roopesh Singh Gangwar, Ulrich Gerstel, Katharina Jahn, Piia Karisola, Lee Yan, Britta Hansmann, Shintaro Katayama, Stephan Meller, Max Bylesjö, Philippe Hupé, Francesca Levi-Schaffer, Dario Greco, Annamari Ranki, Jens M. Schröder, Jonathan Barker, Juha Kere, Sophia Tsoka, Antti Lauerman, Vassili Soumelis, Frank O. Nestle, Bernhard Homey, Björn Andersson & Harri Alenius.

1Institute of Environmental Medicine, Karolinska Institutet, Stockholm 17177, Sweden. 2Department of Bacteriology and Immunology, Medicum, University of Helsinki, Helsinki 00014, Finland. 3Department of Informatics, Faculty of Natural and Mathematical Sciences, King’s College London, London WC2R 2LS, UK. 4Cutaneous Medicine Unit, St. John’s Institute of Dermatology and Biomedical Research Centre, Faculty of Life Sciences and Medicine, King’s College London, London SE1 9RT, UK. 5Centre for Translational Microbiome Research (CTMR), Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm 17177, Sweden. 6Institut Curie, 26 rue d’Ulm, 75248 Paris, France. 7INSERM, U900, 75248 Paris, France. 8Mines ParisTech, 77300 Fontainebleau, France. 9INSERM, U932, 75248 Paris, France. 10Department of Dermatology, University Hospital Duesseldorf, Duesseldorf 40225, Germany. 11Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs 7632, Hungary. 12Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm 17177, Sweden. 13Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs 7632, Hungary. 14Department of Cell and Molecular Biology, Science for Life Laboratory, Karolinska Institutet, Stockholm 17177, Sweden. 15Department of Dermatology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen 6525, The Netherlands. 16Department of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Duesseldorf, Duesseldorf 40225, Germany. 17St John’s Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences and Medicine, King’s College London, London SE1 9RT, UK. 18Department of Dermatology, Allergology and Venereology, University of Helsinki and Helsinki University Hospital, Inflammation Centre, Helsinki 00250, Finland. 19Pharmacology Unit, School of Pharmacy, The Institute for Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel. 20Department of Dermatology, University Hospital Schleswig-Holstein, Kiel 24105, Germany. 21Fios Genomics, Edinburgh EH9 3JL, UK. 22CNRS, UMR144, 75248 Paris, France. 23Faculty of Medicine and Life Sciences, University of Tampere, Tampere 33520, Finland. 24Institute of Biomedical Technology, University of Tampere, Tampere 33520, Finland. 25Institute of Biotechnology, University of Helsinki, Helsinki 00014, Finland. 26School of Basic and Medical Biosciences, King’s College London, London SE1 9RT, UK. 27These authors contributed equally: Nanna Fyhrquist, Gareth Muirhead, Stefanie Prast-Nielsen, Marine Jeanmougin.