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RESEARCH ARTICLE

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Biosignatures of defective sebaceous gland activity in sebum-rich and sebum-poor skin areas in adult atopic dermatitis

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Abstract

Atopic dermatitis (AD) is a composite disease presenting disruption of the skin permeability barrier (SPB) in the stratum corneum (SC). Recent evidence supports derangement of the sebaceous gland (SG) activity in the AD pathomechanisms. The objective of this study was to delineate profiles of both sebaceous and epidermal lipids and of aminoacids from SG-rich (SGR) and SG-poor (SGP) areas in AD. Both sebum and SC were sampled from SGR areas, while SC was sampled also from SGP areas in 54 adult patients with AD, consisting of 34 and 20 subjects, respectively with and without clinical involvement of face, and in 44 age and sex-matched controls. Skin biophysics were assessed in all sampling sites. Disruption of the SBP was found to be associated with dysregulated lipidome. Abundance of sapienate and lignocerate, representing, respectively, sebum and the SC type lipids, were decreased in sebum and SC from both SGR and SGP areas. Analogously, squalene was significantly diminished in AD, regardless the site. Extent of lipid derangement in SGR areas was correlated with the AD severity. The abundance of aminoacids in the SC from SGR areas was altered more than that determined in SGP areas. Several gender-related differences were found in both controls and AD subgroups. In conclusion, the SG activity was differently compromised in adult females and males with AD, in both SGR and SGP areas. In AD, alterations in the aminoacidome profiles were apparent in the SGR areas. Lipid signatures in association with aminoacidome and skin physical properties may serve the definition of phenotype clusters that associate with AD severity and gender.

KEYWORDS
aminoacids, cholesterol, epidermal lipids, fatty acids, fatty alcohols, sapienate, sebaceous gland, squalene

1 | INTRODUCTION

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease becoming highly prevalent in adults from industrialized and developing countries.[1,2] Environmental, genetic and immunologic factors play a role in AD pathogenesis.[3] The AD hallmarks include disruption of the skin permeability barrier (SPB) in the stratum corneum (SC) and increased trans-epidermal water loss (TEWL).[4] The deficiency of filaggrin (FLG) and its breakdown metabolites, altogether known as natural moisturizing factors (NMFs), contribute to the decreased epidermal hydration and increased TEWL. FLG deficiency only partly explains why patients develop AD.[5,6] The release of NMFs in the SC, including urocanic acid (UCA) and pyroglutamate (PGA), assist the water retention.[7] Depletion of FLG and NMFs increases pH and serine protease activity, contributing to the generation of inflammatory cytokines.[8] Free fatty acids (FFAs) concur to the skin pH.[9,10] AD manifests at predilection sites.[11] However, it is unclear whether the biochemical modification at the skin surface is a prerequisite for, or an effect of the barrier perturbation.[12] A competent SPB is formed through a complex process wherein biosynthetic pathways yield the lipid matrix composed of FFAs, cholesterol congeners and sphingolipids.[13] While there is consensus on the defective SPB in AD, the knowledge of the impact on the SPB integrity exerted by lipids originating from the sebaceous gland (SG) is rather limited. Decreases in both moisture and lipids concur to skin dryness in AD.[14] Wirth et al.[15] have reported sebocyte hypoproliferation in AD and reduced overall surface occupied by the SGs. The main function of the SG is to produce and secrete sebum, which lubricates skin, shields environmental insults and shapes the skin microbiota.[16] Sebum is a complex mixture of triglycerides (TGs), wax esters (WEs), squalene, cholesterol and FFAs.[17] SG lipids deploy both pro- and anti-inflammatory activity and modulate immune cells functions.[18,19] The epidermis displays different biochemical profiles depending on the sebum secretion.[20–23] Sebum production is activated at puberty in both genders. Generally, the sebum output is higher in males than in females.[24] Regional differences in the sebum production are observed in the T and the U zones of the face, partly due to the unequal distribution of the androgen receptors.[25] AD often resolves at puberty, coincidentally with the hormonal activation of the SG. While evidence is available on the low abundance of sebum in AD,[26] little is known about the effects of the SBP disruption on the SG function and vice versa. Comprehensive investigations on the metabolome and lipidome in non-invasive sampling are made possible by –omics approaches, which offer valuable tools for the identification of perturbations associated with skin disorders.[27,28] The objective of our study was to investigate the biochemical profiles of non-invasive samples from SG-rich (SGR) and SG-poor (SGP) areas in AD.

2 | MATERIALS AND METHODS

2.1 | Study design

The study followed the principles of the Declaration of Helsinki and was approved by the Institutional Review Board. All participants read and undersigned the informed consent before participation. Ninety-eight subjects of both genders were enrolled. Inclusion criteria for the disease group was diagnosis of AD of mild, moderate and severe grade; exclusion criteria were concurrent dermatological disorders and use of UV-beds. Fifty-four AD patients were enrolled. AD severity was assessed with the Eczema Area and Severity Index (EASI).[29] Patients were sub-classified according to the involvement of the face, which physiologically presents a higher number of SGs. Twenty patients showed uninvolved face (face no, fnAD), while 34 patients presented involved face (face yes, fyAD). Forty-four were healthy controls (hC). TEWL and corneometry were measured before collection of sebum and SC after acclimation to the RT of 18–21°C and the relative humidity of 40%–60%. The sampling of sebum and SC, the extraction procedures and the chromatographic analyses, that is, GCMS and HPTLC, and quantification of total protein were performed as described in the supplementary material and methods (Appendix S1) according to previous studies.[21,30,31] A schematic representation of the study workflow is provided in the Scheme S1.

2.2 | Statistical analysis and multivariate statistics

Data were analysed with the statistical and data analysis solutions XLSTAT 2020.1.2 (Addinsoft, New York, USA), MatLab (version 8.6.0 release R2015b; The Mathworks, Natick, MA) and Python custom scripts that leveraged the scikit-learn library. Continuous variables were represented as median values with confidence intervals or mean ± standard deviation (SD). Mann–Whitney and Kruskal–Wallis tests were used for comparisons between two or among more groups. Pearson's coefficient (R) was used to measure the correlation between two quantitative variables. Differences and correlations were considered statistically significant with $p \leq 0.05$. ANOVA-simultaneous component analysis (ASCA),[32] was used to determine the effect of the controlled factors and their interaction in the experimental design on the multivariate chromatographic profiles.[30,31]

3 | RESULTS

The groups presented homogeneous age and gender distribution (Table 1a). AD severity (EASI) was consistent with the involvement of SGR areas, being significantly higher in fyAD. In contrast, SER was significantly lower in the fyAD subgroup. SER on the forehead discriminated between fnAD and fyAD subgroups. TEWL was higher on foreheads in both fnAD and fyAD groups. The fyAD group showed impaired barrier also on cheeks and forearms (Table 1b,c). On the lesional forearms, the elevation of TEWL was significantly different between the two AD subgroups (Table 1b). Gender appeared to influence TEWL, which was higher on the forehead in males, regardless the dermatological conditions. Elevation of TEWL on the cheeks in AD males

TABLE 1 Characteristics of the studied groups (a): Age, EASI and SER on forehead (H) and cheeks (C). Skin biophysics: TEWL (b), corneometry (c), protein amount (μg/tape) (d) in hC, fnAD and fyAD on H, C, healthy or unaffected forearm (A) and lesional A.

(a)

Group	Count	Age	EASI	SER forehead	SER cheek
hC	44	35.6 ± 15.2	NA	5.04 ± 1.91	5.26 ± 2.01
F	27	35.7 ± 16.2	NA	4.81 ± 2.13	4.71 ± 2.09
M	17	35.5 ± 14.1	NA	5.41 ± 1.48	6.12 ± 1.57
M/F ratio	0.629	0.994		1.12	1.30$^{\$\$}$
fnAD	20	29.5 ± 11.1	28.7 ± 10.8	4.93 ± 1.64	4.65 ± 1.55
F	11	30.4 ± 12.9	27.5 ± 11.9	5.30 ± 1.69	5.06 ± 1.67
M	9	28.4 ± 9.08	30.1 ± 9.75	4.48 ± 1.55	4.16 ± 1.31
M/F ratio	0.818	0.934	1.09	0.845	0.822
fyAD	34	31.2 ± 12.9	36.4 ± 10.6$^{\#}$	3.52 ± 2.13$^{\circ\circ\circ\#}$	3.60 ± 2.87$^{\circ\circ\circ}$
fyAD\F	16	32.1 ± 14.2	37.2 ± 10.1	2.89 ± 1.98	3.05 ± 2.45
fyAD\M	18	30.4 ± 12.0	35.7 ± 11.2	4.08 ± 2.17	4.08 ± 3.18*
M/F ratio	1.12	0.947	0.959	1.41	1.33

(b)

Group	TEWL forehead	TEWL cheek	TEWL non lesional forearm	TEWL lesional forearm
hC	16.5 ± 6.73	20.4 ± 7.89	9.68 ± 3.15	NA
F	13.7 ± 2.79	18.9 ± 5.86	9.59 ± 2.15	NA
M	20.8 ± 8.72	22.9 ± 10.0	9.82 ± 4.38	NA
M/F ratio	1.52$^{\$\$\$}$	1.21	1.02	
fnAD	22.9 ± 6.59°	25.8 ± 11.6	11.9 ± 4.28	22.4 ± 10.6
F	19.6 ± 6.33	19.9 ± 8.70	10.6 ± 3.56	23.0 ± 11.5
M	26.9 ± 4.44	33.0 ± 10.8	13.5 ± 4.73	21.7 ± 9.87
M/F ratio	1.37$^{\$}$	1.66$^{\$}$	1.27	0.925
fyAD	33.1 ± 15.6$^{\circ\circ\circ}$	32.3 ± 17.2$^{\circ\circ}$	13.1 ± 6.58°	32.2 ± 13.8$^{\#}$
F	25.7 ± 11.4**	27.7 ± 18.1	11.8 ± 3.43	32.5 ± 10.8
M	39.7 ± 16.1*	36.4 ± 15.8	14.2 ± 8.43	31.9 ± 16.4
M/F ratio	1.54$^{\$}$	1.31	1.20	0.982

(c)

Group	Corneometry forehead	Corneometry cheek	Corneometry non lesional forearm	Corneometry lesional forearm
hC	51.3 ± 14.0	44.3 ± 12.1	38.8 ± 12.2	NA
F	50.6 ± 11.2	43.6 ± 13.0	35.4 ± 11.1	NA
M	52.4 ± 18.0	45.5 ± 10.8	44.1 ± 12.4	NA
M/F ratio	1.04	1.04	1.25$^{\$}$	
fnAD	39.6 ± 9.77°	41.6 ± 12.8	32.1 ± 7.83	22.6 ± 11.8
F	38.4 ± 7.92	45.6 ± 11.4	31.9 ± 7.51	20.8 ± 10.5
M	41.1 ± 12.0	36.8 ± 13.4	32.4 ± 8.65	24.7 ± 13.5
M/F ratio	1.07	0.807	1.02	1.19
fyAD	30.7 ± 14.3$^{\circ\circ\circ\#}$	33.2 ± 33.2°	28.7 ± 8.06$^{\circ\circ\circ}$	18.8 ± 8.90
F	27.5 ± 11.6**	35.3 ± 16.2	30.3 ± 7.40	16.3 ± 6.80
M	33.5 ± 16.1**	31.3 ± 16.6	27.1 ± 8.53**	20.9 ± 10.1

Continues)

TABLE 1 (Continued)

(c)

Group	Corneometry forehead	Corneometry cheek	Corneometry non lesional forearm	Corneometry lesional forearm
M/F ratio	0.893	0.887	0.894	1.28

Abbreviations: F, females; fnAD, atopic dermatitis face not involved; fyAD, atopic dermatitis face involved; hD, healthy controls; M, males; NA, not applicable; SER, sebum excretion rate; TEWL transepidermal water loss.

Data are reported as mean ± standards deviation (SD).

°Differences between controls and AD subgroups and #differences between AD subgroups independent on the gender (Bonferroni corrected significance level: 0.0167; °$p \le 0.0167$, °°$p \le 0.00167$; °°°$p \le 0.000167$).

*Difference between controls and AD subgroups in each gender (Bonferroni corrected significance level: 0.030; *$p \le 0.033$, **$p \le 0.0033$, ***$p \le 0.00033$).

$Differences between F and M (Mann–Whitney significance level: 0.05; $^{\$}p \le 0.05$, $^{\$\$}p \le 0.005$, $^{\$\$\$}p \le 0.0005$).

was significant in the fnAD group only. Corneometry, which indicates skin hydration, was altered on the foreheads more than the cheeks and forearms, in AD. Skin hydration was diminished on the foreheads in both fnAD and in all the analysed areas in the fyAD group. SGP of unaffected males presented higher hydration levels than females (Table 1c).

3.1 | ANOVA-simultaneous component analysis (ASCA) on the relative concentrations of individual lipids in sebum

Sebum was analysed from two SGR areas, that is, forehead and cheeks. ASCA analysis was performed on sebum lipids expressed as pmol/tape to find different lipid profiles in hC, fnAD, and fyAD groups. ANOVA partitioning of the variance for the forehead and cheek data matrices evidenced that the effect of AD status accounted for 11.5%, and 15.6%of the total variability, respectively. Permutation tests allowed to assess that effect of AD grade were significant on both sites, that is, forehead (p-value <0.001) and cheeks (p-value <0.001) (data not shown). The abundance of the sebum components clearly allowed differentiating between healthy and AD conditions, as reported in Figure 1, which refers to the amounts of sebum metabolites determined from each facial area reported in the Tables S1 (forehead) and S2 (cheek). The scores along the first simultaneous component (SC1), which represented the 93.3% and the 96.6% of the total variability of the forehead and cheek data, respectively, were represented in the score plots (A1 and A2) in Figure 1. The separation was almost total for the fyAD group, which had mostly negative scores, opposite to hC that had mostly positive scores. On both SGR sites, hC and fyAD presented positive and negative average scores, respectively. The fnAD group showed an average score that approached zero on the positive side, for both forehead (A1) and cheek (A2) sites. To interpret the score trends, the loadings were investigated according to their magnitude and sign represented in the loadings plots B1 and B2, referred, respectively, to the score plots A1 and A2. Most variables determined in sebum had loadings going into the direction of positive scores, meaning that in hC they were higher than those computed in AD. Noteworthy, among the compounds identified as significantly contributing to the differentiation, lipids belonging to both the sebaceous-type and the epidermal-type were involved. Most sebum lipids presented significantly higher levels in hC on forehead, except for 11Me-C14:0, C18:0, C21:0, FOHC16:0, FOHC18:0 and cholesterol, which were unmodified (Figure 1B1). Similarly, on the cheek site (Figure 1B2), most sebum lipids showed significantly higher levels in hC, except for 9Me-C12:0, 10Me-C12:0, C18:2, C21:0 and three FOHs, that is, FOHC16:0, FOHC18:0 and FOHC18:1, which showed not significantly different levels. Overall, profiles of sebum lipids from forehead and cheeks were comparably discriminative of hC and AD conditions.

Comparison between forehead and cheek and female and males in hC, fnAD, and fyAD are reported in Figures S1 and S2, respectively (Appendix S2). Gender discrimination on cheek was reported in Figure S3 (Appendix S2).

The involvement of FFAs and their precursors in AD is depicted in the box plots in Figure 2. Propionic acid can be the starter of the biosynthesis of odd chain FFAs. C.acnes metabolism and biotransformation of amino acids and valeric acid are sources of propionic acid.[33,34] Acetate is the precursor of even FFAs via the canonical pathway. The straight chain FFAs with even C-number, that is, C14:0, C16:0, C18:0 and C20:0, were present at significantly lower levels in males of both fnAD and fyAD groups. In contrast, female subjects of the fyAD group, presented significantly diminished amounts of even FFAs in cheek sebum. A similar trend was observed for odd straight chain FFAs. Most of these FFAs were unaffected in fnAD females, while they were significantly decreased in males in both AD subgroups. Box plots in Figure 3 depict the abundance of branched chain FFAs (BCFAs) sorted according to their possible origin. BCFAs are synthesized from short, branched chain organic acids, and aminoacids. Valine is reported to be the precursor of the even-BCFAs with isobranching of the C-chain, while isobutyrate and isovalerate acid are the precursors of BCFAs with even and odd number of C-atoms, respectively. Leucine and isoleucine are the precursors of iso- and anteiso branched FFAs with odd C-number, that is, iBCFAs and aiBCFAs, respectively.[35,36] Most of the BCFAs were decreased in severe AD in both females and males, while 12Me-C14:0, 13Me-C16:0

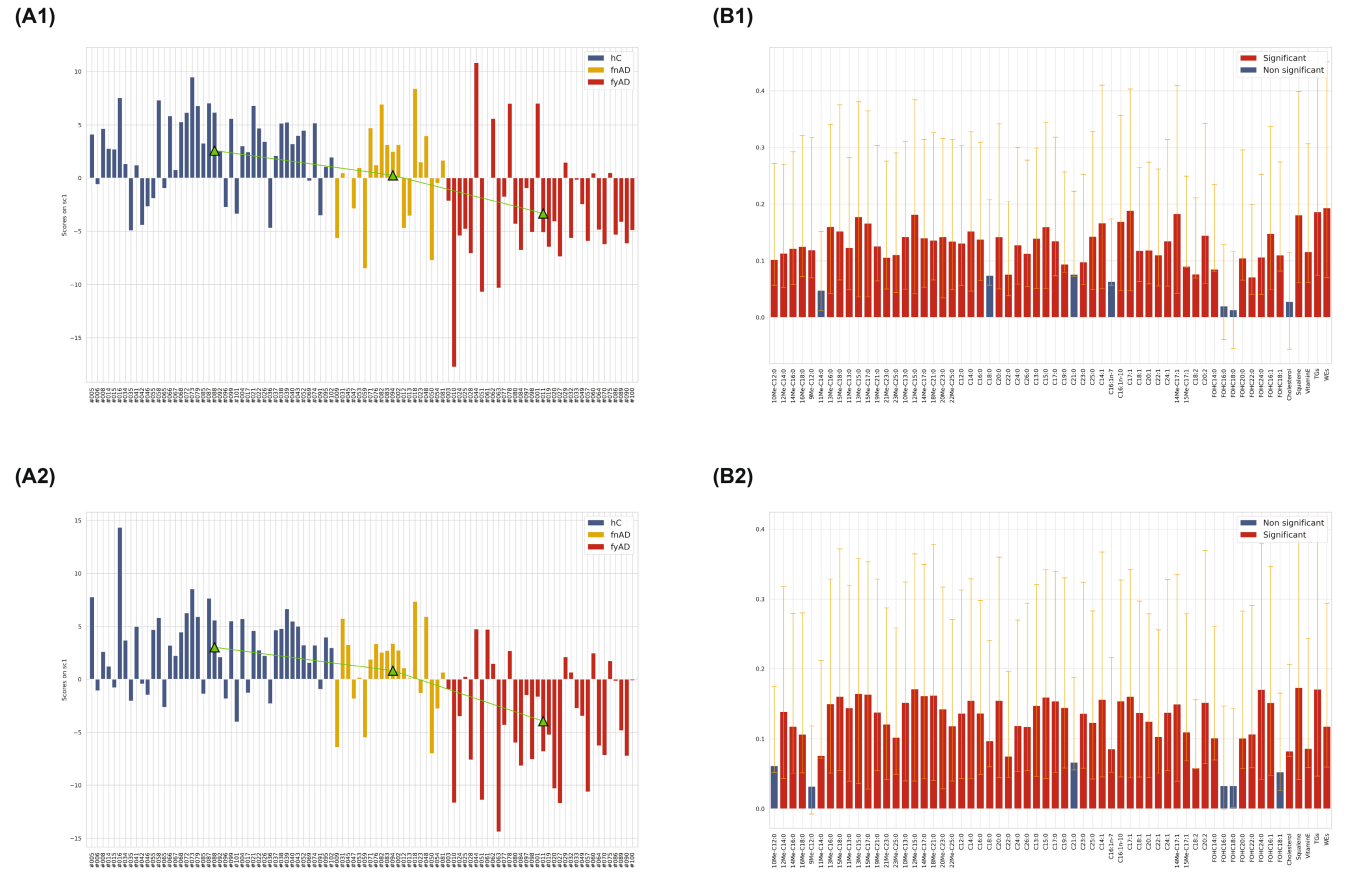

FIGURE 1 ASCA analysis on the effect matrix for lipid metabolites (pmol/tape) investigated in sebum from foreheads (upper panels) and cheeks (lower panels) in hC, fnAD, and fyAD groups. Panels A report scores plots after projection of the residuals onto the space spanned by the first simultaneous component SC1 for forehead (A1), and cheek (A2). Legend of the bar colours: Blue = hC; Yellow=; fnAD; Red = fyAD. The triangles in green indicate the average scores for each subgroup and are connected by a green line to indicate the trend of changes of the AD groups related to controls. Panels B report the variable loadings on SC1, together with their confidence interval, for forehead (B1) and cheek (B2); red and blue bars indicate significantly and not significantly contributing descriptors, respectively.

and 12Me-15:0 were significantly lower at the cheek site in males with uninvolved face. Additionally, MUFAs, squalene, TGs and WEs are represented with box plots for both genders according to the AD subgroups (Figure S4, Appendix S2).

In Table S3 (Appendix S2), we evaluate the extent of lipid transformation processes occurring in the SG. We focused on specific FFAs ratios expressing the FADS2,[37,38] the SCD1[39] and the elongation activity,[40,41] and their contribution to the formation of complex lipids. C18:2 PUFA was examined separately.[42] Cholesterol biosynthesis was studied through squalene/cholesterol ratio.

Lignoceric acid (C24:0) is illustrated in Figure S5 similarly to what observed for shorter saturated FFAs (Appendix S2).

3.2 | ASCA analysis on the concentrations of lipid and non-lipid metabolites in the stratum corneum

Sebaceous and epidermal lipids were determined in the SC extracts together with non-lipid metabolites, of which aminoacids account for the most numerous species. The normalized amounts (pmol/μg protein) were obtained in the SC from SGR areas and SGP areas, that is, forearms. The amounts of all the species detected in the SC are provided in Tables S4 (forehead), S5 (cheek), S6 (lesional forearm) and S7 (non lesional forearm). The scores plots of the resulting ASCA in Figure 4 evidence the differentiation between hC and the two AD subgroups. The separation was more pronounced for severe cases. On the forehead (Figure 4A1), the most lipids and aminoacids in the SC had loadings with positive values, meaning that their abundance was higher in controls. The cheeks site (Figure 4A2) showed a similar pattern; however, the loadings associated with aminoacids were less intense than those obtained on the forehead (Figure 4A1). Notably, on the forearms (Figure 4A3), lipid species most exclusively contributed to the separation, since loadings associated to aminoacids were not significant, except for threonine (Figure 4B3). Noteworthy, the squalene loadings accounted among the highest ones in each of the three areas, regardless their different volume of the SGs.

The effect of site and gender studied is explained in Figure S6 (Appendix S2). Scores and loadings plot show the trend of aminoacids, bioamine, glycerol[43] and sebaceous lipids in the SGP areas compared to SGR ones (Figure S7, Appendix S2). ASCA model also

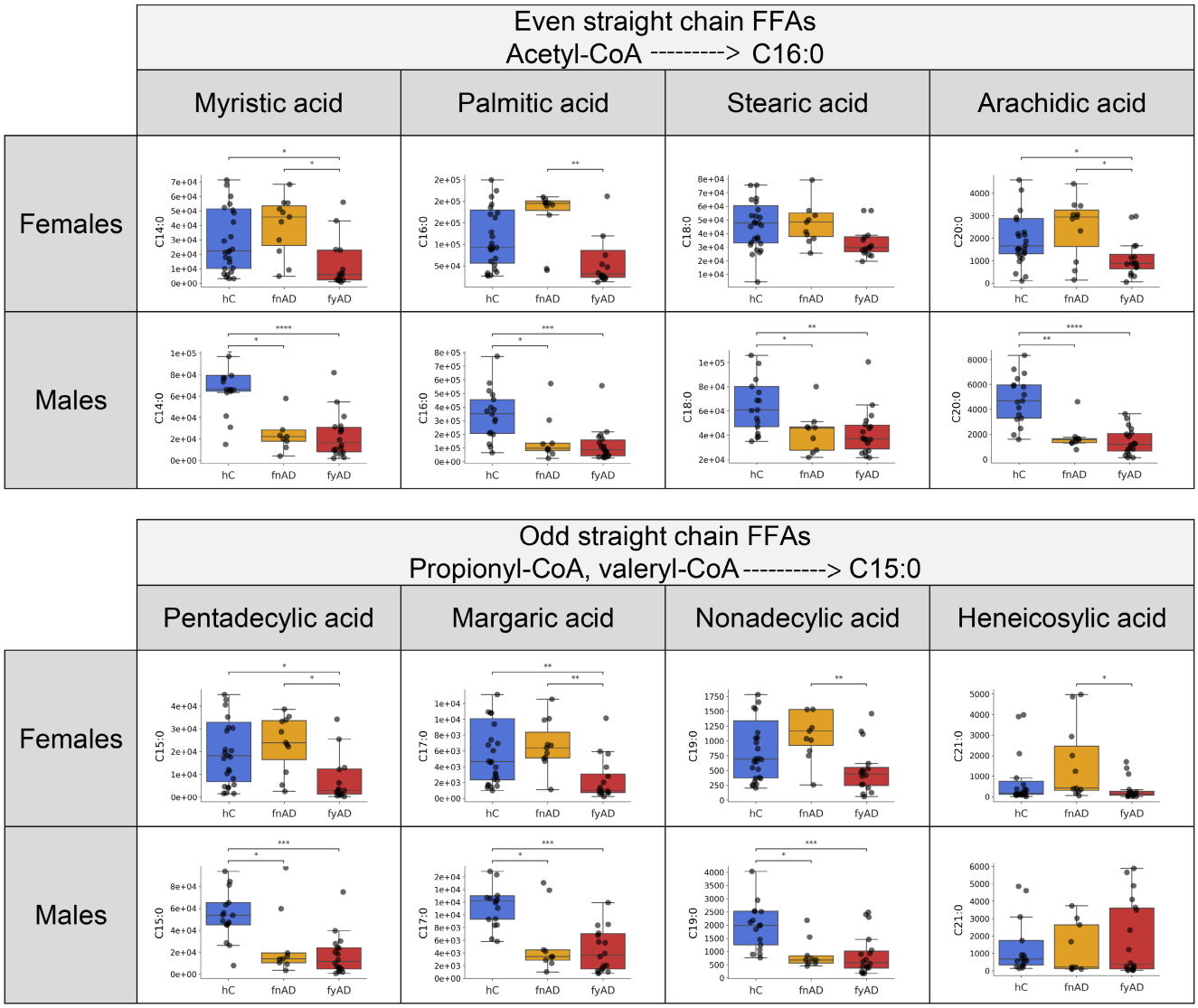

FIGURE 2 Gender-associated differences in the amounts (pmol/tape) of even and odd straight chain FFAs in females and males in healthy controls (hC) and patients with atopic dermatitis (AD), with uninvolved (fnAD) and involved (fyAD) face. Average amounts of FFAs are indicated in box plots, with individual values determined in cheek sebum indicated by dots. Median, interquartile range (IQR), and p-values retrieved by Kruskal–Wallis test are reported in Tables S2.

investigates the effects of the skin condition on the metabolite profiles determined on all sites (Figure S8, Appendix S2). Box plots in Figure S9 (Appendix S2) illustrate the differences among hC, fnAD and fyAD, in females and males. Comparison between non lesional and lesional SGP areas in AD, retrieved minimal significant differences (data not shown). The ASCA analysis highlighted several outlier subjects in all the studied groups.

3.3 | EASI and correlation of stratum corneum metabolites

The associations of EASI and metabolites composition of SC in forehead, cheeks, non-lesional and lesional forearm were investigated by Pearson's correlation (Table S8). Numerous metabolites from the SGR areas were correlated inversely or directly with the EASI. Palmitic acid and histidine were consistently and negatively correlated with EASI in both SGR areas, that is, the forehead and cheeks of fyAD patients.

4 | DISCUSSION

AD is a heterogeneous inflammatory disease presenting with different severity of the skin symptoms and variability of endogenous biomarkers.[23,44,45] Skin lipids and the water content in the SC concur to the integrity of the barrier opposed by the skin to the external environment. Their disruption contributes to xerosis, that is dry skin, in AD.[14,46] The unaffected population also presents a wide range of variability in the lipid abundance and the skin physical properties.

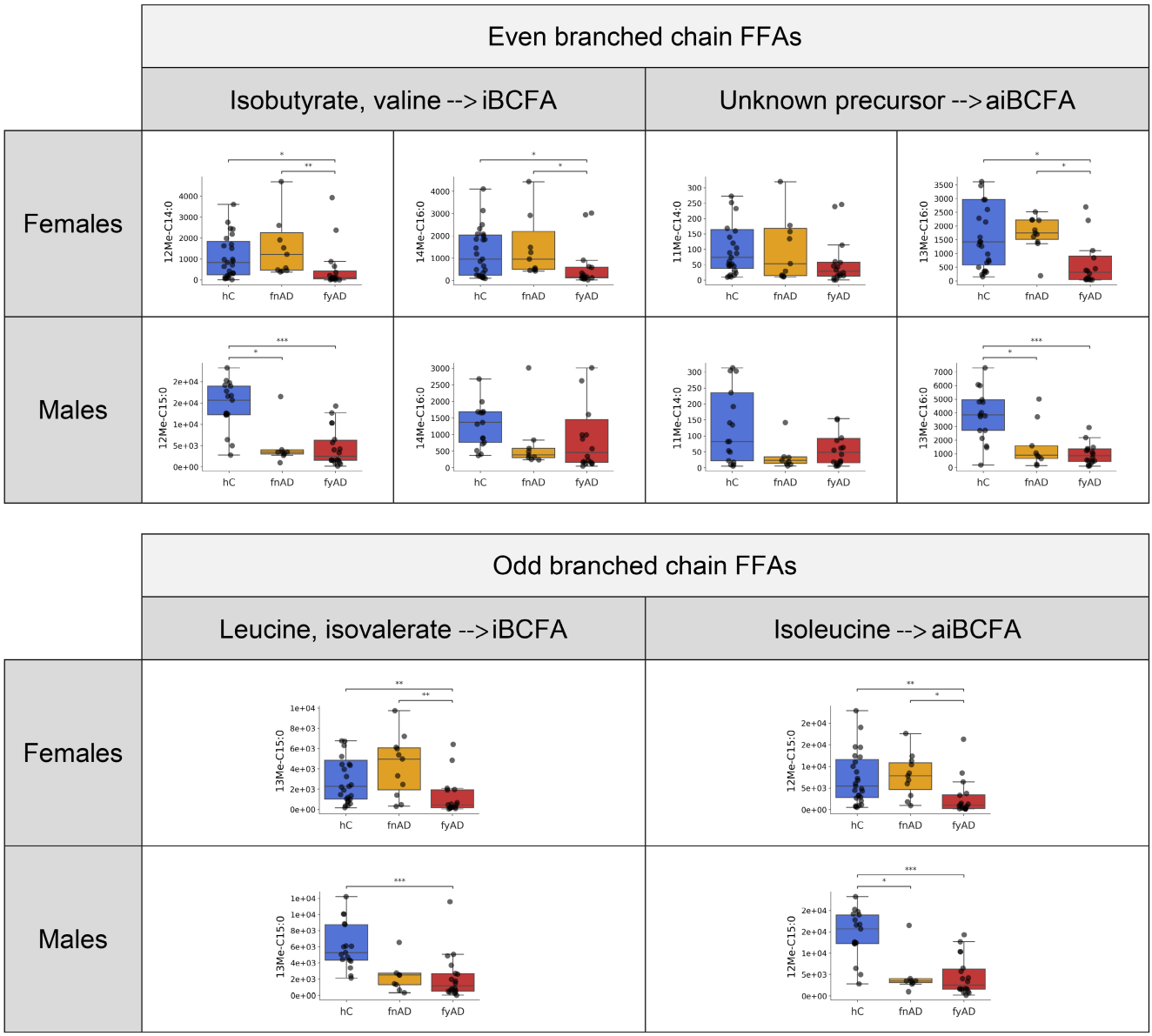

FIGURE 3 Gender-associated differences in the amounts (pmol/tape) of even and odd branched chain FFAs (BCFA) in females and males in healthy controls (hC) and patients with atopic dermatitis (AD), with uninvolved (fnAD) and involved (fyAD) face. Isobranched (iBCFA) and anteisobranched (aiBCFA) FFAs were those with terminal and ante-terminal methyl-branching, respectively. Proposed precursors of each BCFA subgroup are indicated in the boxes above the representative family member. Average amounts of FFAs are indicated in box plots, with individual values determined in cheek sebum marked by dots. Median, interquartile range(IQR), and p-values retrieved by Kruskal–Wallis test are reported in Tables S2.

Diet and smoking can precipitate acute alterations of the lipid barrier in the epidermis.[47,48] Exposome factors, such as food, pollution and solar radiation, can lead to acute stress, which could provoke a temporary different response on skin function. However, any acute stress should be put into perspective against the genetic background of the single subject, particularly in the case of chronic disease. Our study did not include data on diet, environmental exposure, and external factors, since its primary objective was to find association between clinical appearance and the involvement of the SG. Further studies are required to investigate the role of exposome factors in the different phases of the chronic skin diseases.

Omics technologies, which include metabolomics and lipidomics, are strategic in the interpretation of the complexity opposed by the barrier anomalies in AD.[23,49–52] This study focused on the occurrence of AD skin manifestations in facial areas in addition to the forearm, which is a SGP area. We evaluated non-invasive samples of the skin surface lipids from SGR and SGP areas. For the first time, aminoacids were simultaneously evaluated in SC extracts. Aminoacids are key components of the NMFs supporting hydration of the outmost epidermal layer.[53] The NMFs, derived from FLG proteolysis, include a pool of hygroscopic aminoacids. Deficiency of FLG contributes to barrier perturbation and alters the balance between

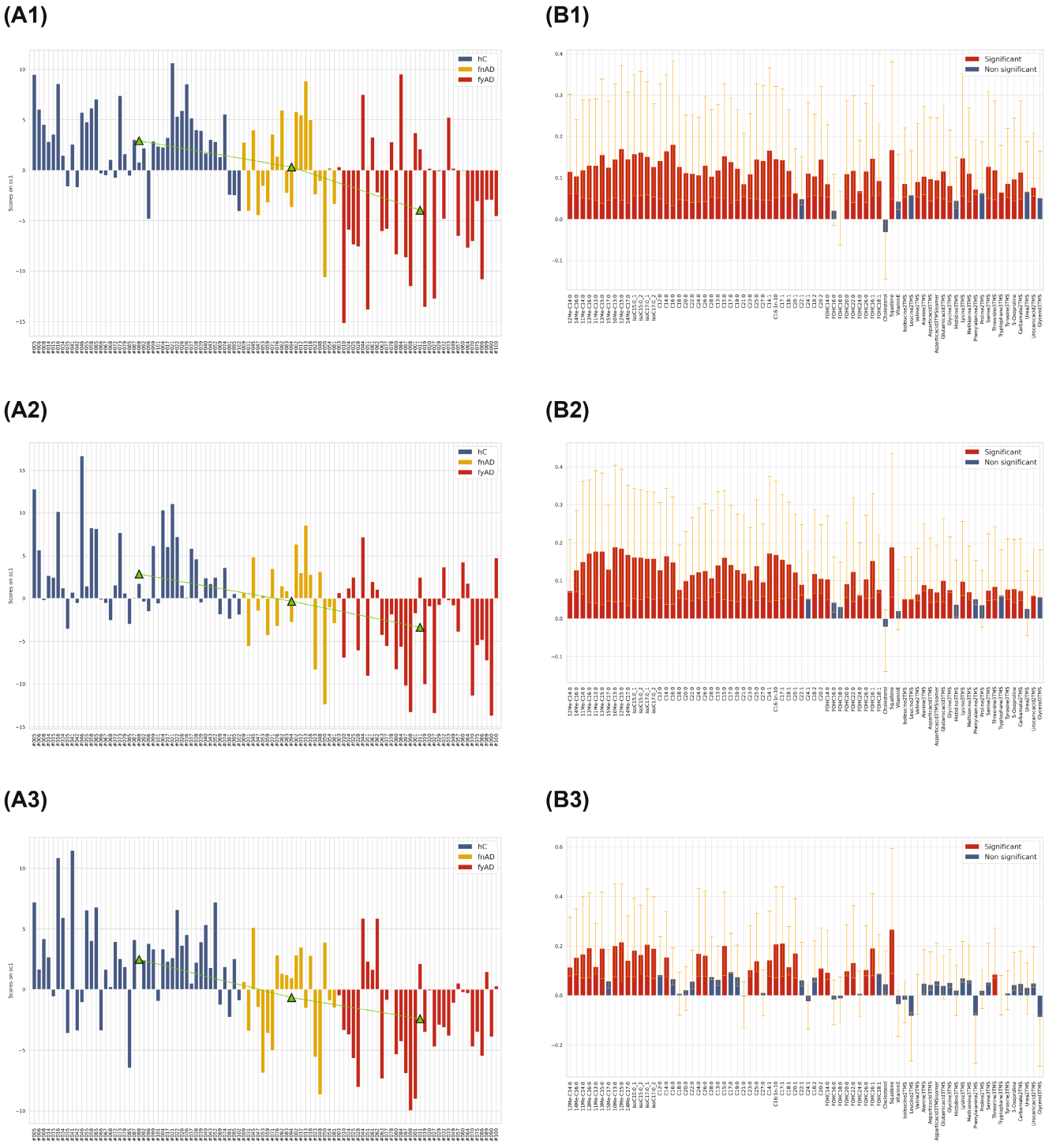

FIGURE 4 ASCA analysis on the effect matrix for lipid and aminoacid amounts (pmol/μg protein) investigated in stratum corneum (SC) from forehead, cheek and forearm in hC, fnAD, and fyAD conditions. Panels A report scores plots after projection of the residuals onto the space spanned by the first simultaneous component SC1 for forehead (A1), cheek (A2), and forearm (A3). Scores for hC, fnAD, and fyAD are represented by blue, yellow and red bars, respectively. The triangles in green indicate the average scores for each subgroup and are connected by a green line to indicate the trend of changes of the AD groups related to controls. Panels B report variable loadings on SC1, together with their confidence interval, for forehead (B1), cheek (B2), and forearm (B3); red and blue bars in panels B indicate significantly and not significantly contributing descriptors, respectively.

the lipid and protein components of the SC.[54] Histidine accounts for around 10% of the aminoacids in FLG and is the precursor of trans-urocanic acid, which contributes to the skin's acid mantle. FLG degradation products have an impact on several essential functions responsible for the maintenance of epidermal homeostasis.[7,55,56] FLG mutations represent the most predominant risk factor in AD,

but not all individuals with the FLG mutation develop AD.[57] The altered composition of the NMFs in the SC may have multiple causes, beyond FLG processing.[58] We detected an extensive perturbation of the aminoacid content in the SC sampled from SGR areas. With the exception of a few aminoacids, which were differently significant in foreheads and cheeks, that is, leucine, phenylalanine, and tryptophane, most of the detected aminoacids presented lower levels in AD subjects with involved face. By contrast, aminoacid profiles were similar between healthy skin and uninvolved areas of AD subjects. Threonine was the only exception. The overall decrease in the serine levels was consistent with previous results in AD.[59–61] Serine condensates with FA-CoAs by serine palmitoyl transferase (SPT), which is the rate-limiting enzyme in sphingolipid synthesis.[62] Whether the lower level of serine in the SC in AD patients is linked with the altered ceramide composition awaits clarification.

Glycine, together with glutamate, is an important neurotransmitter in mammals. A functionally active receptor of glycine has been detected in human keratinocytes.[63] Glycine, topically applied onto the skin, accelerates the barrier repair.[64] Aminoacids acting as precursors of branched FFAs were diminished in the SGR areas. Unequivocal evidence on how aminoacids enter the FA synthetic pathways in SG cells is still lacking. Mitochondria are the main site of aminoacid catabolism and formation of branched FAs precursors. The function of mitochondria in AD has been addressed recently in keratinocytes.[65] Our results demonstrated a significant anomaly of the SG function in patients with AD that presented manifestations on SGR areas. Reduced SER is known to be associated with AD,[26] however, the pathomechanisms involved in the SG-derangement has been largely overlooked. In our procedure we collected the sebum being excreted at the skin surface for 30 min to minimize influences by external factors.[66] Abundance of sebum components was almost universally compromised in severe AD. A few FFAs, FOHs, and cholesterol were an exception. In the SG, sebum-type FFAs, palmitate and sapienate, are highly dependent on the de novo synthesis.[67] Diminished FOHs observed in AD can be linked to the decreased FFAs.[68] Short chain FFAs are key determinants of the skin surface pH.[69] Defective enzymic FAs elongation mediated by elongases, for example, ELOVL3, has been found in AD.[70–72]

The mild dysregulation of sebum abundance observed in fnAD patients suggests that when sebum secretion is normal, the symptoms are mitigated, or that the SG activity is marginally affected. Unlike males, female patients with fnAD presented normal levels of some sebaceous lipids, for example, sapienate. This observation may indicate an effect of the face care routine or female hormones in the mitigation of sebum derangement occurring in AD.

Despite the statistically significant higher value of the EASI index in the fyAD group, the EASI values measured in the fnAD subgroup was indicative of a moderate to severe AD grade.[73,74] This supports the hypothesis that in advanced AD grades, manifestations on the face are minimized if both secretion rate and sebum composition are normal. In hC and both AD groups, the average TEWL values on SGP areas were as half as those on SGR areas, consistent with previous studies.[75] In contrast, skin hydration in SGR and SGP areas presented different ratios in hC and AD groups. While capacitance was higher on the face in hC, the same parameter was comparable in the three sites in AD. Thus, sebum may influence the barrier function and hydration through different mechanisms.

Altered microbiome and fungiome play a pathogenic role in head and neck dermatitis (HND).[76] The lack of data on skin microbiome and dysbiosis in our study limits the conclusions on the factors concurring to the observed sebum dysregulation. Whether the decrease of lipids results from the inefficient lipid machinery alone or from its interplay with commensal bacteria and fungi remains to be clarified.[22,23,77–79] The whole metagenome analysis of skin microbiomes assists classification of patients according to distinct microbiome configurations corresponding to two main dermotypes.[80] The sebum depletion observed in the fyAD subgroup was matched by a notable impoverishment of sebum species in the SC from both SGR and SGP areas. In particular, the decreased amounts of MUFAs suggest that the pathways involved in the FAs desaturation is compromised in AD.[39] It is not known at what extent the FADS2 activity is compromised in AD skin and if the decreased sapienate is a primary or a secondary event in the derangement of the lipogenic cascade. MUFAs were severely decreased in AD. Due to the antimicrobial activity of sapienate, it is likely that the depletion of MUFAs in sebum translates into a permissive environment towards pathogenic microbes.[81,82] Expression of enzymes involved in the elongation of FFAs present with different distribution in AD epidermis.[39] A recent paper has reported dysregulation of sebum production in AD in keeping with aberrant expression of genes regulating lipid biosynthetic pathways, including FA de novo synthesis, elongation and desaturation.[52] While the lipid domains evaluated in previous studies differed from those addressed here, coincident results were obtained on the significant depletion of TGs in AD sebum. Our approach addressed sebum-specific biomarkers, e.g., squalene, sapienate and branched FFAs, indicating compromised early events in the lipid cascade leading to sebaceous end products, including TGs and WEs. In our study, detection of aminoacids and other non-lipid metabolites was accomplished in the SC extracts. This is advantageous in the characterization of metabotypes in AD, as aminoacids participate in the hydration of the SC as part of the NMFs. Differences in the sebum parameter were apparent in the AD group with facial manifestations. In contrast, at the same SGR site, differences in the barrier parameters between controls and AD were found in both AD severity groups. This finding suggests that the alterations detected on the foreheads correlate with the AD grade better than those of the other two sites. The interplay of sebaceous and epidermal metabolism has been hypothesized long time ago,[83] however, there is little evidence about the mechanisms of reciprocal interaction.[21,84] The three sites showed significant derangement of the lipid and NMF arrays. Overall, the forehead area displayed more pronounced decrease of the metabolites' abundance. Interestingly, squalene and branched FFAs, were significantly depleted in the SGP areas. In contrast, C24:0, which is an

epidermal FA, accounted among the significantly depleted species in the SGR areas. Gender differences were observed in both control and AD groups, in keeping with available evidence.[50] While males presented higher amounts of sebum lipids than females in control groups, the effect of AD condition was aggravated in the male gender, translating into a more severe lipid depletion.

5 | CONCLUSION

The results of this study suggest that multiple pathways are disrupted in the normal epidermal differentiation and provide candidate biomarkers in the definition of disease clusters, personalized treatments, and prediction of outcomes. Multi-omics are instrumental to the fine characterization of the biological basis of the AD pathogenesis and support the interplay of different pathways leading to the identification of specific biomarkers.[49] Translation into the clinical routine of biomarkers that define AD severity and development or grading of comorbidities remain limited.[85] Easiness and un-invasiveness of SC sampling, together with direct information on SG activity and NMFs can address simultaneously different compartments affected by the AD pathogenesis. In keeping with the current landscape of multiple endophenotypic variations of AD,[45,86] we gathered evidence that skin lipidomics can assist different expressivity of the disease. The findings demonstrating that the SG activity is impaired together with the NMFs in sebaceous areas of adult patients with AD, create a strong rationale for the deployment of management strategies that incorporate sebum lipids and key aminoacids to improve barrier function in AD.[87,88]

AUTHOR CONTRIBUTIONS

Conceptualization: E.C., M.P., E.P., A.F., M.C.B. Methodology: E.C., A.Ca., M.Mar. G.B.; A.F., E.P. Validation: E.C., A.Ca., M.Mar. G.B., M.T., F.M.; M.C.B. Investigation: A.Ca., Mar. F.P., E.C., M.T., F.M. Writing—original draft preparation: E.C., A.Ca., G.B., M.Mai., M.T. Writing—review and editing: E.C., A.Ca., G.B., M.Mai., M.T., A.F., M.C.B., E.P. Supervision: E.C. and A.Cr. Project administration: E.C., M.P., M.C.B. Funding acquisition: E.C., M.P., M.C.B., A.F., and E.P.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at 10.5281/zenodo.7385410.

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REFERENCES

1. Weidinger S, Novak N. Atopic dermatitis. *Lancet*. 2016;387(10023):1109-1122.
2. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nat Rev Dis Primers*. 2018;4(1):1.
3. Luger T, Amagai M, Dreno B, et al. Atopic dermatitis: role of the skin barrier, environment, microbiome, and therapeutic agents. *J Dermatol Sci*. 2021;102(3):142-157.
4. Pavel P, Blunder S, Moosbrugger-Martinz V, Elias PM, Dubrac S. Atopic dermatitis: the fate of the fat. *Int J Mol Sci*. 2022;23(4):2121. doi:10.3390/ijms23042121
5. Bandier J, Johansen JD, Petersen LJ, Carlsen BC. Skin pH, atopic dermatitis, and filaggrin mutations. *Dermatitis*. 2014;25(3):127-129.
6. Tamari M, Hirota T. Genome-wide association studies of atopic dermatitis. *J Dermatol*. 2014;41(3):213-220.
7. Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol*. 2014;134:792-799.
8. Kezic S, O'Regan GM, Lutter R, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol*. 2012;129(4):1031-1039.
9. Elias PM, Williams ML, Choi EH, Feingold KR. Role of cholesterol sulfate in epidermal structure and function: lessons from X-linked ichthyosis. *Biochim Biophys Acta*. 2014;1841(3):353-361.
10. Elias PM. Stratum corneum acidification: how and why? *Exp Dermatol*. 2015;24(3):179-180.
11. Zainal H, Jamil A, Md Nor N, Tang MM. Skin pH mapping and its relationship with transepidermal water loss, hydration and disease severity in adult patients with atopic dermatitis. *Skin Res Technol*. 2020;26(1):91-98.
12. Fischer CL, Blanchette DR, Brogden KA, et al. The roles of cutaneous lipids in host defense. *Biochim Biophys Acta*. 2014;1841(3):319-322.

13. Feingold KR, Elias PM. The important role of lipids in the epidermis and their role in the formation and maintenance of the cutaneous barrier. *Biochim Biophys Acta*. 2014;1841(3):279.
14. Sator PG, Schmidt JB, Honigsmann H. Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. *J Am Acad Dermatol*. 2003;48(3):352-358.
15. Wirth H, Gloor M, Stoika D. Sebaceous glands in uninvolved skin of patients suffering from atopic dermatitis. *Arch Dermatol Res*. 1981;270(2):167-169.
16. Picardo M, Mastrofrancesco A, Biro T. Sebaceous gland—a major player in skin homoeostasis. *Exp Dermatol*. 2015;24(7):485-486.
17. Picardo M, Ottaviani M, Camera E, Mastrofrancesco A. Sebaceous gland lipids. *Dermatoendocrinology*. 2009;1(2):68-71.
18. Lovaszi M, Szegedi A, Zouboulis CC, Törocsik D. Sebaceous-immunobiology is orchestrated by sebum lipids. *Dermatoendocrinology*. 2017;9(1):e1375636.
19. Choi CW, Kim Y, Kim JE, et al. Enhancement of lipid content and inflammatory cytokine secretion in SZ95 sebocytes by palmitic acid suggests a potential link between free fatty acids and acne aggravation. *Exp Dermatol*. 2019;28(2):207-210.
20. Sadowski T, Klose C, Gerl MJ, et al. Large-scale human skin lipidomics by quantitative, high-throughput shotgun mass spectrometry. *Sci Rep*. 2017;7:43761.
21. Ludovici M, Kozul N, Materazzi S, Risoluti R, Picardo M, Camera E. Influence of the sebaceous gland density on the stratum corneum lipidome. *Sci Rep*. 2018;8(1):11500-11507.
22. Baurecht H, Rühlemann MC, Rodríguez E, et al. Epidermal lipid composition, barrier integrity, and eczematous inflammation are associated with skin microbiome configuration. *J Allergy Clin Immunol*. 2018;141(5):1668-1676.
23. Emmert H, Baurecht H, Thielking F, et al. Stratum corneum lipidomics analysis reveals altered ceramide profile in atopic dermatitis patients across body sites with correlated changes in skin microbiome. *Exp Dermatol*. 2021;30(10):1398-1408.
24. Luebberding S, Krueger N, Kerscher M. Age-related changes in male skin: quantitative evaluation of one hundred and fifty male subjects. *Skin Pharmacol Physiol*. 2014;27(1):9-17.
25. Seo YJ, Li ZJ, Choi DK, et al. Regional difference in sebum production by androgen susceptibility in human facial skin. *Exp Dermatol*. 2014;23(1):70-72.
26. Furuichi M, Makino T, Matsunaga K, Hamade E, Yokoi H, Shimizu T. The usefulness of sebum check film for measuring the secretion of sebum. *Arch Dermatol Res*. 2010;302(9):657-660.
27. Koppes SA, Brans R, Ljubojevic HS, Frings-Dresen MHW, Rustemeyer T, Kezic S. Stratum corneum tape stripping: monitoring of inflammatory mediators in atopic dermatitis patients using topical therapy. *Int Arch Allergy Immunol*. 2016;170(3):187-193.
28. Smirnov VV, Egorenkov EA, Myasnikova TN, et al. Lipidomic analysis as a tool for identifying susceptibility to various skin diseases. *Fortschr Med*. 2019;10(11):1871-1874.
29. Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. *Exp Dermatol*. 2001;10(1):11-18.
30. Okoro OE, Adenle A, Ludovici M, Truglio M, Marini F, Camera E. Lipidomics of facial sebum in the comparison between acne and non-acne adolescents with dark skin. *Sci Rep*. 2021;11(1):16591. doi:10.1038/s41598-021-96043-x:16591
31. Briganti S, Truglio M, Angiolillo A, et al. Application of sebum lipidomics to biomarkers discovery in neurodegenerative diseases. *Metabolites*. 2021;11(12):819. doi:10.3390/metabo11120819
32. Smilde AK, Jansen JJ, Hoefsloot HC, Lamers RJ, van der Greef J, Timmerman ME. ANOVA-simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data. *Bioinformatics*. 2005;21(13):3043-3048.
33. Crown SB, Marze N, Antoniewicz MR. Catabolism of branched chain amino acids contributes significantly to synthesis of odd-chain and even-chain fatty acids in 3T3-L1 adipocytes. *PLoS One*. 2015;10(12):e0145850.
34. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018;16:143-155.
35. Green CR, Wallace M, Divakaruni AS, et al. Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat Chem Biol*. 2016;12(1):15-21.
36. Wallace M, Green CR, Roberts LS, et al. Enzyme promiscuity drives branched-chain fatty acid synthesis in adipose tissues. *Nat Chem Biol*. 2018;14(11):1021-1031.
37. Park HG, Kothapalli KSD, Park WJ, et al. Palmitic acid (16:0) competes with omega-6 linoleic and omega-3 a-linolenic acids for FADS2 mediated Delta6-desaturation. *Biochim Biophys Acta*. 2016;1861(2):91-97.
38. Flori E, Mastrofrancesco A, Ottaviani M, Maiellaro M, Zouboulis CC, Camera E. Desaturation of sebaceous-type saturated fatty acids through the SCD1 and the FADS2 pathways impacts lipid neosynthesis and inflammatory response in sebocytes in culture. *Exp Dermatol*. 2023;32(6):808-821.
39. Danso M, Boiten W, van Drongelen V, et al. Altered expression of epidermal lipid bio-synthesis enzymes in atopic dermatitis skin is accompanied by changes in stratum corneum lipid composition. *J Dermatol Sci*. 2017;88(1):57-66.
40. van Smeden J, Janssens M, Kaye ECJ, et al. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. *Exp Dermatol*. 2014;23(1):45-52.
41. Zwara A, Wertheim-Tysarowska K, Mika A. Alterations of ultra long-chain fatty acids in hereditary skin diseases-review article. *Front Med (Lausanne)*. 2021;8:730855.
42. Mihaly J, Marosvolgyi T, Szegedi A, et al. Increased FADS2-derived n-6 PUFAs and reduced n-3 PUFAs in plasma of atopic dermatitis patients. *Skin Pharmacol Physiol*. 2014;27(5):242-248.
43. Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. *Br J Dermatol*. 2008;159(1):23-34.
44. Bakker DS, Nierkens S, Knol EF, et al. Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. *J Allergy Clin Immunol*. 2021;147(1):189-198.
45. Tokura Y, Hayano S. Subtypes of atopic dermatitis: from phenotype to endotype. *Allergol Int*. 2022;71(1):14-24.
46. Elias PM. Lipid abnormalities and lipid-based repair strategies in atopic dermatitis. *Biochim Biophys Acta*. 2003;1841(3):323-330.
47. Passeron T, Zouboulis CC, Tan J, et al. Adult skin acute stress responses to short-term environmental and internal aggression from exposome factors. *J Eur Acad Dermatol Venereol*. 2021;35(10):1963-1975. doi:10.1111/jdv.17432
48. Maintz L, Welchowski T, Herrmann N, et al. Machine learning-based deep phenotyping of atopic dermatitis: severity-associated factors in adolescent and adult patients. *JAMA Dermatol*. 2021;157(12):1414-1424. doi:10.1001/jamadermatol.2021.3668
49. Ghosh D, Bernstein JA, Khurana Hershey GK, Rothenberg ME, Mersha TB. Leveraging multilayered "omics" data for atopic dermatitis: a road map to precision medicine. *Front Immunol*. 2018;9:2727.
50. Agrawal K, Hassoun LA, Foolad N, et al. Effects of atopic dermatitis and gender on sebum lipid mediator and fatty acid profiles. *Prostaglandins Leukot Essent Fatty Acids*. 2018;134:7-16.
51. Ilves L, Ottas A, Kaldvee B, et al. Metabolomic analysis of skin biopsies from patients with atopic dermatitis reveals hallmarks of inflammation, disrupted barrier function and oxidative stress. *Acta Derm Venereol*. 2021;101(2):adv00407.
52. Yin H, Qiu Z, Zhu R, et al. Dysregulated lipidome of sebum in patients with atopic dermatitis. *Allergy*. 2022;78:1524-1537.
53. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol*. 2009;129(8):1892-1908.

54. Irvine AD, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med*. 2011;365(14):1315-1327.
55. Čepelak I, Dodig S, Pavić I. Filaggrin and atopic march. *Biochem Med (Zagreb)*. 2019;29(2):20501. doi:10.11613/BM.2019.020501
56. Suarez-Farinas M, Tintle SJ, Shemer A, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol*. 2011;127(4):954.
57. Gupta J, Margolis DJ. Filaggrin gene mutations with special reference to atopic dermatitis. *Curr Treat Options Allergy*. 2020;7(3):403-413.
58. Kezic S, O'Regan GM, Yau N, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy*. 2011;66(7):934-940.
59. Wong LS, Otsuka A, Tanizaki H, et al. Decrease of superficial serine and lactate in the stratum corneum due to repetitive frictional trauma. *Int J Dermatol*. 2018;57(3):299-305.
60. Amin R, Lechner A, Vogt A, Blume-Peytavi U, Kottner J. Molecular characterization of xerosis cutis: a systematic review. *PLoS One*. 2021;16(12):e0261253.
61. Yoshihisa Y, Rehman MU, Nakagawa M, et al. Inflammatory cytokine-mediated induction of serine racemase in atopic dermatitis. *J Cell Mol Med*. 2018;22(6):3133-3138.
62. Wang Y, Niu Y, Zhang Z, et al. Structural insights into the regulation of human serine palmitoyltransferase complexes. *Nat Struct Mol Biol*. 2021;28(3):240-248.
63. Inoue K, Takei K, Denda M. Functional glycine receptor in cultured human keratinocytes. *Exp Dermatol*. 2015;24(4):307-309.
64. Denda M, Fuziwara S, Inoue K. Influx of calcium and chloride ions into epidermal keratinocytes regulates exocytosis of epidermal lamellar bodies and skin permeability barrier homeostasis. *J Invest Dermatol*. 2003;121(2):362-367.
65. Leman G, Pavel P, Hermann M, et al. Mitochondrial activity is upregulated in nonlesional atopic dermatitis and amenable to therapeutic intervention. *J Invest Dermatol*. 2022;142(10):2623-2634.
66. Mukherjee S, Mitra R, Maitra A, et al. Sebum and hydration levels in specific regions of human face significantly predict the nature and diversity of facial skin microbiome. *Sci Rep*. 2016;6:36062.
67. Esler WP, Tesz GJ, Hellerstein MK, et al. Human sebum requires de novo lipogenesis, which is increased in acne vulgaris and suppressed by acetyl-CoA carboxylase inhibition. *Sci Transl Med*. 2019;11(492). doi:10.1126/scitranslmed.aau8465
68. Rizzo WB. Fatty aldehyde and fatty alcohol metabolism: review and importance for epidermal structure and function. *Biochim Biophys Acta*. 2014;1841(3):377-389.
69. Danby SG, Cork MJ. pH in atopic dermatitis. *Curr Probl Dermatol*. 2018;54:95-107.
70. Sassa T, Kihara A. Metabolism of very long-chain fatty acids: genes and pathophysiology. *Biomol Ther (Seoul)*. 2014;22(2):83-92.
71. Kihara A. Synthesis and degradation pathways, functions, and pathology of ceramides and epidermal acylceramides. *Prog Lipid Res*. 2016;63:50-69.
72. Brunner PM, Israel A, Zhang N, et al. Early-onset pediatric atopic dermatitis is characterized by T(H)2/T(H)17/T(H)22-centered inflammation and lipid alterations. *J Allergy Clin Immunol*. 2018;141(6):2094-2106.
73. Leshem YA, Hajar T, Hanifin JM, Simpson EL. What the eczema area and severity index score tells us about the severity of atopic dermatitis: an interpretability study. *Br J Dermatol*. 2015;172(5):1353-1357.
74. Chopra R, Vakharia PP, Sacotte R, et al. Severity strata for eczema area and severity index (EASI), modified EASI, scoring atopic dermatitis (SCORAD), objective SCORAD, atopic dermatitis severity index and body surface area in adolescents and adults with atopic dermatitis. *Br J Dermatol*. 2017;177(5):1316-1321.
75. Ishikawa J, Shimotoyodome Y, Ito S, et al. Variations in the ceramide profile in different seasons and regions of the body contribute to stratum corneum functions. *Arch Dermatol Res*. 2013;305(2):151-162.
76. Chu H, Kim SM, Zhang K, et al. Head and neck dermatitis is exacerbated by malassezia furfur colonization, skin barrier disruption, and immune dysregulation. *Front Immunol*. 2023;14:1114321. doi:10.3389/fimmu.2023.1114321
77. Szabo K, Erdei L, Bolla BS, Tax G, Biro T, Kemeny L. Factors shaping the composition of the cutaneous microbiota. *Br J Dermatol*. 2017;176(2):344-351.
78. Li S, Villarreal M, Stewart S, et al. Altered composition of epidermal lipids correlates with staphylococcus aureus colonization status in atopic dermatitis. *Br J Dermatol*. 2017;177(4):e125-e127.
79. Zheng Y, Hunt RL, Villaruz AE, et al. Commensal staphylococcus epidermidis contributes to skin barrier homeostasis by generating protective ceramides. *Cell Host Microbe*. 2022;30(3):301-313.
80. Tay ASL, Li C, Nandi T, et al. Atopic dermatitis microbiomes stratify into ecologic dermotypes enabling microbial virulence and disease severity. *J Allergy Clin Immunol*. 2021;147(4):1329-1340.
81. Wille JJ, Kydonieus A. Palmitoleic acid isomer (C16:1delta6) in human skin sebum is effective against gram-positive bacteria. *Skin Pharmacol Appl Ski Physiol*. 2003;16(3):176-187.
82. Moran JC, Alorabi JA, Horsburgh MJ. Comparative transcriptomics reveals discrete survival responses of *S. aureus* and *S. epidermidis* to sapienic acid. *Front Microbiol*. 2017;8:33.
83. Wheatley VR. Cutaneous lipogenesis. Major pathways of carbon flow and possible interrelationships between the epidermis and sebaceous glands. *J Invest Dermatol*. 1974;62(3):245-256.
84. Jia Y, Gan Y, He C, Chen Z, Zhou C. The mechanism of skin lipids influencing skin status. *J Dermatol Sci*. 2018;89(2):112-119.
85. Broderick C, Ziehfreund S, van Bart K, et al. Biomarkers associated with the development of comorbidities in patients with atopic dermatitis: a systematic review. *Allergy*. 2022;78:84-120.
86. Nomura T, Wu J, Kabashima K, Guttman-Yassky E. Endophenotypic variations of atopic dermatitis by age, race, and ethnicity. *J Allergy Clin Immunol Pract*. 2020;8(6):1840-1852.
87. Araviiskaia E, Pincelli C, Sparavigna A, Luger T. The role of a novel generation of emollients, 'emollients plus', in atopic dermatitis. *Clin Cosmet Investig Dermatol*. 2022;15:2705-2719.
88. Simpson EL, Chalmers JR, Hanifin JM, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol*. 2014;134(4):818-823.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Scheme S1. Representation of (1) The SG rich (SGR) and SG poor (SGP) areas investigated for the skin biophysics, that is, TEWL, and corneometry and for the lipid and metabolite profiling in samples of sebum and stratum corneum (SC); (2) Sampling and extraction of sebum with SEBUTAPE from SGR areas and of SC with CORNEOFIX from both SGR and SGP areas; (3) Analyses by GCMS of lipids and metabolites in sebum and SC extracts; (4) Statistical analysis by ANOVA-simultaneous component analysis (ASCA).

Appendix S1. Supplementary materials and methods.

Appendix S2. Supplementary results.

Figure S1. Significance of differences between SGR areas, that is, forehead and cheek, determined with the ASCA model on abundance of lipid metabolites in sebum sampled from the forehead and the cheek areas. Sum of squares of the effect matrix (red line) compared to the corresponding distribution under the

null hypothesis estimated by permutation tests (blue histograms). Legend: Comparison between forehead and cheek in hC (a), fnAD (b), and fyAD (c).

Figure S2. Significance of differences in sebum composition between females and males determined with the ASCA model on abundance of lipid metabolites in sebum sampled from SGR areas (forehead and cheek). Sum of squares of the effect matrix (red line) compared to the corresponding distribution under the null hypothesis estimated by permutation tests (blue histograms). Legend: Comparison between females and males in hC (a), fnAD (b), and fyAD (c).

Figure S3. ASCA on the effect matrix for gender based on metabolites determined in cheek sebum in females (pink) and males (blue) in all subjects from hC, fnAD, and fyAD groups. Scores plot obtained from data on cheek sebum after projection of the residuals onto the space spanned by the first simultaneous component SC1 (a). Variable loadings on SC1 (b), together with their confidence interval (red and blue bars indicate significantly and not significantly contributing descriptors, respectively) in the gender differences on cheek.

Figure S4. Gender-associated differences in the amounts (pmol/tape) of sebum-specific lipids in females and males in healthy controls (hC) and patients with atopic dermatitis (AD), with uninvolved (fnAD) and involved (fyAD) face. Average amounts of lipids are indicated in box plots, with individual values determined in cheek sebum from indicated by dots. Median, interquartile range (IQR), and Kruskal–Wallis test are reported in Table S2.

Figure S5. Gender-associated differences in the amounts (pmol/tape) of the epidermal type FFA lignoceric acid (C24:0) in females and males in healthy controls (hC) and patients with atopic dermatitis (AD), with uninvolved (fnAD) and involved (fyAD) face. Average amounts of FFAs are indicated in box plots, with individual values determined in forehead and cheek sebum indicated by dots. Median, interquartile range (IQR), and *p*-values retrieved by Kruskal–Wallis test are reported in Tables S1 and S2.

Figure S6. Significance of (a) differences between SGR, and SGP areas, and (b) difference between genders determined with the ASCA model on the abundance of lipid and non-lipid metabolites assessed in the stratum corneum sampled from the forehead, the cheek, and the forearm areas in healthy controls (hC). Sum of squares of the effect matrix (red line) compared to the corresponding distribution under the null hypothesis estimated by permutation tests (blue histograms).

Figure S7. ASCA analysis on the effect matrix for the normalized amounts of lipid and non-lipid metabolites (pmol/μg protein) investigated in stratum corneum extracts from SGR (forehead and cheek) and SGP (forearm) areas in healthy controls (hC), regardless gender. Left panel: Scores plot after projection of the residuals onto the space spanned by the first two simultaneous components SC1 and SC2 when considering forehead, cheek, and forearm sites in healthy controls (hC). Right panel: Variable loadings on SC1 (full lines), and variable loading on SC2 (dotted lines); only the significant loadings are displayed, and the variables are coloured according to the lipid species described in the legend. Further details on the species grouping are reported in the supporting information.

Figure S8. Significance of differences in the dermatological condition based on chemometric analysis of stratum corneum extracts from (a) forehead, (b) cheek, and (c) forearm assessed with the ASCA model on abundance of lipid and non-lipid metabolites determined in the SC sampled in hC, fnAD, and fyAD groups.

Figure S9. Gender-associated differences in the amounts (pmol/μg protein) of aminoacids and natural moisturizing factors (NMFs) in the stratum corneum in females and males in healthy controls (hC) and patients with atopic dermatitis (AD), with uninvolved (fnAD) and involved (fyAD) face. Average amounts of aminoacids and NMFs are indicated in box plots, with individual values determined in SC extracts from forehead indicated by dots. A grey background marks metabolites that presented significant differences. Median, interquartile range (IQR), and *p*-values retrieved by Kruskal–Wallis test are reported in Table S4.

Table S3. Changes of ratios between precursor and product in biosynthetic pathways in the subjects with AD compared to unaffected individuals, in the respective SG-rich areas, that is, forehead (head) and cheek. Upward (↑) and downward (↓) arrows indicated statistically significant increase or decrease, respectively, of the index in AD compared to unaffected controls. No changes or statistically insignificant changes were indicated with the equal (=) key.

Table S1. Amounts (pmol/tape) of lipid metabolites in sebum samples from forehead in healthy controls (hC) and patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median pmol/tape, and inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis). Statistically significant differences were indicated by *p*-values ≤0.05 in bold.

Table S2. Amounts (pmol/tape) of lipid metabolites in sebum samples from the cheek area in healthy controls (hC) and patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median pmol/tape, and inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis). Statistically significant differences were indicated by *p*-values ≤0.05 in bold.

Table S4. Abundance of lipid and non-lipid metabolites in the stratum corneum (SC) from the forehead in healthy controls (hC) and patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median pmol/μg protein, inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis).

Table S5. Abundance of lipid and non-lipid metabolites in the stratum corneum (SC) from the cheek area in healthy controls (hC) and patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median pmol/μg protein, inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis).

Table S6. Abundance of lipid metabolites in the stratum corneum (SC) from forearm areas in healthy controls (hC) and clinically unaffected forearm areas of patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median

pmol/μg protein, inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis).

Table S7. Abundance of lipid metabolites in the stratum corneum (SC) from the involved areas on forearms in patients with AD presenting uninvolved (fnAD) and involved (fyAD) facial areas. The table reports median pmol/μg protein, inter-quartile range (IQR) in both genders in each group, and *p*-values determined by non-parametric significant testing (Kruskal–Wallis).

Table S8. Correlation matrix of EASI and SC metabolites of forehead, cheek, non-lesional and lesional forearm. Pearson's correlation coefficients (*R*) are listed with their respective *p*-values. Results were analysed for statistical significance, with a significance level of $p \leq 0.05$ (values in bold).

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