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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

The skin microbiome in the first year of life and its association with atopic dermatitis

Alexis Rapin[1,2] | Eva Maria Rehbinder[3,4] | Matthew Macowan[5] | Céline Pattaroni[2,5] | Karin C. Lødrup Carlsen[4,6] | Nicola L. Harris[1,5] | Christine M. Jonassen[7] | Linn Landrø[3,4] | Astrid H. Lossius[3,4] | Björn Nordlund[8,9] | Knut Rudi[10] | Håvard O. Skjerven[4,6] | Anne Cathrine Staff[4,11] | Cilla Söderhäll[8,9] | Niki Ubags[2] | Riyas Vettukattil[4,6] | Benjamin J. Marsland[2,5]

[1]Global Health Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[2]Service de Pneumologie, Département de Médecine, Centre Hospitalier Universitaire Vaudois (CHUV), Epalinges, Switzerland

[3]Department of Dermatology, Oslo University Hospital, Oslo, Norway

[4]Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[5]Department of Immunology, Monash University, Melbourne, Victoria, Australia

[6]Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway

[7]Genetic Unit, Centre for Laboratory Medicine, Østfold Hospital Trust, Kalnes, Norway

[8]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

[9]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

[10]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway

[11]Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway

Correspondence
Benjamin J. Marsland, Department of Immunology and Pathology, Monash University, Level 6, Burnet Institute 89 Commercial Road, Melbourne, Vic. 3004, Australia.
Email: benjamin.marsland@monash.edu

Present address
Alexis Rapin, Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract

Background: Early-life microbial colonization of the skin may modulate the immune system and impact the development of atopic dermatitis (AD) and allergic diseases later in life. To address this question, we assessed the association between the skin microbiome and AD, skin barrier integrity and allergic diseases in the first year of life. We further explored the evolution of the skin microbiome with age and its possible determinants, including delivery mode.

Methods: Skin microbiome was sampled from the lateral upper arm on the first day of life, and at 3, 6, and 12 months of age. Bacterial communities were assessed by 16S rRNA gene amplicon sequencing in 346 infants from the PreventADALL

Abbreviations: AD, atopic dermatitis; ASV, amplicon sequence variant; AUC, area under the curve; CCA, constrained correspondence analysis; CS, caesarean section; LD, linear discriminant; MCA, multiple correspondence analysis; NMDS, non-metric multidimensional scaling; OOB, out-of-bag; perMANOVA, permutational multivariate analysis of variance; PFC, portion of falsely classified values; PreventADALL, Preventing Atopic Dermatitis and ALLergies in Children; RCT, randomized clinical trial; RF, random forest; ROC, receiver operating characteristic; SDI, Shannon diversity index; SPLS-DA, sparse partial least squares discriminant analysis; SPT, skin prick test; TEWL, trans-epidermal water loss; VD, vaginal delivery.

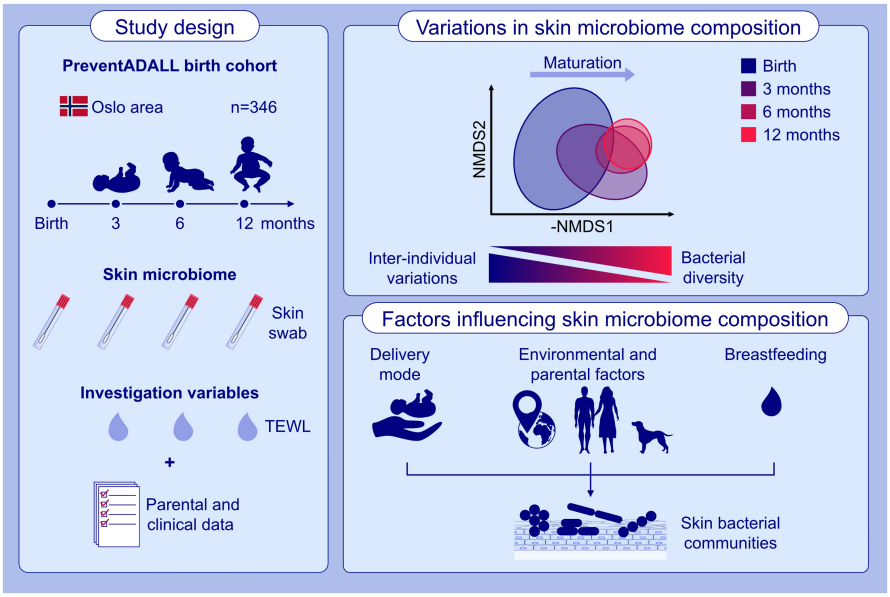
Alexis Rapin and Eva Maria Rehbinder should be considered joint first author.

population-based birth cohort study, representing 970 samples. Clinical investigations included skin examination and skin barrier function measured as trans-epidermal water loss (TEWL) at the site and time of microbiome sampling at 3, 6, and 12 months. Parental background information was recorded in electronic questionnaires, and delivery mode (including vaginal delivery (VD), VD in water, elective caesarean section (CS) and emergency CS) was obtained from maternal hospital charts.

Results: Strong temporal variations in skin bacterial community composition were found in the first year of life, with distinct patterns associated with different ages. Confirming our hypothesis, skin bacterial community composition in the first year of life was associated with skin barrier integrity and later onsets of AD. Delivery mode had a strong impact on the microbiome composition at birth, with each mode leading to distinct patterns of colonization. Other possible determinants of the skin microbiome were identified, including environmental and parental factors as well as breastfeeding.

Conclusion: Skin microbiome composition during infancy is defined by age, transiently influenced by delivery mode as well as environmental, parental factors and breastfeeding. The microbiome is also associated with skin barrier integrity and the onset of AD.

KEYWORDS
atopic dermatitis, cohort study, infancy, microbiome, skin



GRAPHICAL ABSTRACT

The skin bacterial community composition varies between individuals after birth, then progressively converges during the first year of life. The skin microbiome composition and diversity at birth is associated with later onset of atopic dermatitis with associations found between skin barrier function, dry skin, and bacterial communities. Delivery mode and location are major determinants of the skin microbiome at birth.

Abbreviations: NMDS, non-metric multidimensional scaling; PreventADALL, Preventing Atopic Dermatitis and ALLergies in Children; TEWL, trans-epidermal water loss

1 | INTRODUCTION

Atopic dermatitis (AD), which affects one fifth of the world population and often starts in infancy, is characterized by a dysfunctional skin barrier, inflammation, and a dysbiotic microbiome on lesional skin.[1] Skin barrier impairments may precede AD development, suggesting that preventive strategies could be implemented based on early risk identification.[2,3]

Recent advances have revealed a strong relationship between early-life microbial colonization and the development of the immune system,[4,5] and the associations between the gut and skin microbiome and allergic diseases such as asthma and AD have been extensively studied.[4,6,7]

Although the skin microbiome development is influenced by many factors including genetics, antibiotics, diet, and environmental exposures, maternal transfer during delivery is the most obvious source of a newborn skin microbial colonization.[8] While studies have addressed the impact of delivery mode, in particular caesarean section (CS), on the microbiome, few distinguished between elective and acute/emergency CS and the possible role of various delivery modes on the development of allergic diseases remains unclear.[9–11]

In AD, lesional skin is typically colonized by *Staphylococcus aureus*, whose abundance decreases upon treatment as skin microbial diversity is restored.[12–16] While *S. aureus* outgrowth seems to contribute to disease recurrence it remains unclear whether it is first a consequence or a cause of AD. In contrast, skin colonization by commensal *Staphylococci* species has been associated with lower incidence of AD in babies, suggesting these bacteria may also play a role in the disease development.[17] High transepidermal water loss (TEWL) is also characteristic of AD, reflecting an impaired skin barrier, both in lesional and non-lesional skin.[18] Few studies have investigated the relationship between TEWL and skin microbiome and to our knowledge there are no previous human studies focusing on infants.

In this context, we hypothesized that the skin microbiome in early life may be associated with the later development of allergic diseases. To verify this we explored associations between the skin bacterial communities of the lateral upper arm and variables covering atopic dermatitis and skin barrier at four timepoints in the first year of life. To gain further insight into the determinants of the skin microbiome in infancy, we also assessed associations with delivery mode, genetics, environmental and parental factors, and breastfeeding. Vaginal delivery (VD) and CS have different impact on the skin microbiome at birth, and we further hypothesize that different types of VD and CS may also differently affect it. In particular, we set out to compare normal VD to VD in water, elective CS and emergency CS, the main difference between the two later being the level of exposure to the vaginal flora.

To our knowledge this is the largest longitudinal study assessing the skin microbiome in the first year of life and the first to investigate the impact of delivery mode while considering VD in water and distinguishing between elective and emergency CS.

2 | METHODS

See Appendix S1 for detailed methods.

2.1 | Study population

The present study included 346 newborns selected from the Preventing atopic dermatitis and allergies in children (PreventADALL) study,[19] a population-based randomized control trial (RCT) and observational study enrolling 2396 mother–child pairs in Oslo, Østfold (both Norway) and Stockholm, Sweden. Children were recruited antenatally and randomized at birth into four groups: observation only (no intervention), food intervention (early introduction of peanut, milk, wheat, and egg from 3 to 6 months), skin intervention (oil bath and facial cream from 2 weeks to 9 months), or both food and skin interventions.[20,21] The primary outcomes of the study was the prevention of food allergy at 3 years and the prevention of AD at 1 year. The present nested study includes the first participants from whom clinical data and skin microbiome samples were available longitudinally. The study size was estimated to include at least 50 AD patients based on an anticipated prevalence of 23%.[22] Although only participants born in Norway were included in the nested cohort, its characteristics were similar to the ones of the larger RCT (Table S1). Informed written consent was obtained from all pregnant mothers upon inclusion, and again from both parents upon inclusion of the child. The PreventADALL study has been approved by the Regional Ethical Committee for Medical and Health Research Ethics in South-Eastern Norway (2014/518) as well as in Sweden (2014/2242-31/4) by the Regional Ethical Trial Committee of Stockholm. It is an ongoing study that has been approved for data collection until 2044.

2.2 | Microbiome sampling and TEWL measurement

Skin microbiome sampling was performed on the left lateral upper arm using an ESwab Copan 490 CE (Copan Diagnostics, California, USA) during the RCT follow-up sessions at 1 day after birth, 3, 6, and 12 months. TEWL ($g/m^2/h$) was measured at the same location at 3, 6, and 12 months using an open chamber DermaLab USB (Cortex, Hadslund, Denmark).

2.3 | Skin examination

Health personnel examined the skin by visual inspection and palpation as previously described.[23] Eleven predefined skin areas were rated in terms of no, mild, moderate, or severe dry skin.[3] Atopic dermatitis was defined using either the diagnostic criteria of the UK Working Party[24] and/or those of Hanifin and Rajka[25] during examinations at 3, 6, and 12 months.[26] Eczema was defined as the

presence of eczematous lesions observed by a medical doctor, with the exclusion of differential diagnoses to AD.

2.4 | Bacterial 16S rRNA gene amplicon sequencing

Skin bacterial communities were assessed by high-throughput sequencing of the V4 hyper-variable region of the bacterial 16S rRNA gene as previously described,[27] following the alternate protocol "Bacterial 16S rRNA sequencing of bacterial communities present on the surface of mouse or human skin". Samples were processed in 16 sequencing runs on an Illumina MiSeq platform using Paired End (PE) v2 2×250 chemistry. Raw nucleotide sequences were deposited on the European Nucleotide Archive database under accession number PRJEB42268.

2.5 | Investigation variables

Investigation variables were selected from the PreventADALL databank to cover the skin barrier integrity (including AD, eczema, dry skin, and TEWL), delivery mode, genetics, environmental and parental factors, breastfeeding, and RCT intervention. These data are stored exclusively at the Service for Sensitive Data at University of Oslo in accordance with the Norwegian legislation on personal data protection. Only variables showing at least 10 individuals per group with corresponding skin microbiome samples available at all four timepoints were kept (Figure S3A). Food allergy, determined by skin prick test for peanut, milk, wheat, or eggs at 36 months, was observed in 12 subjects yet did not meet this criteria, yet it possible association with skin microbiome was assessed separately. Missing values were imputed (R package missForest) and individuals were grouped into two clusters based on skin barrier integrity data using partitioning around medoids (R package fpc) on multiple correspondence analysis (MCA) dimensions (R package FactoMineR).

2.6 | Data analysis

Analyses were performed using R and RStudio. The entire code is available in Appendix S1 (data_analysis.zip) as well as at https://github.com/alexisrapin/skin-microbiome-and-atopic-dermatitis-in-babies and is archived on Zenodo (DOI: https://doi.org/10.5281/zenodo.7660269).

2.6.1 | Amplicon sequences processing and quality control

Bacterial 16S rRNA gene amplicon sequences were processed into a table of amplicon sequence variants (ASV) using the dada2 pipeline (R package dada2 version 1.18.0).[28] Samples with fewer than 20,000 reads were excluded from the dataset (Figure S1A) and ASVs below 1% prevalence or unassigned at Phylum level were filtered out. Reads were rarefied at 20,000 reads per sample using random sampling without replacement (hereafter referred to as the rarefied ASV counts) and a Hellinger transformation was applied (hereafter referred to as the transformed ASV counts). Rarefaction analysis showed a sufficient sequencing depth to detect most taxa (Figure S1B). Eleven samples collected at 6 and 12 months and displaying a diversity lower than the minimum observed at day 1 were also removed (Figure S1C,D). These samples were not associated with any sign of disease, based on the collected clinical data (Table S2). *Burkholderiaceae* was identified as a potential contaminant based on 11 negative PCR controls that led to read assignments (encompassing 717,721 reads assigned to 300 ASVs, 231 of which found in less than three samples and only eight of which found in more than five samples) (Figure S2). Given the relatively high proportion of this taxa in samples collected at day 1, it was not ignored when comparing bacterial communities between ages yet was ignored, together with ASVs assigned to chloroplasts and mitochondria, in all associative analyses.

2.6.2 | Statistical analysis and visualization

All boxplots represent first and third quartiles, with the median as a middle line and whiskers at the last value within a 1.5×IQR distance respectively from the upper or lower quartile, where IQR is the interquartile range. Lines in all violin plots represent the first, second, and third quartiles.

Evolution of the skin microbiome in the first year of life

The skin bacterial community composition was compared across ages using perMANOVA accounting for repeated sampling, on complete longitudinal sample sets ($n = 124$). Non-metric multidimensional scaling (NMDS) analysis was applied on the transformed ASV counts. Shannon diversity index (SDI) and richness were assessed based on rarefied ASV counts. A random forest (RF) classifier was trained to predict the age and extract associated features based on transformed ASV counts (R package Boruta).

Associations between skin microbiome and investigation variables

Associations between the skin bacterial community composition and categorical investigation variable was assessed separately at each age based on imputed values using perMANOVA in an additive model accounting for interactions with delivery mode and RCT interventions (day 1: $n = 225$, 3 months: $n = 273$, 6 months: $n = 242$, 12 months: $n = 230$). For each variable showing a significant association with the skin bacterial community composition ($p < .05$), associated ASVs were identified in a two-step process including a first feature selection based on truncated the Kruskal–Wallis test for zero-inflated data[29] ($p < .01$) and second a sparse partial least squares discriminant analysis (SPLS-DA) (R package spls).[30] Associations between ASVs and TEWL was assessed separately at each age using sparse partial least squares (SPLS) regression (R

package spls). Associations between food allergy and the skin bacterial community composition and diversity was assessed separately.

Associations between skin microbiome and delivery mode

Constrained correspondence analysis (CCA) based on transformed ASV counts and constrained along age was applied and its first component was used to compare samples collected from babies born through different delivery modes. SDI and richness based on rarefied ASV counts, as well as TEWL were compared between delivery modes. Correlations between SDI at day 1 and TEWL were assessed within samples from the same delivery modes using the Kendall rank coefficient.

3 | RESULTS

3.1 | Microbiome and investigation variables

Following quality filtering, 16S rRNA gene amplicon sequences were available from skin swabs of 346 individuals all born in Oslo and Østfold, Norway, representing 225 to 273 samples for each of the four sampling timepoints and 124 complete longitudinal sets (Figure 1 and Figure S3B; Table 1 and Table S3). Infants were born at a mean (SD, min–max) gestational age of 39.7 (1.5, 35.2–42.9) with a mean (SD, min–max) birth weight of 3.6 (0.5, 2.0–5.6) kg and 45% were females. PCR controls did not pass the quality control, yet allowed identification of *Burkholderiaceae* as the main potential contaminant (Figure S2). In total, 2730 ASVs were observed, encompassing 21 bacterial phyla with *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* being the most abundant (Figure 2A,B).

Investigation variables included 30 categorical variables with at least 10 individuals per group, plus TEWL measurement collected at same location and time as microbiome samples (Figure S4 and Table 1 and Tables S4–S6). Individuals were grouped into two clusters globally reflecting impaired or intact skin barrier (Figure S5A). The first cluster was characterized by higher frequencies of eczema and AD as well as higher TEWL at 3 and 6 months (Figure S5B,C).

3.2 | Evolution during the first year of life

Age influenced the composition and diversity of skin bacterial communities in the first year of life (Table S7). In particular, the relative abundance of *Burkholderiaceae* and *Staphylococcus*, highly abundant after birth, decreased progressively, to the benefit of newly colonizing taxa such as *Streptococcus*, *Veillonella*, and *Enhydrobacter aerosaccus* (Figure 2A).

A core group of 1018 ASVs was detected at all timepoints (Figure 2B). While 61 and 64 unique ASVs were detected at day 1 and 3 months, respectively, only 7 and 21 ASVs were specific to 6 months and 12 months, respectively. NMDS analysis based on bacterial community composition highlighted a greater dispersion of samples at early timepoints, which then converged between 6 and 12 months (Figure 2C). This was reflected by a decrease in both within-age inter-individual variations and within-individual temporal variations (Figure S6A), and an increase in bacterial diversity with age (Figure 2D and Figure S6B,C). These variations in composition were sufficient to predict the age, as demonstrated by training a RF classifier with ROC curves AUC ranging from 0.85 for 6 months to 0.99 for day 1 (Figure S6D). Temporal patterns of colonization showed

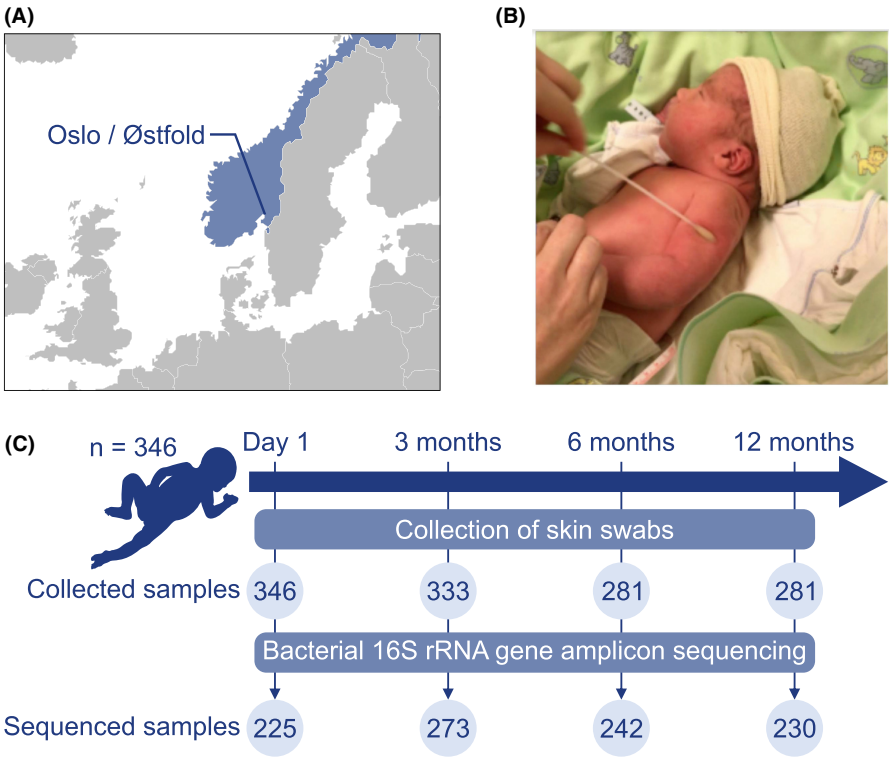


FIGURE 1 Skin microbiome samples from the first year of life were collected in a nested longitudinal study on babies born in the Oslo area in Norway. (A) All subjects were born in the Oslo area in Norway. (B) Microbiome samples were collected on the left lateral upper arm. (C) Skin swabs were collected longitudinally and the bacterial community composition was assessed using 16S rRNA gene amplicon sequencing.

TABLE 1 Summary of cohort characteristics and investigation variables.

Factor	Variable	Label	*N* (%)	Available (%)	Missing (%)
Sex	Sex	F	145 (44%)	326 (100%)	0 (0%)
		M	181 (56%)		
Delivery mode	Delivery mode	VD	248 (76%)	323 (99%)	3 (1%)
		VD in water	27 (8%)		
		Emergency CS	31 (10%)		
		Elective CS	17 (5%)		
Environment	Birth location	Oslo	294 (90%)	326 (100%)	0 (0%)
		Ostfold	32 (10%)		
	Dog	Yes	33 (10%)	261 (80%)	65 (20%)
		No	228 (70%)		
	Cat	Yes	30 (9%)	258 (79%)	68 (21%)
		No	228 (70%)		
Skin barrier	Dry skin at 3 months	Yes	207 (63%)	326 (100%)	0 (0%)
		No	119 (37%)		
	Dry skin at 6 months	Yes	244 (75%)	307 (94%)	19 (6%)
		No	63 (19%)		
	Dry skin at 12 months	Yes	146 (45%)	289 (89%)	37 (11%)
		No	143 (44%)		
	Dry skin on limbs at 3 months	Moderate–severe	16 (5%)	206 (63%)	120 (37%)
		Mild	97 (30%)		
		None	93 (29%)		
	AD by 12 months	Yes	23 (7%)	326 (100%)	0 (0%)
		No	303 (93%)		
	AD by 36 months	Yes	63 (19%)	326 (100%)	0 (0%)
		No	263 (81%)		
	Possible AD	Yes	110 (34%)	288 (88%)	38 (12%)
		No	178 (55%)		
	Eczema at 3 months	Yes	34 (10%)	326 (100%)	0 (0%)
		No	292 (90%)		
	Eczema at 6 months	Yes	61 (19%)	307 (94%)	19 (6%)
		No	246 (75%)		
	Eczema at 12 months	Yes	45 (14%)	289 (89%)	37 (11%)
		No	244 (75%)		
	Eczema by 12 months	Yes	102 (31%)	288 (88%)	38 (12%)
		No	186 (57%)		
	TEWL at 3 months (Qs)	(1.47, 4.48)	64 (20%)	257 (79%)	69 (21%)
		(4.48, 5.97)	64 (20%)		
		(5.97, 8.1)	61 (19%)		
		(8.1, 46.2)	68 (21%)		
	TEWL at 6 months (Qs)	(0.6, 4.57)	67 (21%)	285 (87%)	41 (13%)
		(4.57, 5.93)	71 (22%)		
		(5.93, 7.53)	71 (22%)		
		(7.53, 39.4)	76 (23%)		

TABLE 1 (Continued)

Factor	Variable	Label	N (%)	Available (%)	Missing (%)
	TEWL at 12 months (Qs)	(1, 4.1)	50 (15%)	208 (64%)	118 (36%)
		(4.1, 5.27)	55 (17%)		
		(5.27, 6.47)	51 (16%)		
		(6.47, 24.1)	52 (16%)		
Breastfeeding	Breastfeeding at 6 months	Yes	261 (80%)	293 (90%)	33 (10%)
		No	32 (10%)		
Genetics	FLG mutation	Yes	20 (6%)	274 (84%)	52 (16%)
		No	254 (78%)		
Parental	Maternal AD	AD	50 (15%)	276 (85%)	50 (15%)
		Other atopy	118 (36%)		
		No atopy	108 (33%)		
	Paternal AD	AD	23 (7%)	281 (86%)	45 (14%)
		Other atopy	110 (34%)		
		No atopy	148 (45%)		
	Maternal allergic rhinitis	Allergic rhinitis	60 (18%)	253 (78%)	73 (22%)
		Other atopy	85 (26%)		
		No atopy	108 (33%)		
	Paternal allergic rhinitis	Allergic rhinitis	67 (21%)	258 (79%)	68 (21%)
		Other atopy	43 (13%)		
		No atopy	148 (45%)		
	Maternal asthma	Asthma	42 (13%)	288 (88%)	38 (12%)
		Other atopy	138 (42%)		
		No atopy	108 (33%)		
	Paternal asthma	Asthma	31 (10%)	289 (89%)	37 (11%)
		Other atopy	110 (34%)		
		No atopy	148 (45%)		
	Maternal food allergy	Food allergy	35 (11%)	256 (79%)	70 (21%)
		Other atopy	113 (35%)		
		No atopy	108 (33%)		
	Paternal food allergy	Food allergy	25 (8%)	273 (84%)	53 (16%)
		Other atopy	100 (31%)		
		No atopy	148 (45%)		
Skin and dietary intervention	RCT group	1 – Observation	83 (25%)	326 (100%)	0 (0%)
		2 – Food intervention	76 (23%)		
		3 – Skin intervention	99 (30%)		
		4 – Food & skin intervention	68 (21%)		

Note: Factor and Variable: type and description of the investigation variable. Label: variable label. *N* (%): number and percentage of available individuals per label. Available (%): total number and percentage of available individuals. Missing (%): total number and percentage of missing values. Percentages are rounded to the nearest integer.

substantial differences (Figure 2E and Figure S7). For example, *Lactobacillus iners* were most abundant at day 1 and dropped afterward. *Enhydrobacter aerosaccus* were in low abundance at day 1 and increased thereafter, while certain *Streptococcus* and *Acinetobacter* gradually increased from birth to 6 and 12 months, respectively. In comparison, *Bifidobacterium* and *Veillonella* showed transient peaks at 3 and 6 months, respectively.

3.3 | Associations with atopic dermatitis, skin barrier, and other variables

Twenty significant associations ($p < .05$) between the skin bacterial composition and investigation variables were identified, which included the delivery mode and the birth location, but not the sex, the defined skin barrier clusters, nor the RCT interventions (Figure 3A

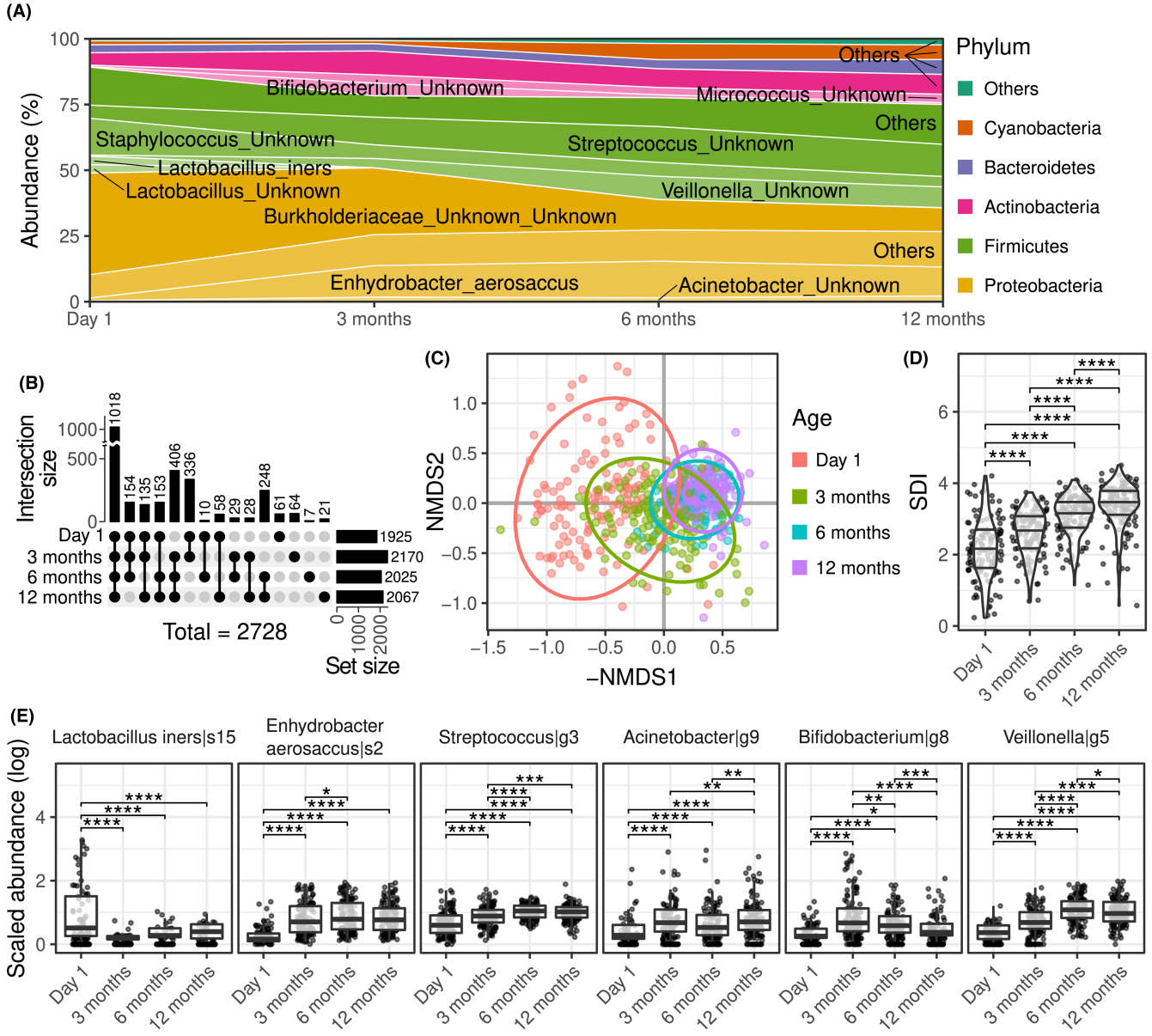


FIGURE 2 The skin bacterial community shows strong temporal variations along the first year of life. (A) General overview of the evolution of the relative abundances of skin bacterial Phyla and Species with age. Phyla are represented in different colors and Species are represented in different shades. Phyla and Species showing a maximal relative abundance lower than 2% across all ages are included as "Others". The deepest resolved taxonomic classification is included in the label of unresolved Species and deeper unresolved taxonomic levels are labeled as "Unknown". (B) Number of different skin bacterial amplicon sequence variants (ASVs) observed at different ages. (C) Non-metric multidimensional scaling (NMDS) analysis of the skin bacterial community, with 95% data ellipses shown for each age. (D) Skin bacterial diversity across age represented in terms of Shannon diversity index (SDI). (E) Relative abundance of selected ASVs across ages shown as rarefied, standardized, scaled, and log-transformed read counts. Boxplots are drawn on non-zero values only. All plots represent complete longitudinal sample sets across timepoints ($n = 124$). Statistical significance assessed by paired Wilcoxon test with p-values adjusted for multiple comparisons using the Benjamini and Hochberg method. ns: $p > .05$ (not shown), *: $p \leq .05$, **: $p \leq .01$, ***: $p \leq .001$, ****: $p \leq .0001$.

and Table 2 and Table S8). Among these associations, nine reflected variations in skin bacterial diversity, including birth location, dry skin, AD as well as maternal AD, and food allergy (Figure 3B and Figure S8A). Seven variables were found associated with sets of 10–187 ASVs at day 1 and 3, 6 or 12 months (Table 2 and Table S9): Birth location was associated with 86, 59, and 22 taxa at day 1, 3 months, and 6 months, respectively, including a same *Betaproteobacteriales* at both day 1 and 3 months (Figure 3C and Figure S9). The presence of dogs during pregnancy was associated with 10 and 11 ASVs at 3 and 6 months, respectively. This included higher abundances and frequencies of *Betaproteobacteriales* and *Ralstonia* at 3 months (Figure 3C and Figure S10). Breastfeeding was associated with 15 and 18 ASVs at 3 and 6 months, respectively, including lower abundances and frequencies of members of the *Prevotellaceae* family at

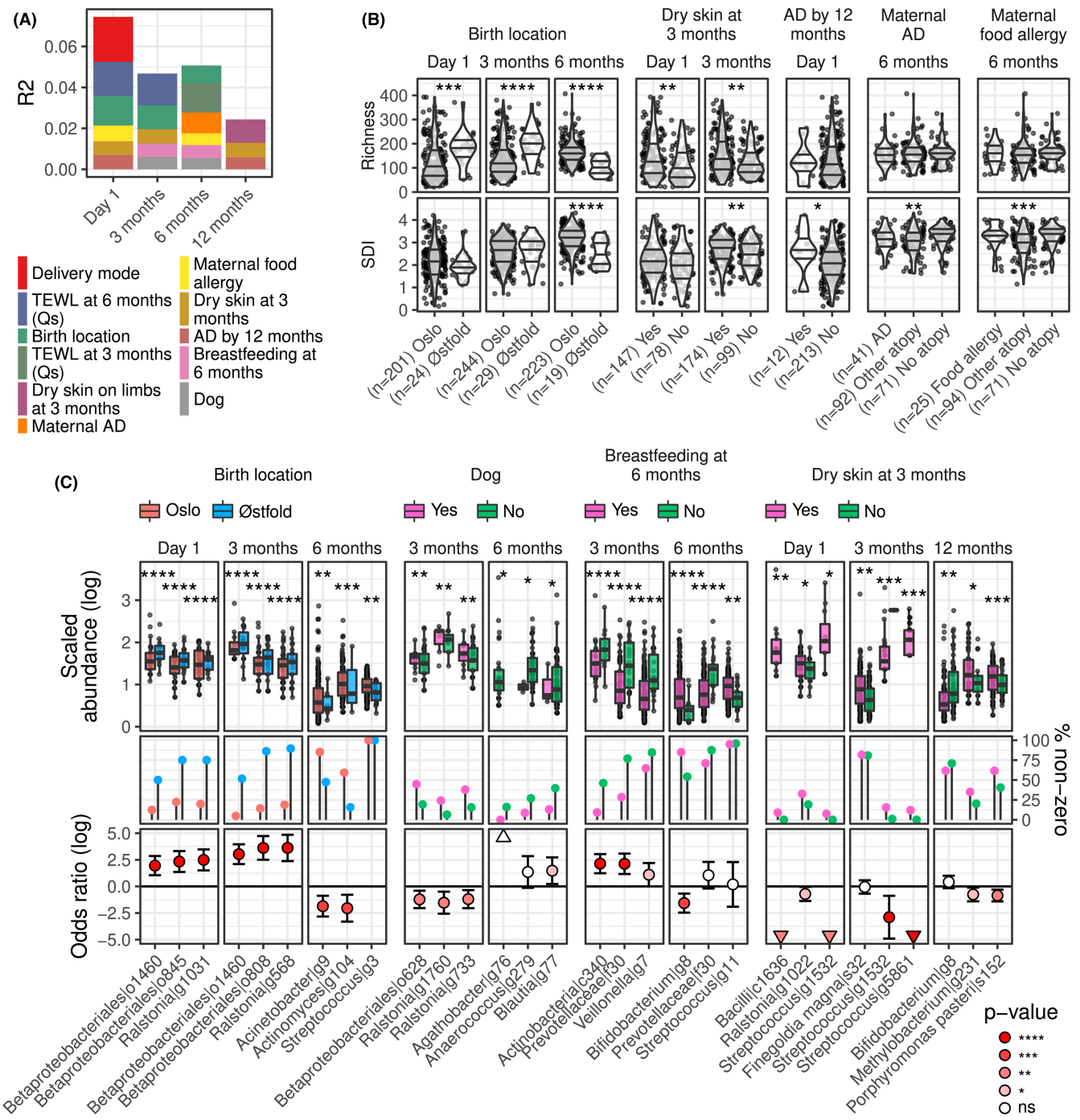


FIGURE 3 The skin bacterial community is associated with delivery mode, environment, skin health, breastfeeding, and parental factors. (A) Proportions of skin bacterial community composition variance explained by investigation variables. Contributions shown for variables significantly associated with skin bacterial community composition ($p < .05$), as assessed by perMANOVA independently at each time point. (B) Association between skin bacterial diversity in terms of richness and Shannon diversity index (SDI), and variables significantly associated with skin bacterial community composition ($p < .05$). Statistical significance assessed by the Kruskal–Wallis test. (C) Relative abundance of a curated set of amplicon sequence variants (ASV) selected by truncated the Kruskal–Wallis test ($p < .01$) and sparse partial least squares discriminant analysis (SPLS-DA). ASV abundance shown as rarefied, standardized, scaled, and log-transformed read counts. Boxplots in the top facet are based on non-zero abundances, with statistical significance assessed by the Wilcoxon test. Percentages and odds ratio of non-zero values are shown below, with statistical significance assessed by the Fisher's exact test and whiskers depicting a 95% confidence interval. ns: $p > .05$ (not shown), *: $p \leq .05$, **: $p \leq .01$, ***: $p \leq .001$, ****: $p \leq .0001$. All plots represent independent analyses at each time point (Day 1: $n = 225$, 3 months: $n = 273$, 6 months: $n = 242$, 12 months: $n = 230$).

TABLE 2 Summary of evidence for associations between investigatory variables and the skin microbiome.

Age	Factor	Variable	Comp.	SDI	Richness	TEWL	Ass. taxa
Day 1	Delivery mode	Delivery mode	***	**	ns	NA	187
	Environment	Birth location	****	ns	***	NA	86
	Skin barrier	AD by 12 months	*	*	ns	NA	0
		Dry skin at 3 months	*	ns	**	NA	24
		TEWL at 6 months (Qs)	*	ns	ns	NA	15
	Parental	Maternal food allergy	**	ns	ns	NA	121
3 months	Environment	Birth location	****	ns	****	ns	59
		Dog	**	ns	ns	ns	10
	Breastfeeding	Breastfeeding at 6 months	**	ns	ns	ns	15
	Skin barrier	Dry skin at 3 months	**	**	**	ns	59
		TEWL at 6 months (Qs)	**	ns	ns	NA	21
6 months	Environment	Birth location	***	****	****	****	22
		Dog	*	ns	ns	*	11
	Breastfeeding	Breastfeeding at 6 months	**	ns	ns	ns	18
	Skin barrier	TEWL at 3 months (Qs)	*	ns	ns	NA	0
	Parental	Maternal AD	*	**	ns	ns	0
		Maternal food allergy	*	***	ns	ns	187
12 months	Skin barrier	AD by 12 months	*	ns	ns	ns	0
		Dry skin at 3 months	**	ns	ns	ns	23
		Dry skin on limbs at 3 months	*	ns	ns	ns	0

Note: Age: age at microbiome sampling, Factor and Variable: type and description of the investigation variable, Comp.: *p*-values derived from groups comparisons of the skin bacterial community composition based on permutational multivariate analysis of variance (perMANOVA) (Table S8), SDI, and Richness: the skin bacterial diversity in terms of Shannon diversity index (SDI) and richness (Figure S8A), TEWL: trans-epidermal water loss (TEWL) (Figure S8C), Ass. taxa: number of skin bacterial taxa associated with the investigatory variable, identified based on truncated the Kruskal–Wallis test and sparse partial least squares discriminant analysis (SPLS-DA) (Table S9). SDI, Richness and TEWL statistical significance assessed by the Kruskal–Wallis test. ns: $p > .05$, *: $p \leq .05$, **: $p \leq .01$, ***: $p \leq .001$, ****: $p \leq .0001$.

Abbreviation: NA, not available.

both timepoints (Figure 3C and Figure S11). Dry skin observed at 3 months was associated with 24, 59, and 23 ASVs at day 1, 3 months, and 12 months, respectively, including higher abundances and frequencies of *Streptococcus* at both day 1 and 3 months (Figure S12). Maternal food allergies were associated with 121 and 187 ASVs at day 1 and 6 months, respectively, including higher representations of *Prevotella* and *Alloprevotella* at day 1 and 6 months, respectively (Figure S13). Measurement of TEWL at 6 months was associated with 15 and 21 ASVs at day 1 and 3 months, respectively, including *Ralstonia* taxa which representations correlated with higher TEWL (Figure S14). Among the variables associated with the skin bacterial community, five were also associated with TEWL: birth location, the presence of dogs during pregnancy, dry skin on limbs at 3 months of age, AD, and maternal food allergy (Table 2 and Figure S8C). SPLS regression did not identify any strong association between ASV abundance and TEWL (Figure S15). Seven associations between microbiome composition and investigation variables that involved interactions between either delivery mode or RCT intervention were observed (Table S8). Food allergy was found associated with the skin bacteria community composition, as well as with higher TEWL, at 3 months (Table S11 and Figure S19).

3.4 | Comparisons between delivery modes

Differences in skin bacterial community composition were observed in the first day of life between infants born either through VD, VD in water, emergency CS or elective CS ($p < .05$). A significant association was also observed between delivery mode and the skin bacterial community at 6 months (Table 2 and Table S8). The skin bacterial community of babies born by CS and VD in water were, on day 1, more similar to communities found at 3 months, as estimated by age-constrained CCA (Figure 4A,B and Figure S16A). At day 1, lower SDI was found in babies born through emergency and elective CS compared to VD and VD in water (Figure 4C). One hundred eighty seven ASVs were associated with different delivery modes at day 1 (Table 2 and Table S9), including *Lactobacillus*, *Staphylococcus*, and *Pseudomonas* found underrepresented in elective CS as well as a *Streptococcus* found in higher abundance in elective CS and VD in water compared to normal VD (Figure S16C). Inter-individual variations were observed in the pattern of colonization at this age, and no consistent, global combinations of taxa associated with specific groups were observed (Figure S17).

At 3 months, higher TEWL was found in babies born through emergency CS compared to babies born through elective CS

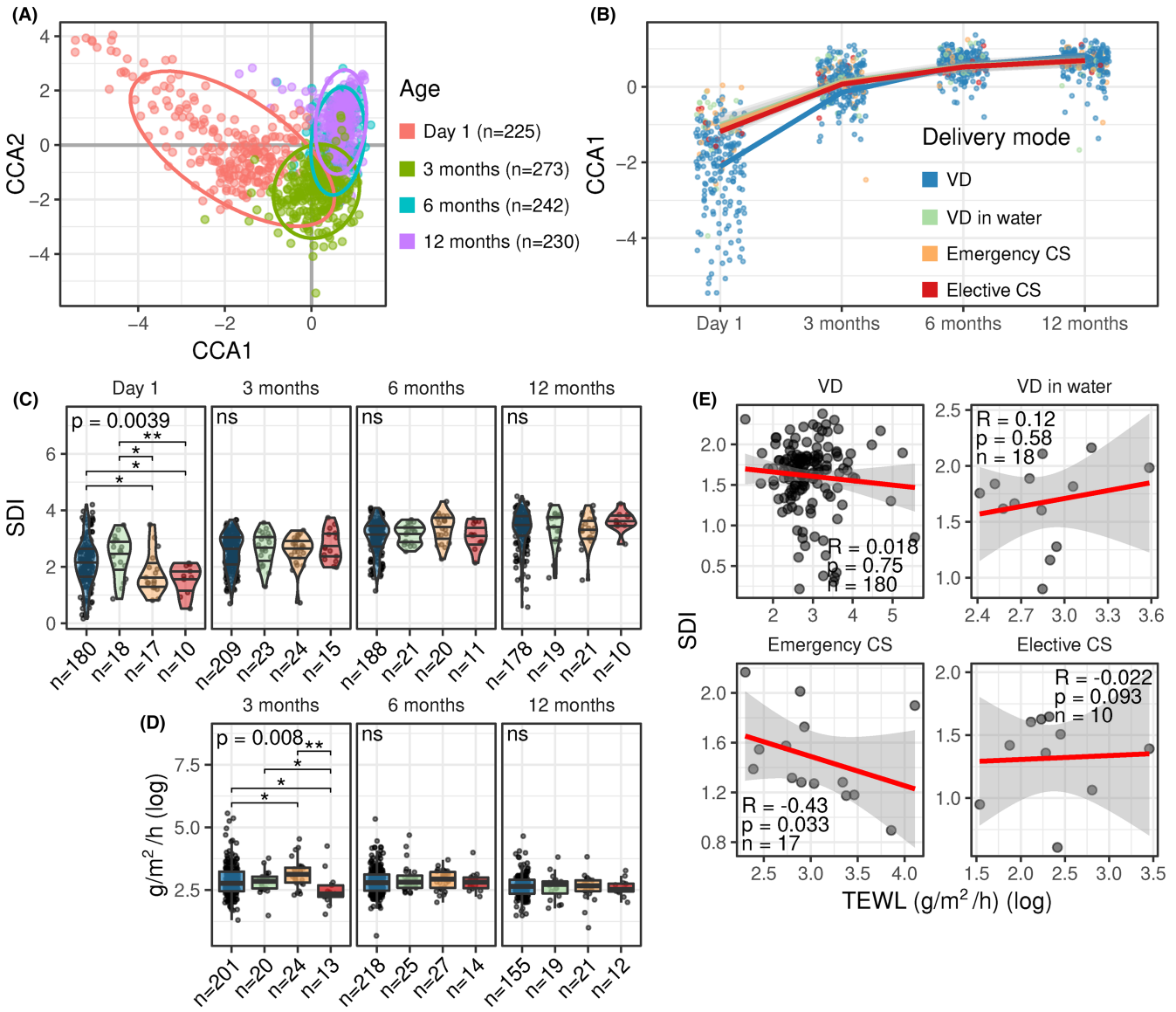


FIGURE 4 The delivery mode influences the skin bacterial community in the first day of life. (A) Constrained correspondence analysis (CCA) of the skin bacterial amplicon sequence variants (ASVs) relative abundances constrained by age. (B) Maturation of the skin microbiome of subjects born through different delivery modes depicted as a fitted negative exponential curve on the first CCA component from (A) along age. (C) Skin bacterial diversity in terms of Shannon diversity index (SDI) and (D) transepidermal water loss (TEWL) across age and different delivery modes. Statistical significance assessed by the Kruskal–Wallis test and post-hoc Wilcoxon test with *p*-values adjusted for multiple comparisons using the Benjamini and Hochberg method. ns: $p > .05$ (not shown), *: $p \leq .05$, **: $p \leq .01$, ***: $p \leq .001$, ****: $p \leq .0001$. (E) Correlation between TEWL at 3 months and SDI at day 1. Correlation assessed by the Kendall rank coefficient.

(Figure 4D). In emergency CS, the skin bacterial diversity at birth was correlated with TEWL values observed at 3 months – lower SDI being associated with higher TEWL, and thus weaker skin barrier function (Figure 4E).

Caesarean section, and in particular elective CS, was associated with a higher frequency of eczema in the first year of life. A similar trend was observed for AD and the individuals cluster reflecting weaker skin barrier, although this trend was lost when considering AD diagnosed by 3 years of age. Dry skin was observed in all infants born through elective CS (Figure S18 and Table S10).

4 | DISCUSSION

4.1 | The skin microbiome evolves in the first year of life

In line with previous studies, we show that the skin microbiome of babies changes enormously in the first year of life, following a gradual maturation characterized by the loss of some key members of the vaginal microbiome, such as *Lactobacillus*, and increasing diversity (Figure 5A).[31–33]

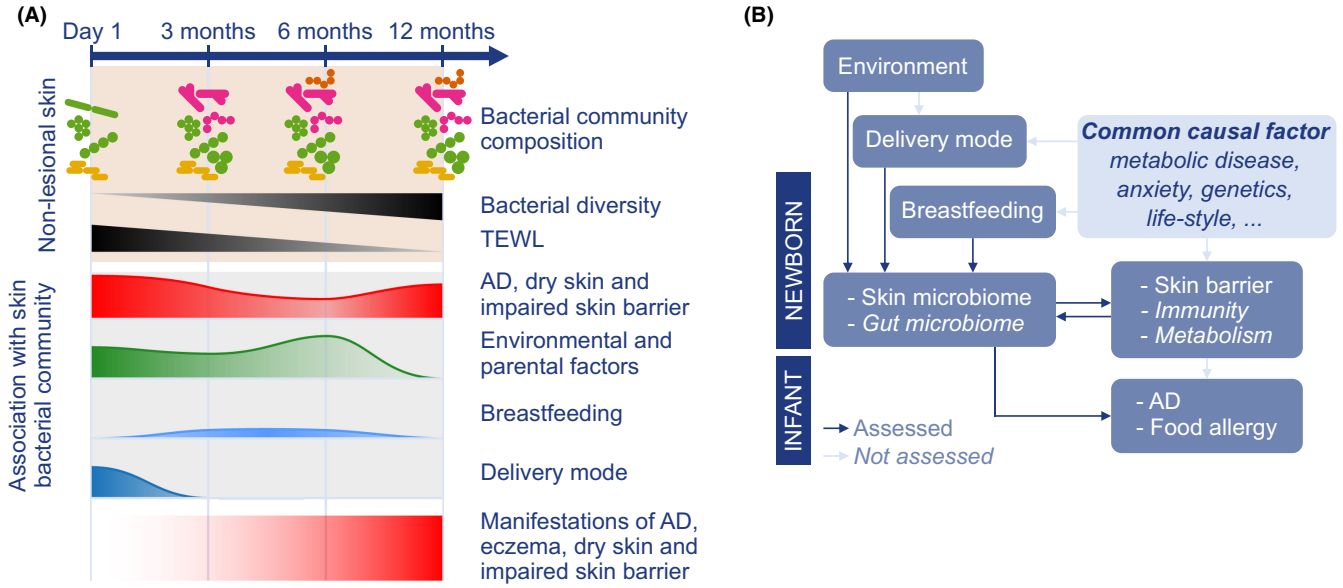


FIGURE 5 Summary of results and possible causal links involving the skin microbiome. (A) Qualitative summary of the key features observed in non-lesional skin and the evidence for associations between the skin bacterial community and investigation variables along age. (B) Proposed causal links between the skin microbiome in the first year of life, the environment, delivery mode, breastfeeding, skin barrier, atopic dermatitis (AD), and allergic diseases. Shaded elements were not investigated in the present study.

4.2 | The skin microbiome is associated with AD and skin barrier integrity

Variations in the skin bacterial community in early life were associated with later manifestations of AD, dry skin, and reduced skin barrier integrity (Figure 5A and Table 2) as well as food allergy (Table S11), suggesting that the skin microbiome in early life may play an important role for the later development of allergic disease. Given that (1) the skin barrier is weaker at birth and strengthen with age and (2) the skin at birth constitutes an empty niche which can be seeded by distinct sets of microbes, we hypothesize that the skin of newborn, and their immune system, is likely to be significantly impacted by microbial colonization. Large differences between individuals may therefore be expected, perhaps leading some to get primed for the later development of AD (Figure 5B). Our results suggest that the freshly seeded newborn skin constitutes an ecosystem that converges toward stability and reduced inter-individual variations, which altogether may explain why the immature microbiome of newborns is more easily associated with AD than the more mature one of 1 year old babies. The role of the microbiome at other sites, like the gut, as well as the interplay with the immune system and metabolism, remain to be investigated.

Our finding of associations between bacterial community composition and dry skin at any location and specifically on the limb extensors is novel. The observation of a higher skin bacterial diversity in newborns and 3-month-old infants with dry skin contrasts with a previous report where emollients increased the skin microbial diversity in AD patients, who are prone to dry skin.[34] However, most of the participants presenting dry skin in the present study did not develop AD before 12 months. The association between skin bacterial communities and diagnosed AD in the first year of life is in line with previous findings from a smaller Polish infant study[35] and studies including older children and adults.[12,36–38] No specific bacterial taxa was found associated with AD, which is supported by previous findings in the Polish study,[35] yet contrasts with other findings in infants, where the commensal *Staphylococcus epidermidis* was found protective against AD,[17] as well as in older populations,[36,38] where *Staphylococcus aureus* was associated with both lesional and non-lesional skin in AD. Although based on a small sample size (nine positive skin prick tests at 36 months), our results suggest a link between the skin microbiome composition, an impaired skin barrier function at 3 months, and the development of food allergy, which is compatible with the dual allergen exposure hypothesis.[39]

4.3 | The skin microbiome is associated with environmental and parental factors and breastfeeding

Associations involving environmental or parental factors, including birth location, pets, and parental allergic diseases were observed from birth to 6 months. Infants born at different locations showed strong differences in skin bacterial populations from day 1 to 6 months, which we hypothesize may be due to differences in the bacteria found in the hospital environment.

The finding that the presence of dogs during pregnancy was linked to variations in the skin microbiome of 3- and 6-month-old infants is novel. Interestingly, a previous study on the same cohort showed that the presence of pets during pregnancy decreased the risk of dry skin at 3 months. Although these results may reflect the colonization by part of the animal microbiome, a study reporting a

protective effect against atopic dermatitis from pets and farm animals during pregnancy suggests it may be mediated by the stimulation of the newborn innate immunity.[3,40]

The associations found between breastfeeding until 6 months and certain taxa at 3 and 6 months are in line with a previous smaller study and may reflect either the direct contact of the mother's milk on the skin, or an indirect mechanism involving the infant metabolism and immunity.[41] Interestingly, this association seems transient and limited to the typical period of breastfeeding, as weaning often occurs before 12 months.

4.4 | Delivery mode shapes the skin microbiome at birth

In line with previous findings, we confirm that delivery mode impacts on the skin bacterial community at birth.[42–44] In particular, vaginally delivered newborns harbored a higher proportion of bacteria belonging to a typical vaginal microbiome (e.g., *Lactobacilli*) and had higher bacterial diversity compared to CS delivered babies. While babies born through elective CS have no direct contact with the mother's vaginal flora, babies born through emergency CS can have various levels of exposure to it, as in this case CS is often performed after the baby started entering the birth canal. To the best of our knowledge, only a few previous microbiome studies differentiated between acute and elective CSs, and none focused on the skin. The present study found no significant difference in bacterial skin diversity between elective and emergency CS. This contrasts with a small study including six newborns reporting a lower bacterial diversity in the feces of babies born through elective compared to emergency CS.[45] We observed a lower *Lactobacillus* abundance in elective CSs compared to emergency CSs, in line with our previous reporting of labor-induced exposure to vaginal flora during emergency CS in the PreventADALL cohort.[46] Interestingly, in the first day of life, the skin bacterial community of babies born through CS or VD in water resembled more a skin microbiome of a 3-month old baby compared to that of vaginally-born babies, whose skin bacterial composition was more clearly distinct from the one of older babies. Overall, this highlights that the skin of vaginally-delivered newborns is mainly seeded by the vaginal flora of the mother while the skin of CS-delivered newborns is seeded by more typical skin bacteria, likely through contact with the mother's skin. We hypothesize that VD in water may have a diluting effect on the bacteria acquired in the birth canal and that a larger contact with fecal microbiota may occasionally occur through contamination of the water. Therefore, babies born this way may exhibit a pattern of bacterial colonization where the place of the vaginal flora is less prominent. Altogether, these findings highlight that all four modes of delivery are associated with distinct patterns of microbial colonization at birth.

To our knowledge, for the first time, we show that the delivery mode impacted on the newborn's skin microbiome and that variations of the latter correlated with variations of the skin barrier function (Figure 4E). However, it is not clear whether the skin microbiome was impacting the skin barrier function, or whether a common causal factor exists which increases the risk of CS delivery while also affecting the newborn's skin barrier, independently of the skin microbiome. This may involve parental factors such as metabolic diseases and anxiety as well as genetic, environmental, and life-style factors that may affect both mothers and children.[47–49]

4.5 | No impact of skin intervention nor genetics were detected in the first year of life

No associations between the RCT interventions and skin bacterial composition was found, which contrasts with other studies reporting higher bacterial diversity in skin treated with emollients.[34,50,51]

Surprisingly, no association between filaggrin functional deficiency and the skin microbiome were detected, despite it being responsible for impaired skin barrier and a major risk factor for AD.[52,53] This suggests that while genetics can shape the skin microenvironment, its importance in the selection of the colonizing bacteria may be out-competed by other factors, such as exposure to different sets of microbes from the mother's flora and the environment.

Overall, our study provides a strong motivation to further investigate the role of distinct skin microbiomes and the role of delivery mode in the development of allergic diseases.

4.6 | Strengths and limitations

The strengths of the present study include its longitudinal and prospective design, its relatively large sample size, detailed clinical information, and TEWL measured at the exact same location as the skin microbiota sampling. The limitations include investigating the skin microbiome at a unique location and the inherent drawbacks of prospective designs, including varying levels of missing values and group size imbalance across investigation variables. Although interactions between investigation variables, delivery mode, and RCT intervention are reported, these were not characterized and further studies on their own will be required in this direction. The present study included only 20 individuals with FLG mutations, which may not be enough to draw conclusions regarding the role of genetics on the skin microbiome. Also, the method used to compare microbiomes is based on relative abundance only and may not detect functional, or small yet biologically relevant variations. Finally, the heterogeneity of AD may impair the detection of association between sub-categories of diseases and particular bacteria. For instance, one could hypothesize that only a subcategory of AD is mainly driven by the colonization of bacteria. In such a case, the AD diagnosis methods used in the present study would fail to distinguish between a more bacterial-driven AD and the other, microbiome-independent cases. Therefore, studies including larger groups of AD patients may be needed to represent the heterogeneity of the disease and discover microbiome patterns associated with its distinct sub-categories.

5 | CONCLUSION

Using a cohort including over 340 infants, we show that the skin bacterial community undergoes dramatic changes during the first year of life, with different sets of taxa colonizing the skin at different timepoints. We confirmed our hypothesis that early life skin microbiome can be associated with later onsets of AD. We also confirm that the delivery mode influences the skin microbiome in the first day of life and that biological differences exist between different types of VD and CS. Our results suggest that while this impact appears transient, it may still play a role in the priming and development of the immune system. Using a data-driven approach, we identified possible other determinants of the skin microbiome in early life, including environmental and parental factors as well as breastfeeding. Overall, our results provide impetus to gain further insights into the mechanisms underlying the development of allergic diseases and hope for the development of preventive strategies.

AUTHOR CONTRIBUTIONS

AR, EMR, CP, KCLC, and BJM designed the nested study. EMR, KCLC, CMJ, LL, BN, KR, HOS, ACS, CS, and RV participated either in the entirety or in part of the conception and design of the PreventADALL study, its conduction and the data collection and preparation. EMR and RV participated in the clinical data collection and preparation for the nested study. AR and CP managed and participated in the processing and sequencing of the microbiome samples. AR, EMR, and MM performed the data analysis. AR, EMR, and MM wrote the main manuscript with inputs from KCLC, NLH, CMJ, LL, AHL, BN, KR, HOS, ACS, CS, NU, RV, and BJM. NLH and BJM provided resources for the microbiome sequencing and data analysis. KCLC is the principal investigator of the PreventADALL cohort. All authors have read, revised and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

EMR reports personal fees for presentations from Sanofi Genzyme, Novartis, Leo Pharma. MEDA and Omega Pharma outside the submitted work.

ORCID

Karin C. Lødrup Carlsen https://orcid.org/0000-0002-9257-1198
Cilla Söderhäll https://orcid.org/0000-0002-8397-3080

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SUPPORTING INFORMATION

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

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