Reduced Skin Microbiome Diversity in Infancy Is Associated with Increased Risk of Atopic Dermatitis in High-Risk Children

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It is currently unknown whether alterations in the skin microbiome exist before development of atopic dermatitis (AD). In this prospective Danish birth cohort of 300 children, we examined whether skin microbiome alterations during the first 2 months of life were associated with an increased risk of AD in the first 2 years and its severity after adjustment for environmental factors and selected skin chemokine and natural moisturizing factor levels. We found no overall association between the skin microbiome at birth and age 2 months and AD during the first 2 years of life. However, when restricting the analysis to children with at least one parent with atopy, a lower alpha diversity at age 2 months was associated with an increased risk of AD (adjusted hazard ratio = 1.7, 95% confidence interval = 1.1−2.6). We observed a stronger association in children where both parents had atopy (adjusted hazard ratio = 4.4, 95% confidence interval = 1.1−18.2). The putative pathogenic role of changes in the skin microbiome on AD risk remains uncertain but may play a role in those with an atopic predisposition.

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INTRODUCTION

Atopic dermatitis (AD) is a common chronic and itchy skin disease in childhood (Langan et al., 2020). It is caused by a complex interplay between epidermal barrier impairment and immune dysfunction. Loss-of-function mutations in the *FLG* gene represent the strongest genetic risk factor in Northern Europeans and Asians (Palmer et al., 2006), but important genetic variants in the T helper 2 signaling immune pathway have also been identified (Paternoster et al., 2011). Together with shared environmental exposures and acquired skin barrier defects, these gene variants may explain why parental atopy, including AD, asthma, and hay fever, is a strong risk factor for AD (Ravn et al., 2020).

In a recently published birth cohort study on 300 term and 150 preterm children, increased skin levels of thymus and activation-regulated chemokine (TARC)/CCL17 and decreased levels of natural moisturizing factor (NMF) collected from the dorsal aspect of the hand in children aged 2 months preceded the onset of AD (Halling et al., 2022). In a subgroup of children (n = 88) from the same cohort, free sphingoid base phytosphingosine levels were strongly reduced in children who later developed AD during the first year of life compared with the levels in those who did not (Rinnov et al., 2023).

It is currently unclear whether the skin microbiome is involved in the etiopathogenesis of AD. Modern lifestyle changes, for example, increased rates of births by cesarean delivery, early and widespread use of antibiotics and cosmetics, use of chemicals for cleaning and washing, as well as urban living, have been associated with microbial dysbiosis (Renz and Skevaki, 2021), which at least in part may explain the increased occurrence of AD and allergic diseases (Akdis, 2021). Only one Irish case-control study of 20 children examined the association between the skin microbiome and the development of AD within the first year of life but found no association between alpha or beta diversity at ages 0, 2, 6, and 12 months and AD (Kennedy et al., 2017). However, a severity-dependent lower microbial diversity and altered

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Abbreviations: AD, atopic dermatitis; CI, confidence interval; EASI, Eczema Area and Severity Index; FDR, false discovery rate; HR, hazard ratio; NMF, natural moisturizing factor; TARC, thymus and activation-regulated chemokine

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Table 1. Baseline Demographics

Demographics	All Children N = 300	Children with Parental Atopy n = 188[1]	Children with No Parental Atopy n = 100[1]
Sex, male, % (n_{cases}/n_{total})	43.0 (129/300)	44.7 (84/188)	40.0 (40/100)
Birth weight (g), mean (SD)	3544.9 (459.6)	3548.5 (470.0)	3537.2 (445.6)
Birth length (cm), mean (SD)	51.9 (2.2)	52.0 (2.3)	51.8 (2.1)
Season of birth, % (n_{cases}/n_{total})			
Spring	21.0 (63/300)	19.2 (36/188)	24.0 (24/100)
Summer	22.3 (67/300)	24.5 (46/188)	19.0 (19/100)
Autumn	31.3 (94/300)	30.3 (57/188)	32.0 (32/100)
Winter	25.3 (76/300)	26.1 (49/188)	25.0 (25/100)
Delivery method, % (n_{cases}/n_{total})			
Vaginal	59.7 (179/300)	63.3 /119/188)	57.0 (57/100)
C-section, planned	27.3 (82/300)	23.4 (44/188)	31.0 (31/100)
C-section, acute	13.0 (39/300)	13.3 (25/188)	12.0 (12/100)
Parental atopy[2] (self-reported), % (n_{cases}/n_{total})	63.3 (153/240)	100.0 (188/188)	0.0 (0/100)
FLG gene mutation, % (n_{cases}/n_{total})	9.1 (27/297)	9.2 (17/185)	8.0 (8/100)
R501X	3.0 (9/297)	2.7 (5/185)	3.0 (3/100)
R2447X	1.3 (4/297)	0.5 (1/185)	3.0 (3/100)
2282del4	4.7 (14/297)	5.9 (11/185)	2.0 (2/100)
Antibiotic use in first 2 months, % (n_{cases}/n_{total})	9.7 (26/267)	12.6 (22/174)	4.3 (4/93)
Topical antibiotics	19.2 (5/26)	18.2 (4/22)	25.0 (1/4)
Nontopical antibiotics	80.8 (21/26)	81.8 (18/22)	75.0 (3/4)
Emollient therapy in first 2 months, % (n_{cases}/n_{total})	32.6 (87/267)	35.6 (62/174)	26.9 (25/93)
Breastfed in first 2 months, % (n_{cases}/n_{total})	95.3 (266/279)	96.7 (174/180)	92.9 (92/99)
High parental education, % (n_{cases}/n_{total})	64.2 (95/265)	71.2 (121/170)	51.6 (49/95)
Domestic dog exposure in first 2 months, % (n_{cases}/n_{total})	5.9 (17/286)	4.8 (9/186)	8.0 (8/100)

Abbreviations: AD, atopic dermatitis; C-section, Cesarian section.

[1] Owing to imputation in a parental history of atopy, these numbers are higher than the actual numbers presented for all children.

[2] Parental atopy defined as self-reported physician-diagnosed AD, asthma, hay fever, and/or self-reported allergy to selected allergens (birch, grass, mugwort, horse, dog, cat, house dust mites, or molds).

microbiome community composition on both lesional and nonlesional skin were found in infants with AD compared with that in healthy controls in the first 30 months of life (Olesen et al., 2022).

Owing to the sparse data on the skin microbiome before the onset of AD, it is currently unknown whether the reduced microbial diversity and altered community composition observed in the skin of patients with established AD is secondary to skin barrier impairment and immune dysfunction or whether skin microbiome alterations precede the onset of AD. In a prospective Danish birth cohort of 300 newborns, we examined whether skin microbiome alterations at birth and age 2 months were associated with an increased risk of onset and severity of AD during the first 2 years of life.

RESULTS

A total of 300 children born to term were included (43.0% male), of whom 95.3% (286 of 300) were followed until the age of 2 years. A total of 9.1% (27 of 297) were *FLG* mutation carriers, and 63.3% (153 of 240) had a parental history of atopy (Table 1). At 2 months of age, a total of 32.6% (67 of 276) of children had used emollients. The presence of *FLG* mutations or a history of parental atopy was more common among children who used emollients than among children with no reported use (*FLG* mutation prevalence was 11.5% vs. 6.7%, and prevalence of history of parental atopy was 70.4% vs. 60.6%).

During the first 2 years of life, 34.6% (99 of 286) developed AD, of whom 23.2% (23 of 99) had moderate-to-severe AD at examination (Supplementary Table S1). The mean Eczema Area and Severity Index (EASI) score was 4.2 (interquartile range = 2.0−7.9), and the mean age of AD onset was 6 months (interquartile range = 3.0−11.0 months). The prevalence of *FLG* mutations in children with AD was 17.3% (17 of 98) compared with 4.3% (8 o 187) in children without AD ($P < 0.001$), and the prevalence of parental history of atopy in children with AD was 78.6% (66 of 84) compared with 55.2% (85 of 154) in those without AD ($P < 0.001$).

The association between skin microbiome alpha diversity at birth and the development of AD

At birth, the mean Shannon index was 2.61 (SD = 0.76) among children who later developed AD and 2.60 (SD = 0.80) among children who did not develop AD during the first 2 years of life ($P = 0.9$) (Supplementary Table S2). There was no association between alpha diversity and subsequent AD during the first 2 years of life in crude analysis (hazard ratio [HR] = 0.97, 95% confidence interval [CI] = 0.74−1.27], $P = 0.8$) or fully adjusted analysis (adjusted HR = 0.97, 95% CI = 0.74−1.27, $P = 0.8$) (Figure 1 and Table 2). Analyses restricted to children with and without parental history of atopy also showed no associations (Figure 1 and Table 2 and Supplementary Table S2).

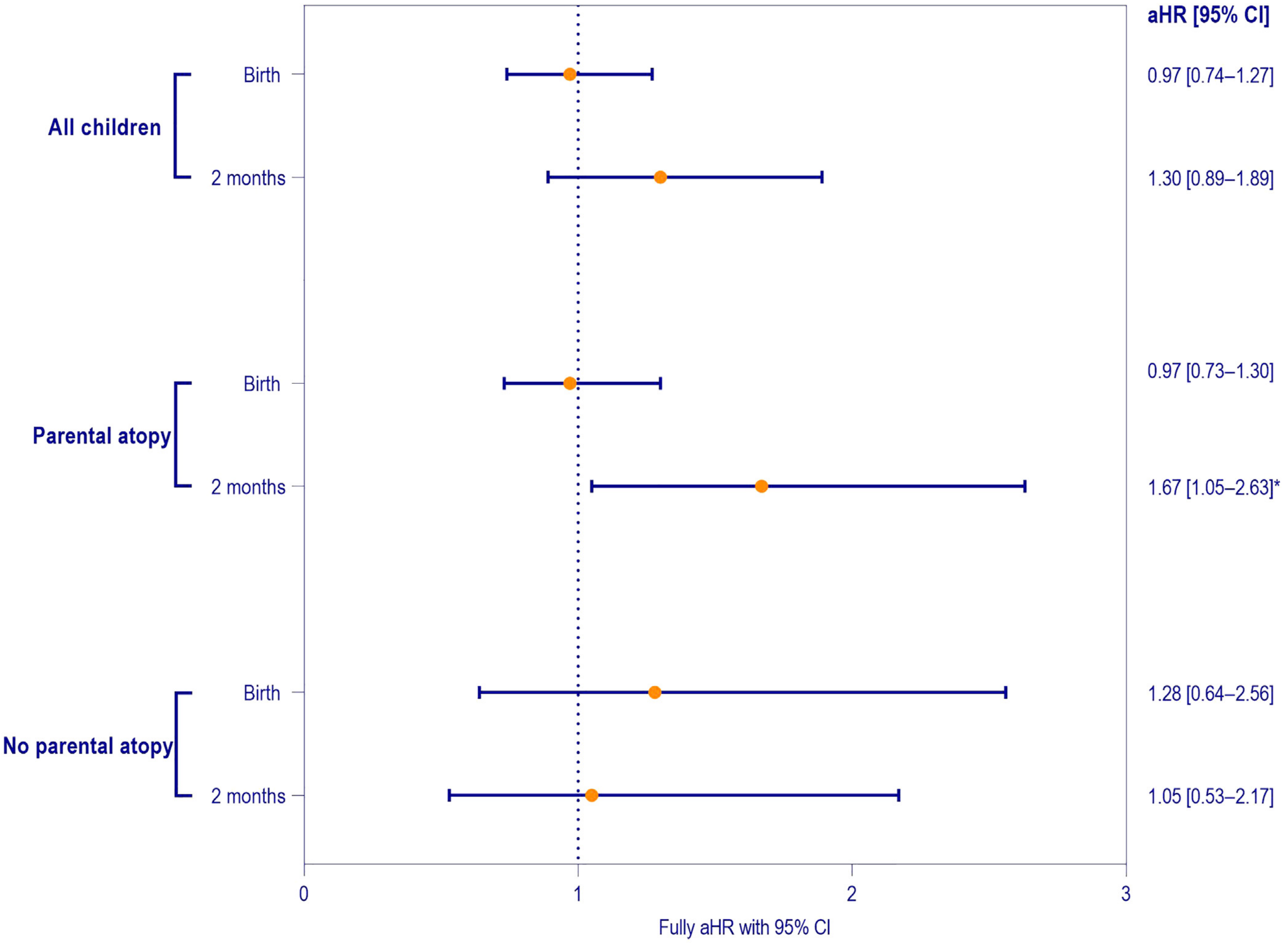

Figure 1. Fully adjusted hazard ratios and 95% CI for the inverse association between alpha diversity in the skin at birth and age 2 months and the risk of atopic dermatitis and stratified by parental history of atopy. aHR, adjusted hazard ratio; CI, confidence interval. *Significant association defined as $P < 0.05$.

The association between skin microbiome alpha diversity at age 2 months and the development of AD

At age 2 months, the mean Shannon index was 2.30 (SD = 0.60) among children who later developed AD and 2.45 (SD = 0.65) among children who did not develop AD during the first 2 years of life ($P = 0.1$) (Figure 2). No inverse association was observed between alpha diversity and development of AD during the first 2 years of life when including all children (crude HR = 1.37, 95% CI = 0.95−1.96, $P = 0.09$; adjusted HR = 1.30, 95% CI = 0.89−1.89; $P = 0.2$) (Figure 1 and Table 2).

In an analysis restricted to children with parental history of atopy, the mean Shannon index was 2.32 (SD = 0.55) for children who later developed AD and 2.57 (SD = 0.65) for children who did not develop AD during the first 2 years of life ($P = 0.02$) (Figure 2). Among these children with parental history of atopy, a lower alpha diversity at age 2 months was associated with an increased risk of AD during the first 2 years of life in crude analysis (HR = 1.64, 95% CI = 1.08−2.05, $P = 0.02$). The association remained significant after adjusting for sex, birth method, season of birth, current NMF levels or *FLG* mutation (data not shown for *FLG* mutation), use of antibiotics during the first 2 months of life, and levels of TARC/CCL17 at age 2 months (adjusted HR = 1.67, 95% CI = 1.05−2.63, $P = 0.03$) (Figure 1 and Table 2). In a sensitivity analysis adjusting for educational status of the parents, domestic dog exposure during the first 2 months, and breastfeeding during the first 2 months, the association remained essentially the same (adjusted HR = 1.61, 95% CI = 1.02−2.55, $P = 0.04$). No correlation was observed between alpha diversity and the severity of AD ($P = 0.7$). In an analysis restricted to children where both parents had a history of atopy, a lower alpha diversity at age 2 months was even more strongly associated with an increased risk of AD during the first 2 years of life (crude HR = 3.46, 95% CI = 1.11−10.87, $P = 0.03$; adjusted HR = 4.44, 95% CI = 1.08−18.18, $P = 0.04$).

In an analysis restricted to children with no parental history of atopy, the mean Shannon index was 2.23 (SD = 0.74) among children who later developed AD and 2.29 (SD = 0.61) among children who did not develop AD during the first 2 years of life ($P = 0.8$) (Figure 2). No inverse association between alpha diversity and risk of AD during the first 2 years of life was observed (crude HR = 1.15, 95% CI = 0.55−2.38, $P = 0.7$; adjusted HR = 1.05, 95% CI = 0.53−2.17, $P = 0.9$) (Figure 1 and Table 2).

Table 2. The Inverse Association between Alpha Diversity and Risk of Atopic Dermatitis during the First 2 Years of Life Stratified for a Parental History of Atopy

	All Children N = 288				Children with Parental Atopy n = 188				Children with No Parental Atopy n = 100			
Shannon Index	Crude HR[1] (95% CI)	*P*-Value	aHR[2,3] (95% CI)	*P*-Value	Crude HR[4] (95% CI)	*P*-Value	aHR[2,5] (95% CI)	*P*-Value	Crude HR[6] (95% CI)	*P*-Value	aHR[2,7] (95% CI)	*P*-Value
At birth	0.97 (0.74−1.27)	0.8	0.97 (0.74−1.27)	0.8	0.95 (0.70−1.28)	0.7	0.97 (0.73−1.30)	0.9	1.16 (0.65−2.08)	0.6	1.28 (0.64−2.56)	0.5
At age 2 mo	1.37 (0.95−1.96)	0.09	1.30 (0.89−1.89)	0.2	1.64 (1.08−2.05)[8]	0.02[8]	1.67 (1.05−2.63)[8]	0.03[8]	1.15 (0.55−2.38)	0.7	1.05 (0.53−2.17)	0.9

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; NMF, natural moisturizing factor; TARC, thymus and activation-regulated chemokine.

[1] A total of 250 children included in the analysis at birth and 222 children included in the analysis at age 2 months.

[2] Hazard ratio analysis adjusted for sex, birth method, season of birth, antibiotic use and emollient use during the first 2 months of life (only at age 2 months), current NMF, and TARC levels.

[3] A total of 249 children included in the analysis at birth and 222 children included in the analysis at age 2 months.

[4] A total of 162 children included in the analysis at birth and 139 children included in the analysis at age 2 months.

[5] A total of 161 children included in the analysis at birth and 139 children included in the analysis at age 2 months.

[6] A total of 88 children included in the analysis at birth and 83 children included in the analysis at age 2 months.

[7] A total of 88 children included in the analysis at birth and 83 children included in the analysis at age 2 months.

[8] Significant association defined as $P < 0.05$.

The association between skin microbiome beta diversity at birth and age 2 months and the development of AD

At birth and age 2 months, there was no significant association between the skin microbiome community composition and development of AD during the first 2 years of life in crude analysis (birth: $R^2 = 0.003$, $P = 0.9$; age 2 months: $R^2 = 0.004$, $P = 0.8$) (Figure 3) or when adjusting for sex, birth method, season of birth, *FLG* mutation (or NMF levels), use of antibiotics during the first 2 months of life, and levels of TARC/CCL17 at age 2 months (birth: $R^2 = 0.003$, $P = 0.9$; age 2 months: $R^2 = 0.003$, $P = 0.8$). When stratifying according to parental history of atopy, the association remained insignificant (data not shown).

The association between the relative abundance of bacterial genera in the skin at birth and age 2 months and the development of AD

The five most common genera in the skin at birth were *Streptococcus, Staphylococcus, Cutibacterium, Corynebacterium,* and *Acinetobacter*. No association was observed between the relative rarefied abundance of any genera at birth and the development of AD during the first 2 years of life among all children and children stratified by parental history of atopy (false discovery rate [FDR] > 0.10 for all genera) (Supplementary Table S3).

The five most common genera in the skin at age 2 months were *Streptococcus, Staphylococcus, Gemella, Cutibacterium*, and *Acinetobacter* (Figure 4). No association between the relative rarefied abundance of any genera in the skin and the development of AD during the first 2 years of life was found. When stratifying children by a parental history of atopy, we observed that the relative rarefied abundance of *Bifidobacterium* was borderline significantly associated with the development of AD among children with a parental history of AD (FDR = 0.1). No bacterial genera were significantly associated with the development of AD among children with and without a parental history of AD (FDR > 0.1) (Supplementary Table S3).

DISCUSSION

Main findings

In this prospective birth cohort study, we found no overall association between the skin microbiome at birth and age 2 months and subsequent development of AD during the first 2 years of life. However, in analyses restricted to high-risk children, a lower alpha diversity in the skin at age 2 months was significantly associated with the development of AD in the first 2 years and in a dose-dependent manner.

Strengths and limitations

The prospective design of the study, the overall high number of children included, and the close follow-up are major study strengths. AD was diagnosed by a physician, decreasing the risk of misclassification. *FLG* mutations, multiple environmental exposures, and selected predictive skin barrier and immune biomarkers of AD (Rinnov et al., 2023) were adjusted for. However, it is still possible that residual confounding occurred or that it is simply a chance finding. A major limitation is that we were not able to analyze the skin microbiome at the species level. There are

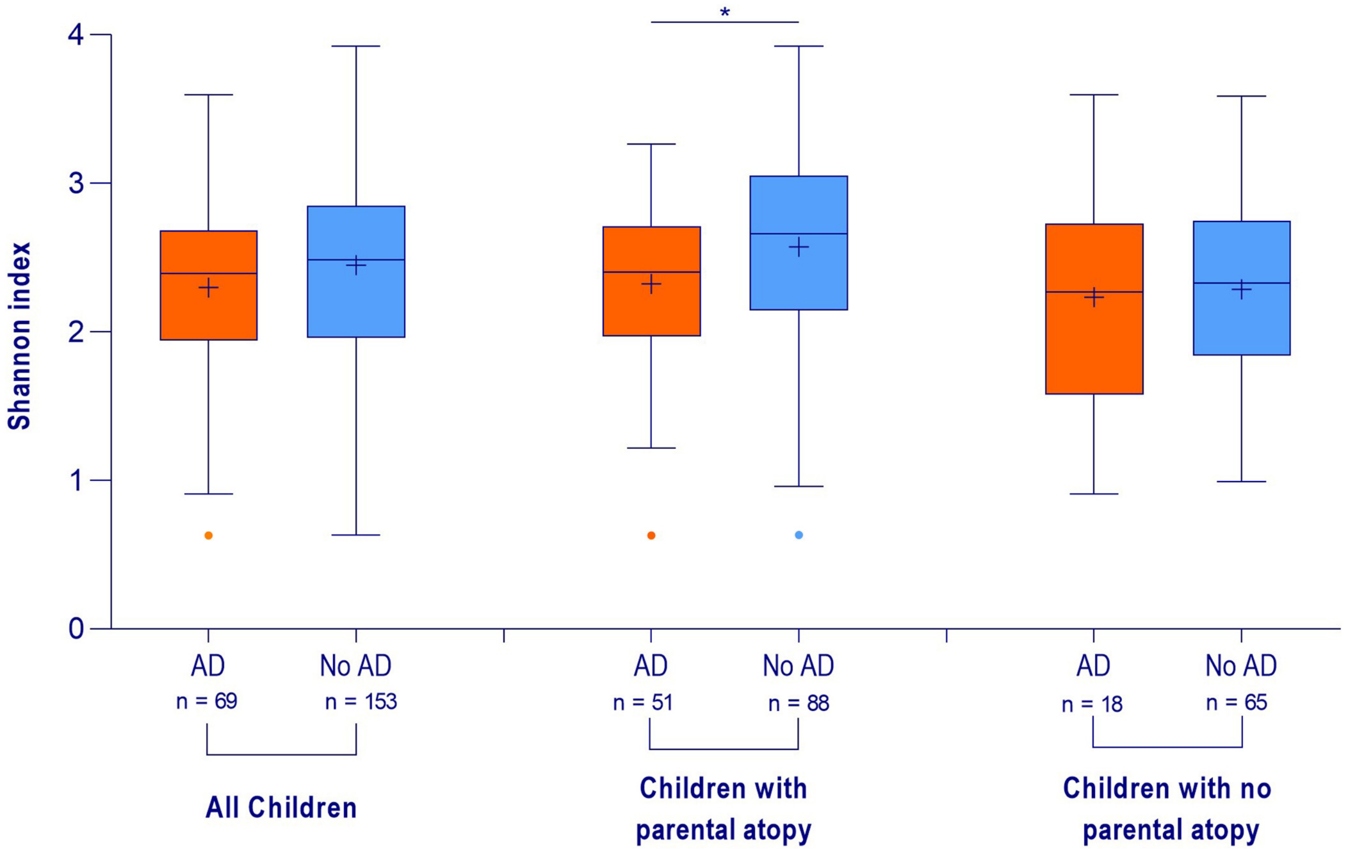

Figure 2. Alpha diversity (Shannon index) at age 2 months stratified by AD development in the first 2 years of life.[1] The superscript 1 denotes that only children with no development of AD at the time of collecting the stratum corneum samples were included in these analyses. *$P < 0.05$. AD, atopic dermatitis.

differences in the skin microbiome according to anatomical skin location (Kennedy et al., 2017), but because our analyses were limited to the skin on the dorsal aspect of the hand, it is possible that other skin sites may have yielded different results. The generalizability of our findings was limited by the majority of study participants being Caucasians and from Denmark. Finally, no mechanistic data were provided but only the association estimate.

Interpretation

The microbial diversity in the skin is lower in both lesional and nonlesional skin of patients with active AD and in a severity-dependent manner (Edslev et al., 2020; Kong et al., 2012; Olesen et al., 2022; Shi et al., 2016; Zheng et al., 2019). We observed a reduction in microbial diversity on the dorsal hand skin of high-risk children aged 2 months before any visible signs of AD during the first 2 years of life. No correlation was observed between the alpha diversity and severity of AD. This might be due to most children who developed AD having mild disease. It is also possible that early alterations in the skin microbiome are only associated with the onset of AD and not the course of AD. Although the primary analysis, including all children, showed no association between low alpha diversity and increased risk of AD, the tendency was the same, suggesting a 30% increased risk.

It is unknown whether the reduced skin microbial diversity before the onset of AD in high-risk infants was a result of possible parent-to-infant transmission of the skin microbiome, environmental exposures, or primary skin barrier or immune alterations. Mother-to-infant gut microbial transmission continues during the first 4 months of life (Ferretti et al., 2018), and it is possible that a similar transmission occurs for the skin during the first months of life. Along this line, a study observed that children and their caregivers living in the same household had skin microbiome similarities (Chia et al., 2022). Hence, children of mothers or fathers with active AD may both have an increased risk of developing AD owing to shared genetics and environment but also owing to putative pathogenic effects of the microbiome after parent-to-infant transmission of reduced microbial diversity. Thus, an important study limitation is that we did not examine the skin microbiome of the parents. Although both antibiotic and emollient use during the first 2 months of life were more common among children with parental history of atopy, adjustment for these exposures did not change the significance of the analysis. Importantly, using the same cohort, we have previously found that alterations in the skin immune response and skin barrier properties preceded the onset of AD (Halling et al., 2022; Rinnov et al., 2023). However, adjustment for TARC/CCL17 and NMF levels in the current analysis did not change the significance level.

We are only aware of one previous study on the putative association between the skin microbiome and AD in infants (Kennedy et al., 2017). No association was found between alpha diversity in the skin of 20 children at ages 2 days, 2

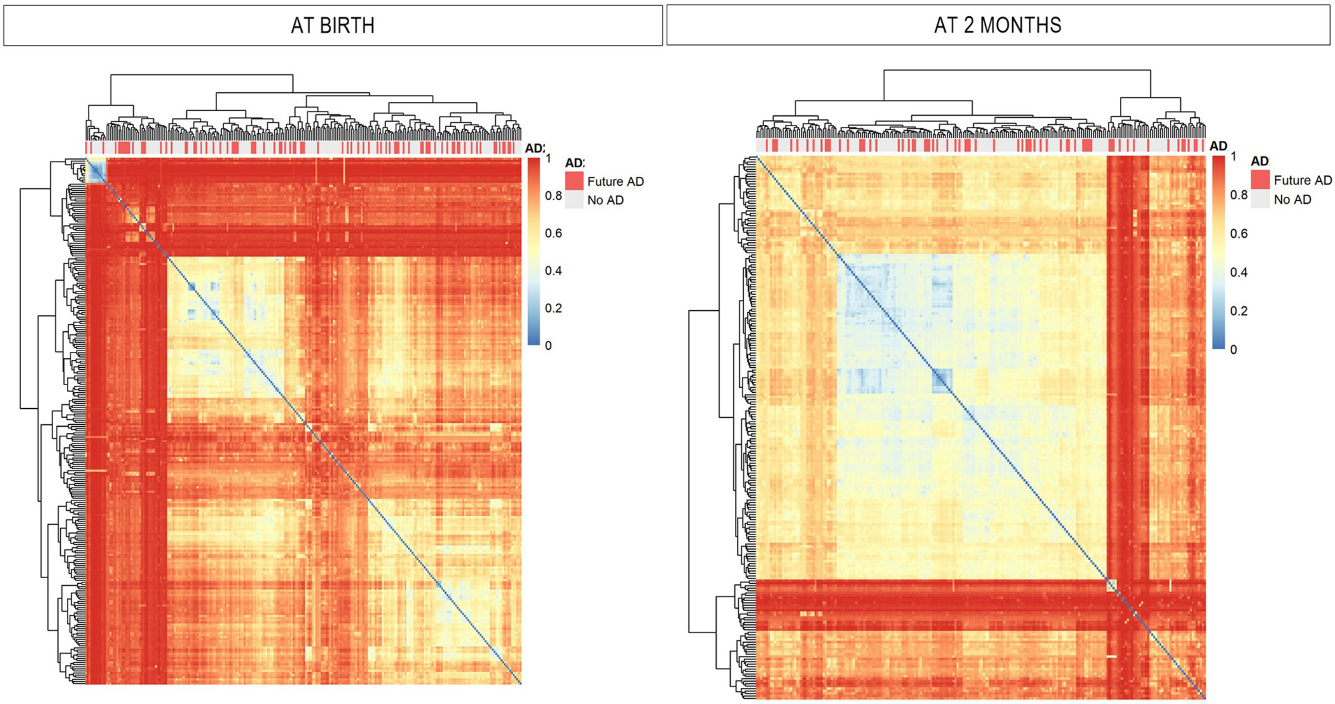

Figure 3. Heatmap of Bray—Curtis beta diversity in the skin at birth and age 2 months according to later onset of AD during the first 2 years of life. AD, atopic dermatitis.

months, and 6 months and the subsequent development of AD during the first year of life. However, they did not examine high-risk children, and only 20 children were included. Studies have also examined *S. aureus* colonization in the skin before AD (Kennedy et al., 2017; Lebon et al., 2009; Meylan et al., 2017; Nakamura et al., 2020; Skov et al., 2009). A Swiss study (N = 149) showed that colonization with *S. Aureus* in axillary skin at least once during the first 2 years of life was associated with an increased risk of AD (adjusted HR = 4.5, 95% CI = 1.5—13, $P = 0.003$) (Meylan et al., 2017). Moreover, one Japanese study (N = 268) found an association between *S. aureus* colonization on the cheek at age 6 months and development of AD during the first 2 years of life (adjusted OR = 4.67, 95% CI = 2.05—10.65, $P < 0.001$) (Nakamura et al., 2020). Furthermore, one Dutch study (N = 1,079) found an association between nasal *S. aureus* colonization at age 6 months and development of AD during the first 2 years of life (adjusted OR = 2.88, 95% CI = 1.60—5.19) (Lebon et al., 2009). In contrast, a Danish study (N = 356 children) (Skov et al., 2009) found no association between *S. aureus* colonization on the vestibulum nasi and perineum at age 1 month and AD during the first 3 years of life. Furthermore, one Irish study (N = 20) examined *S. aureus* colonization on the cheeks, nasal tip, antecubital fossa, and popliteal fossa at age 2 days, 2 months, and 6 months and observed no association with the development of AD during the first year of life (Kennedy et al., 2017).

The most common genera observed at birth and age 2 months are in accordance with a review on the infant skin microbiome, which found that *Staphylococcus* and *Streptococcus* are the predominant bacterial genera on the skin of children aged 1—3 months (Schoch et al., 2019). No association was observed between beta diversity or composition of any genera at age 2 months and subsequent development of AD. Moreover, no association was observed between the skin microbiome at birth and the development of AD, which is in line with the findings of our previous studies from the same birth cohort, where we also found predictive immune and barrier biomarkers of AD at age 2 months but not at birth (Halling et al., 2022; Rinnov et al., 2023). This emphasizes that changes in the skin before AD starts during the first 2 months of age. Moreover, the recently published Short-term Topical Application to Prevent Atopic Dermatitis trial showed that preventive treatment with emollients containing ceramides during the first 8 weeks of life was associated with a decreased 1-year prevalence of AD (Ní Chaoimh et al., 2023). Hence, there appears to be a time window for intervention of AD during the first 2 months of life.

In this Danish birth cohort of 300 children, we observed that a lower alpha diversity in the skin of high-risk children aged 2 months was associated with an increased risk of AD during the first 2 years of life and in a dose-dependent manner. Although additional studies are needed to confirm these results, they suggest that there is an association between skin microbiome alterations and the development of AD in high-risk children.

MATERIALS AND METHODS

Ethical approvals

The barrier dysfunction in Atopic newBorns study (Baby skin) (Gerner et al., 2022) was conducted in accordance with the Declaration of Helsinki and approved by the Scientific Ethical Committee of the Capital Region (H-16042289) and the local data

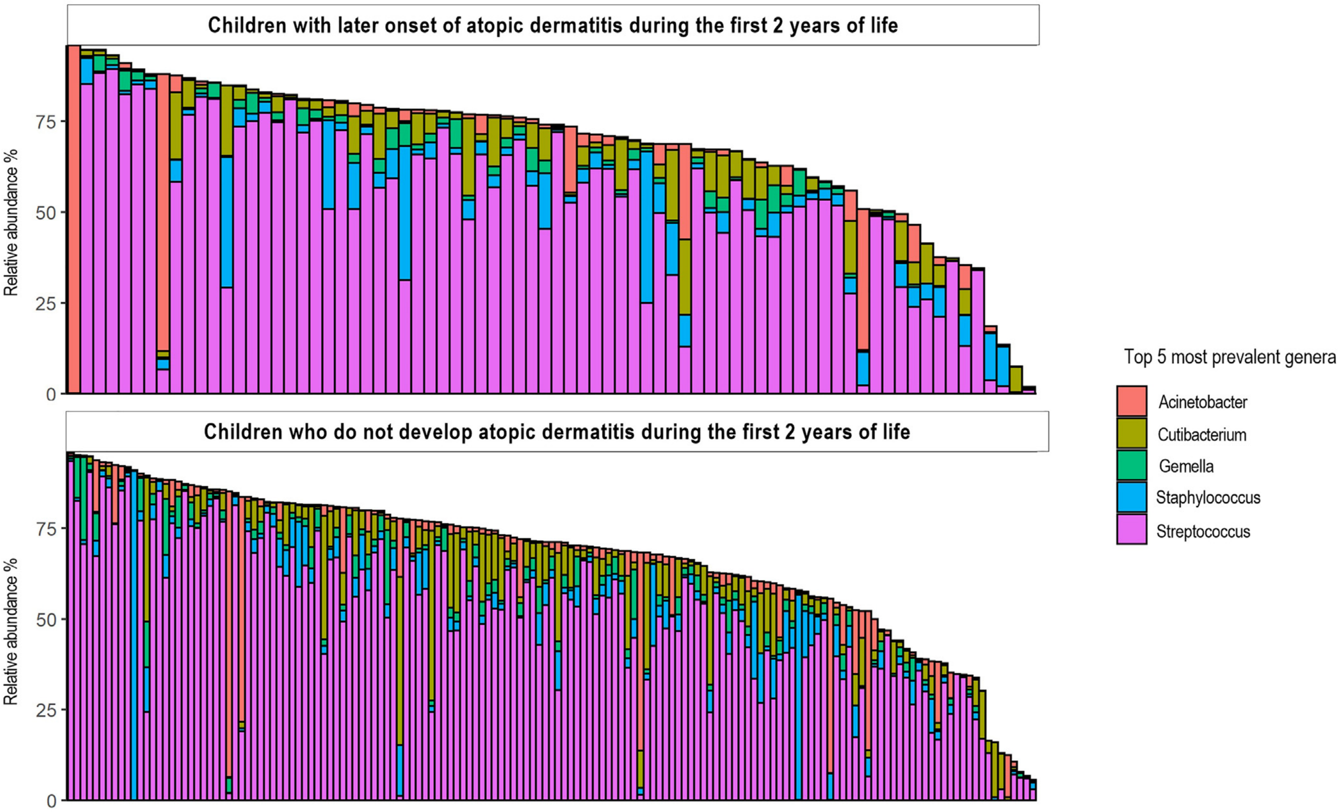

Figure 4. The relative abundance of the five most prevalent genera in the skin of children aged 2 months stratified according to later onset of atopic dermatitis during the first 2 years of life.

protection agency (identification number HGH-3017−040, I-suite number 05578). All parents of children included in this cohort have provided written informed consent.

Study population

Children were recruited from the maternity ward at Copenhagen University Hospital − Rigshospitalet (Copenhagen, Denmark). Children were eligible for enrolment if they were singletons, were born to term (gestational age 37+0 to 41+6), and had no history of treatment with antenatal corticosteroids for fetal lung maturation. All children were enrolled during the first 3 days of life and followed until the age of 2 years.

Clinical visits, questionnaire, and telephone interviews

Children attended a clinical visit with a physician during the first 3 days of life and at ages 2 and 12 months. An interview about the method of feeding, previous systemic and skin infections, antibiotic use, and application of emollients was conducted at each study visit (see Supplementary Materials and Methods). A universal skin examination was performed to identify any clinical signs of AD. If the child was diagnosed with AD, the severity was assessed using the EASI. Almost clear AD was defined as an EASI score of 0.1−1.0, mild AD was defined as an EASI score of 1.1−7.0, and moderate-to-severe AD was defined as an EASI score >7.0 (Leshem et al., 2015).

After attending the clinical visit at age 2 months, parents completed a questionnaire. Parents were asked questions regarding atopic diseases, domestic dog exposure, and educational status, among others (see Supplementary Materials and Methods).

When children were aged 1.5 and 2 years, parents participated in a structured and scheduled telephone interview, which included a modified version of the United Kingdom Working Party criteria for diagnosing AD (Supplementary Table S4) (Williams et al., 1996). If a child developed signs of AD between the clinical visits and/or telephone interviews or if a child fulfilled the United Kingdom Working Party criteria or had any signs of AD at ages 1.5 or 2 years, families attended an additional visit to verify the diagnosis and assess the severity of AD.

Tissue sampling and analysis

At ages 0−3 days, DNA was collected using a buccal swab (Isoehelic, Harrietsham, United Kingdom) and analyzed for three common *FLG* mutations (R501X, 2282del4, and R2447X) using TaqMan genotyping assay (Meldgaard et al., 2012). At ages 0−3 days and 2 months, stratum corneum cells were collected by tape stripping the skin eight consecutive times from the dorsal aspect of the hand (tape strips: 22 mm in diameter; Cuderm, Dallas, TX). No application of emollients or topical therapy was allowed on the day of tape strip sampling. Tapes 1 and 2 were analyzed for the skin microbiome using V3−V4 16s ribosomal RNA gene sequencing. The skin microbiome was assessed by analyzing alpha diversity measured as the Shannon index, beta diversity, and the relative abundance of all genera. For information on library preparation and bioinformatics in relation to the skin microbiome, see Supplementary Materials and Methods. Tape 6 was analyzed for NMF using liquid chromatography (Kezic et al., 2009). Tape 7 was analyzed for TARC/CCL17 using Meso Scale Discovery assay (Clausen et al., 2020).

Statistical analysis

Children who developed AD before collection of skin tapes at age 2 months were excluded from analyses. HR presented with 95% CI was calculated using Cox regression analysis for the inverse association between alpha diversity (Shannon index) and the development of AD. Differences in skin microbiome community composition (beta diversity) according to AD status were assessed using a permutational multivariate ANOVA test. Differences in the relative rarefied abundance of bacterial genera according to AD status were tested using a type 2 ANOVA and corrected for multiple testing by controlling for FDR (FDR < 0.10 was considered significant). For all analyses, stratification was made for parental atopy. Imputation of parental atopy was made for 50 children owing to missing data. For further information on statistical analysis, see Supplementary Materials and Methods.

Data availability statement

Datasets related to this article can be shared after making a Data Protection Agreement that needs to be approved by the Danish Data Protection Agency.

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CONFLICT OF INTEREST

ASH reports grants from Herlev and Gentofte Hospital Research Foundation, Kgl Hoftbundsmager Aage Bangs Foundation, and CAG-Allergy during the conduct of the study. Outside the submitted work, ASH has been a speaker for Leo Pharma and a consultant for Coloplast. TG reports grants from Herlev and Gentofte Hospital Research Foundation and Kgl Hoftbundsmager Aage Bangs Foundation during the conduct of the study. MRR reports grants from Herlev and Gentofte Hospital Research Foundation and Kgl Hoftbundsmager Aage Bangs Foundation during the conduct of the study. MHK reports grants from Herlev and Gentofte Hospital Research Foundation during the conduct of the study. NHR reported a grant from The Novo Nordisk Foundation during the conduct of the study. Outside the submitted work, CO works as an industrial postdoc at Leo Pharma and Bispebjerg Hospital. Outside the submitted work, LS has received research grants from Leo Foundation, the Kgl Hofbundtmager Aage Bang Foundation, Leo Pharma, Janssen-Cilag, Bristol-Myers Squibb, UCB, Almirall, and Sanofi-Genzyme. She has served as an advisor for AbbVie, Novartis, Almirall, Leo Pharma, Pfizer, Bristol-Myers Squibb, Boehringer Ingelheim, UCB, Eli Lilly, and Sanofi-Genzyme and served as a speaker for AbbVie, Leo Pharma, Pfizer, Novartis, AbbVie, and Sanofi-Genzyme. Outside the submitted work, SFT has been a speaker or advisor for Sanofi, AbbVie, Leo Pharma, Pfizer, Eli Lilly, Novartis, UCB Pharma, Almirall, and Janssen Pharmaceuticals and received research support from Sanofi, AbbVie, Leo Pharma, Novartis, UCB Pharma, and Janssen Pharmaceuticals, outside the submitted work. Outside of the submitted work, AE has received research funding from Pfizer, Eli Lilly, Novartis, Bristol-Myers Squibb, AbbVie, Boehringer Ingelheim, Janssen Pharmaceuticals, the Danish National Psoriasis Foundation, the Simon Spies Foundation, and the Kgl Hofbundtmager Aage Bang Foundation and honoraria as consultant and/or speaker from AbbVie, Almirall, Boehringer Ingelheim, Leo Pharma, Zuellig Pharma, Galápagos NV, Sun Pharmaceuticals, Samsung Bioepis, Pfizer, Eli Lilly and Company, Novartis, Union Therapeutics, Galderma, Dermavant, UCB, Mylan, Bristol-Myers Squibb, and Janssen Pharmaceuticals. TB reports a grant from The Novo Nordisk Foundation during the conduct of the study. JPT reports grants from Leo Foundation, Novo Nordisk Foundation, and Lundbeck Foundation and grants from Savværksejer Jeppe Juhl og hustru Ovita Juhls Mindelegat, during the conduct of the study. Outside of this study, TB has served as an advisor for AbbVie, Almirall, Arena Pharmaceuticals, Coloplast, OM Pharma, Aslan Pharmaceuticals, Union Therapeutics, Eli Lilly & Co, Leo Pharma, Pfizer, Regeneron, and Sanofi-Genzyme; served as a speaker for AbbVie, Almirall, Eli Lilly & Co, Leo Pharma, Pfizer, Regeneron, and Sanofi-Genzyme; and received research grants from Pfizer, Regeneron, and Sanofi-Genzyme. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: ASH, MRR, TG, LS, JPT; Data Curation: ASH; Formal Analysis: ASH, BGF, NS, IDPC; Funding Acquisition: ASH, MRR, TG, JPT; Investigation: MRR, TG, ASH, NHR, MHK, JPT; Methodology: ASH, JPT; Writing − Original Draft Preparation: ASH, JPT; Writing − Review and Editing: ASH, BGF, TG, MRR, LB, MHK, NHR, ST, IFR, CO, IDPC, LS, NS, ATMR, SFT, AE, IJ, SK, TB, JPT

Disclaimer

The study was conducted independently of these supports, and the funders had no influence on the study design or data analysis.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.03.1682.

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SUPPLEMENTARY MATERIALS AND METHODS

Definition of variables

Any antibiotic treatment was defined as an affirmative response to the following question: "Has your child received antibiotic treatment since last study visit?" In case of an affirmative response, parents were asked what kind of antibiotics the child was treated with. Emollient therapy was defined as an affirmative response to the following question: "Do you apply moisturizer on your child's skin?" In the case of an affirmative response, parents were asked about the frequency of emollient application. Breastfeeding was defined as answering breastfeeding to the following question: "Primary method of feeding?" A parental history of atopic disease was defined as an affirmative answer from the father or the mother to one of the following questions: "Has a doctor ever told you, that you have childhood eczema/atopic eczema/asthma/hay fever?" or "Has a doctor ever told you, you have allergy?" followed by a yes to either birch, grass, mugwort, horse, dog, cat, house dust mites, or molds. Domestic dog exposure during the first 2 months was defined as an affirmative response to the following questions: "Have you lived together with a dog during your pregnancy?" and "Is a dog currently living in your home?" Parents with a higher educational level were defined as at least one parent answering "Long-cycle higher education" to the following question: "What is your highest completed level of education?"

Statistical analysis

For the inverse association between alpha diversity (Shannon index) and development of atopic dermatitis, adjustment was made for sex, season of birth (spring, summer, autumn, and winter), method of birth (vaginal vs. Cesarian section), current thymus and activation-regulated chemokine/CCL17 levels, and use of any antibiotics and emollients during the first 2 months of age. In these analyses, adjustments were made for either *FLG* mutation status or natural moisturizing factor levels, but these gave essentially similar results. Proportionality test was performed for all Cox regression analyses. In a sensitivity analysis, adjustment was made for breastfeeding during the first 2 months, domestic dog exposure from birth until age 2 months, and the parent's educational status. For significant associations, a correlation between alpha diversity and severity of atopic dermatitis (Eczema Area and Severity Index score) was calculated using Spearman's correlation. For differences in skin microbiome community composition (beta diversity) according to atopic dermatitis status, adjustment was made for sex, *FLG* mutation or natural moisturizing factor levels, season of birth (spring, summer, autumn, and winter), method of birth (vaginal vs. Cesarian section), current thymus and activation-regulated chemokine/CCL17 levels, and use of any antibiotics and emollients during the first 2 months of age.

Microbiome analysis

DNA extraction. DNA was extracted from the skin tapes using the NucleoSpin 96 Soil (Macherey-Nagel, Oensingen, Switzerland) kit. Bead beating was done horizontally on a Vortex-Genie 2 at 2,700 r.p.m. for 5 minutes. A minimum of one negative control was included per batch of samples from the DNA extraction and throughout the laboratory process (including sequencing). ZymoBIOMICS Microbial Community Standards (Zymo Research, Irvine, CA) were also included in the analysis as a positive (mock) control.

V3–V4 amplicon PCR. PCR was done with the forward primer V3V4_skin_F and reverse primer V3V4_skin_R with Illumina adapters attached: Illumina adapter and S-D-Bact-0341-b-S-17: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCAG-3′ and Illumina adapter and S-D-Bact-0785-a-A-21: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACCVGGGTATCTAAKCC-3′

These are universal bacterial 16S ribosomal DNA primers, which target the V3–V4 region.

Sequencing. Sequencing was done on an Illumina NovaSeq sequencer with 2× 250 base pair paired-end sequencing.

Bioinformatics analysis (amplicon sequence variant pipeline). A customized pipeline based on dada2 was used for bioinformatics processing of the sequence data into an (amplicon sequence variant) ASV abundance table. In the first step, primer sequences were removed from raw reads using cutadapt. Reads without primer match or with ambiguous bases (e.g., Ns) as well as reads shorter or longer than expected from the number of sequencing cycles and the lengths of the primers were filtered out. In an additional filtering and trimming step (dada2::filterAndTrim command), reads were trimmed at the three prime ends on the basis of sample-specific quality scores. Trimmed reads that based on the quality scores of the nucleotides were expected to contain more than one error were removed. The remaining reads were dereplicated into unique sequences and then denoised separately for forward and reverse reads for each sample. In this denoising step, a less abundant sequence can be assigned to a closely related, more abundant sequence on the basis of comparison with a data-based error matrix. In that case, the low abundance sequence is considered a sequencing error of the more abundant sequence. Denoised forward and reverse reads were merged, thereby discarding read pairs without sufficient overlap or with any mismatch in the overlap region. Finally, suspected chimeras (also called bimera) were removed from the generated abundance table by internal abundance and sequence comparisons. The taxonomic assignment of the detected ASVs was done in two steps. First, the ASV sequences were compared with full-length 16S sequences in an internal reference database (CM_16S_27Fto1492R_v1.0.0) using a naive Bayesian classifier. The reference database was generated using in silico extraction from the Genome Taxonomy Database database (release: 06-RS202, https://gtdb.ecogenomic.org/), the rrnDB (version 5.7, https://rrndb.umms.med.umich.edu/), and the Unified Human Gastrointestinal Genome database (version 2.0, https://www.ebi.ac.uk/metagenomics/genomecatalogues/human-gut-v2-0) and subsequent curation. In the second annotation step, the taxonomic assignments were improved using precise sequence identity percentages between the found ASVs and reference amplicons in an internal V3V4 amplicon database (CM_16S_341Fto785R_v1.0.0.rds).

Background signal removal. When working with low biomass samples, the background signal may substantially interfere with the biological signal. To mitigate this, we made an in silico removal of the background signal. To remove ASVs that likely represent background signals, we compared the raw count, relative abundance, and prevalence of each ASV between analytical samples and negative controls. We kept an ASV as a biological signal if the maximum raw count in a sample was at least two times as high as the highest count in a negative control. We also kept an ASV if its prevalence and maximum relative abundance ratios between samples and negative controls were both ≥ 1.1.

Supplementary Table S1. Characteristics of Children Developing AD

Characteristics	Patients with AD N = 99
Median onset of AD (IQR)	6 mo (3.0–11.0)
Severity of AD, % (n_{cases}/n_{total})	
Almost clear AD	6.1 (6/99)
Mild AD	50.5 (50/99)
Moderate-to-severe AD	23.2 (23/99)
Not assessed	20.2 (20/99)
Severity of AD, EASI score, median (IQR)	4.2 (2.0–7.9)
Almost clear AD	0.8 (0.7–0.8)
Mild AD	2.8 (2.0–5.0)
Moderate-to-severe AD	10.80 (8.1–15.0)
FLG gene mutation, % (n_{cases}/n_{total})	17.3 (17/98)
Parental history of atopy, % (n_{cases}/n_{total})	78.6 (66/84)

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IQR, interquartile range.

Supplementary Table S2. Mean Shannon Index at Birth and Age 2 Months according to Later Development of AD during the First 2 Years of Life Stratified for Parental History of Atopy

	All Children N = 288			Children with Parental Atopy n = 188			Children with No Parental Atopy n = 100		
Mean Shannon Index	**Later AD**[1]	**No AD**[2]	***P*-Value**	**Later AD**[3]	**No AD**[4]	***P*-Value**	**Later AD**[5]	**No AD**[6]	***P*-Value**
At birth	2.61 (SD = 0.76)	2.60 (SD = 0.80)	0.9	2.66 (SD = 0.78)	2.62 (SD = 0.81)	0.8	2.46 (SD = 0.67)	2.56 (SD = 0.80)	0.6
At age 2 mo	2.30 (SD = 0.60)	2.45 (SD = 0.65)	0.1	2.32 (SD = 0.55)	2.57 (SD = 0.65)	0.02*	2.23 (SD = 0.74)	2.29 (SD = 0.61)	0.8

Abbreviation: AD, atopic dermatitis.

[1] A total of 85 children included in the analysis at birth and 69 children included in the analysis at age 2 months.

[2] A total of 165 children included in the analysis at birth and 153 children included in the analysis at age 2 months.

[3] A total of 67 children included in the analysis at birth and 51 children included in the analysis at age 2 months.

[4] A total of 95 children included in the analysis at birth and 88 children included in the analysis at age 2 months.

[5] A total of 18 children included in the analysis at birth and 18 children included in the analysis at age 2 months.

[6] A total of 70 children included in the analysis at birth and 65 children included in the analysis at age 2 months.

Supplementary Table S3. The Association between the Relative Rarefied Abundance of Genera Collected at Birth and Age 2 Months and Development of AD during the First 2 Years of Life Stratified by Parental History of Atopy

	At Birth						At Age 2 mo					
	All Children		Children with Parental Atopy		Children with No Parental Atopy		All Children		Children with Parental Atopy		Children with No Parental Atopy	
Taxon	*P*-Value	FDR	*P*-Value	FDR	*P*-Value	FDR	*P*-Value	FDR	*P*-Value	FDR	*P*-Value	FDR
28L	0.8	1	0.5	1	0.8	1	0.2	1	—	—	0.6	1
Abiotrophia	0.9	1	0.2	1	0.4	1	—	—	—	—	—	—
Acidovorax	0.4	1	0.9	1	0.3	1	0.9	1	0.2	0.6	0.05	0.9
Acinetobacter	0.2	1	0.5	1	0.8	1	0.4	1	0.3	0.7	0.05	0.9
Actinomyces	0.2	1	0.8	1	0.5	1	0.7	1	0.2	0.5	0.4	1
Aerococcus	0.4	1	0.9	1	0.6	1	0.6	1	0.9	1.0	0.5	1
Aeromonas	0.7	1	0.3	1	0.2	1	0.01	1	0.3	0.6	0.5	1
Agathobacter	0.2	1	0.6	1	0.2	1	0.7	1	0.7	0.9	0.8	1
Aggregatibacter	0.3	1	0.4	1	1.0	1	0.5	1	0.2	0.6	0.1	1
Agrobacterium	0.5	1	0.4	1	0.5	1	0.7	1	0.6	0.8	0.6	1
Anaerococcus	0.8	1	0.9	1	0.3	1	0.4	1	0.05	0.4	0.6	1
Alloiococcus	0.9	1	0.6	1	0.6	1	—	—	—	—	—	—
Bacillus	0.5	1	0.5	1	0.7	1	0.2	1	0.4	0.7	0.2	1
Bacillus_A	0.9	1	0.8	1	1.0	1	0.5	1	0.8	0.9	0.6	1
Bacillus_J	0.5	1	0.7	1	0.4	1	0.4	1	0.9	0.9	0.7	1
Bacteroides	0.4	1	1.0	1	0.8	1	0.1	1	0.3	0.6	0.4	1
Bifidobacterium	0.3	1	0.3	1	0.4	1	0.1	1	0.001	0.1*	0.2	1
Blautia_A	0.4	1	0.8	1	0.3	1	0.8	1	0.7	0.9	0.4	1
Brachybacterium	0.6	1	0.2	1	0.9	1	0.3	1	0.04	0.4	0.1	1
Brevibacterium	0.9	1	0.6	1	0.3	1	0.1	1	0.05	0.4	0.1	0.9
Brevundimonas	0.7	1	0.5	1	0.8	1	0.3	1	0.1	0.4	0.4	1
Brochothrix	—	—	—	—	—	—	0.3	1	0.9	1.0	0.5	1
Bulleidia	—	—	—	—	—	—	0.9	1	0.4	0.7	0.6	1
Caballeronia	0.4	1	0.6	1	0.3	1	0.1	1	0.04	0.4	0.9	1
Campylobacter_A	0.6	1	0.2	1	0.8	1	0.5	1	0.4	0.7	0.4	1
Campylobacter_B	0.8	1	0.4	1	0.4	1	—	—	—	—	—	—
Capnocytophaga	0.1	1	0.01	1	0.4	1	0.6	1	0.9	1	0.2	1
Cardiobacterium	—	—	—	—	—	—	0.4	1	0.4	0.7	0.7	1
Chryseobacterium	0.3	1	0.4	1	0.4	1	0.02	1	0.6	0.8	0.2	1
Citricoccus	0.3	1	0.5	1	0.9	1	0.5	1	0.1	0.5	0.4	1
Clostridium	0.5	1	1.0	1	0.2	1	0.5	1	0.1	0.4	0.1	0.9
Collinsella	0.4	1	0.9	1	0.8	1	0.6	1	0.8	0.9	0.7	1
Comamonas	0.02	1	0.8	1	0.1	1	0.7	1	0.4	0.7	0.4	1
Corynebacterium	0.3	1	0.7	1	0.7	1	0.2	1	0.1	0.5	0.9	1
Cutibacterium	0.9	1	0.7	1	0.3	1	0.3	1	0.8	0.9	0.6	1
Dermabacter	0.6	1	0.6	1	0.7	1	0.8	1	0.5	0.8	0.9	1
Dialister	1.0	1	0.6	1	0.6	1	—	—	—	—	—	—
Dietzia	0.2	1	0.3	1	0.8	1	0.2	1	0.6	0.8	0.2	1
Dolosigranulum	0.7	1	0.9	1	0.7	1	0.7	1	0.1	0.5	1.0	1
Duncaniella	1.0	1	0.9	1	0.8	1	-	-	0.6	0.8	0.6	1
Enterobacter	0.3	1	0.1	1	0.3	1	0.1	1	0.1	0.5	1.0	1
Enterococcus	0.2	1	0.02	1	0.9	1	0.9	1	0.1	0.5	0.8	1
Enterococcus_D	0.9	1	0.5	1	1.0	1	1.0	1	0.6	0.8	0.03	0.9
Epilithonimonas	0.7	1	0.3	1	0.7	1	0.2	1	0.9	1	0.03	0.9
Escherichia	0.1	1	0.1	1	0.4	1	0.9	1	0.5	0.8	0.4	1
Eubacterium_R	0.3	1	0.9	1	0.8	1	—	—	—	—	—	—
Exiguobacterium	0.7	1	0.6	1	0.5	1	1.0	1	0.1	0.5	0.9	1
Ezakiella	0.6	1	0.6	1	0.2	1	0.4	1	0.4	0.7	0.8	1
F0040	0.8	1	0.5	1	0.3	1	1.0	1	0.7	0.9	0.5	1
F0422	0.8	1	0.8	1	0.8	1	0.7	1	0.05	0.4	0.7	1
Facklamia	0.6	1	0.4	1	0.9	1	—	—	—	—	—	—
Faecalibacterium	0.9	1	0.8	1	0.7	1	0.3	1	0.4	0.7	0.3	1
Fannyhessea	0.6	1	0.9	1	0.6	1	—	—	—	—	—	—

(*continued*)

Supplementary Table S3. Continued

	At Birth						At Age 2 mo					
	All Children		Children with Parental Atopy		Children with No Parental Atopy		All Children		Children with Parental Atopy		Children with No Parental Atopy	
Taxon	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**
Fenollaria	0.7	1	0.7	1	0.04	1	0.9	1	0.2	0.6	0.9	1
Finegoldia	0.1	1	0.9	1	0.3	1	0.7	1	0.7	0.9	0.8	1
Frankia	0.2	1	0.8	1	0.3	1	0.4	1	0.5	0.8	0.5	1
Fusicatenibacter	0.4	1	0.7	1	0.9	1	—	—	—	—	—	—
GCA-900066495	0.8	1	0.2	1	0.5	1	0.7	1	0.1	0.5	0.9	1
Gemella	0.4	1	0.4	1	0.8	1	0.1	1	0.5	0.8	0.2	1
Gemmiger	0.6	1	0.5	1	0.9	1	—	—	—	—	—	—
Granulicatella	0.8	1	0.6	1	0.7	1	0.2	1	0.3	0.6	0.9	1
Haemophilus	0.4	1	0.6	1	0.9	1	0.1	1	0.2	0.5	0.1	1
Haemophilus_A	0.3	1	0.8	1	0.6	1	0.6	1	0.1	0.4	0.9	1
Haemophilus_D	0.2	1	0.2	1	0.7	1	0.7	1	0.1	0.5	0.6	1
Herbaspirillum	0.3	1	1.0	1	0.5	1	0.8	1	0.4	0.7	0.8	1
Hydrogenophilus	0.1	1	0.5	1	0.7	1	0.6	1	0.03	0.4	0.1	0.9
Klebsiella	0.6	1	0.7	1	0.9	1	0.2	1	1.0	1.0	0.4	1
Klebsiella_A	0.7	1	0.9	1	0.7	1	0.1	1	0.7	0.9	0.7	1
Kocuria	0.4	1	0.8	1	0.1	1	0.4	1	0.06	0.4	0.2	1
Lachnoanaerobaculum	0.2	1	0.1	1	0.3	1	0.3	1	0.4	0.7	0.8	1
Lachnospira	0.4	1	0.9	1	0.9	1	—	—	—	—	—	—
Lacticaseibacillus	—	—	—	—	—	—	0.1	1	0.8	0.9	0.5	1
Lactobacillus	1.0	1	0.3	1	0.8	1	0.3	1	0.4	0.7	0.7	1
Lactococcus	0.9	1	0.8	1	1.0	1	0.4	1	0.1	0.5	0.7	1
Lancefieldella	0.2	1	0.3	1	0.9	1	1.0	1	0.4	0.7	0.6	1
Latilactobacillus	0.3	1	0.4	1	0.6	1	0.4	1	0.2	0.5	0.2	1
Lautropia	0.2	1	1.0	1	0.6	1	0.2	1	0.1	0.5	0.5	1
Lawsonella	1.0	1	0.5	1	0.4	1	1.0	1	0.5	0.8	0.8	1
Leptotrichia	0.5	1	0.8	1	0.6	1	0.8	1	0.4	0.7	0.3	1
Ligilactobacillus	0.7	1	0.5	1	0.1	1	—	—	—	—	—	—
Limosilactobacillus	0.7	1	0.4	1	0.6	1	0.5	1	0.4	0.7	0.2	1
Massilia	0.2	1	0.7	1	0.8	1	0.7	1	0.1	0.5	0.8	1
Mediterraneibacter	0.5	1	0.8	1	0.7	1	—	—	—	—	—	—
Methylobacterium	0.3	1	0.8	1	0.8	1	0.4	1	0.2	0.5	0.6	1
Microbacterium	0.4	1	0.9	1	0.7	1	0.5	1	0.2	0.6	0.2	1
Micrococcus	0.6	1	0.5	1	0.1	1	0.1	1	0.1	0.5	0.9	1
Moraxella	0.6	1	1.0	1	0.9	1	0.2	1	0.8	0.9	0.3	1
Moraxella_A	0.5	1	0.9	1	1.0	1	0.7	1	0.8	0.9	0.8	1
Muribaculum	0.5	1	1.0	1	0.9	1	—	—	—	—	—	—
Neisseria	0.3	1	1.0	1	0.1	1	0.3	1	0.04	0.4	0.8	1
Nocardioides	0.7	1	0.6	1	0.4	1	0.1	1	0.4	0.7	0.4	1
Novosphingobium	0.3	1	0.8	1	0.4	1	0.3	1	0.6	0.8	1.0	1
Ochrobactrum_A	0.1	1	0.6	1	0.3	1	—	—	—	—	—	—
Pantoea	0.6	1	0.1	1	0.3	1	0.4	1	0.4	0.7	0.5	1
Parabacteroides	0.1	1	0.8	1	0.3	1	—	—	—	—	—	—
Paracoccus	0.3	1	0.8	1	0.8	1	0.1	1	0.4	0.7	0.4	1
Pauljensenia	0.5	1	0.1	1	0.9	1	0.9	1	0.2	0.6	0.1	1
Peptoniphilus_A	0.6	1	0.6	1	0.1	1	0.1	1	0.05	0.4	1.0	1
Peptoniphilus_B	0.8	1	0.8	1	1.0	1	—	—	—	—	—	—
Peptoniphilus_C	0.5	1	0.5	1	0.2	1	0.4	1	0.7	0.8	0.9	1
Peptostreptococcus	0.9	1	0.4	1	0.02	1	—	—	—	—	—	—
Phocaeicola	0.2	1	0.6	1	0.4	1	0.3	1	0.3	0.6	0.04	0.9
Porphyromonas	0.7	1	0.5	1	0.9	1	0.8	1	0.7	0.9	0.5	1
Porphyromonas_A	0.8	1	0.8	1	1.0	1	0.8	1	0.3	0.7	0.4	1
Prevotella	0.4	1	0.8	1	0.7	1	0.5	1	0.1	0.5	0.3	1
Prevotellamassilia	0.3	1	0.7	1	0.7	1	—	—	—	—	—	—
Pseudomonas	0.4	1	0.6	1	0.6	1	0.3	1	0.1	0.5	0.5	1

(*continued*)

Supplementary Table S3. Continued

	At Birth						At Age 2 mo					
	All Children		Children with Parental Atopy		Children with No Parental Atopy		All Children		Children with Parental Atopy		Children with No Parental Atopy	
Taxon	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**	***P*-Value**	**FDR**
Pseudomonas_B	—	—	—	—	—	—	0.5	1	0.3	0.6	0.4	1
Pseudomonas_E	0.6	1	0.3	1	0.8	1	0.3	1	0.5	0.8	0.3	1
Ralstonia	0.7	1	0.9	1	0.6	1	—	—	—	—	—	—
Roseburia	0.6	1	0.3	1	0.1	1	—	—	—	—	—	—
Rothia	0.8	1	0.2	1	0.4	1	0.1	1	0.7	0.9	0.2	1
Ruminococcus_E	0.7	1	0.5	1	0.3	1	—	—	—	—	—	—
Sneathia	0.2	1	0.04	1	1.0	1	—	—	—	—	—	—
Salmonella	—	—	—	—	—	—	0.2	1	0.2	0.6	0.9	1
Sphingomonas	0.2	1	0.1	1	0.8	1	0.4	1	0.003	0.2	0.6	1
Staphylococcus	1.0	1	0.4	1	0.4	1	0.5	1	0.5	0.8	0.9	1
Stenotrophomonas	0.6	1	0.6	1	0.6	1	0.2	1	0.1	0.4	0.7	1
Streptococcus	0.7	1	0.7	1	0.4	1	0.7	1	0.6	0.8	0.2	1
Streptomyces	0.3	1	0.8	1	0.6	1	0.5	1	0.01	0.4	1.0	1
TM7x	—	—	—	—	—	—	0.2	1	0.4	0.7	0.4	1
Turicibacter	0.5	1	0.9	1	1.0	1	—	—	—	—	—	—
UBA2866	0.2	1	0.4	1	0.6	1	—	—	—	—	—	—
UMGS1907	0.2	1	0.3	1	1.0	1	0.4	1	0.05	0.4	0.8	1
Ureaplasma	1.0	1	0.6	1	0.4	1	—	—	—	—	—	—
Veillonella	0.7	1	0.1	1	0.2	1	0.2	1	0.2	0.6	0.7	1
Weissella	0.7	1	0.5	1	1.0	1	—	—	—	—	—	—
Winkia	0.03	1	0.1	1	0.6	1	0.4	1	0.2	0.5	0.9	1
unclassified	0.6	1	0.7	1	0.6	1	0.6	1	0.2	0.5	0.2	1

Abbreviations: AD, atopic dermatitis; FDR, false discovery rate.

Supplementary Table S4. A Modified Version of the U.K. Working Party Diagnostic Criteria for Atopic Dermatitis

U.K. Working Party Diagnostic Criteria for Atopic Dermatitis

Major criteria (required)

- An itchy skin condition in the last 12 mo

Minor criteria (three or more)

- History of flexural involvement
- History of a generally dry skin
- History of first degree relative with atopic disease
- Visible flexural dermatitis

Abbreviation: U.K., United Kingdom.