Skin microbial signatures for eczema: a birth cohort study (abridged secondary publication)

TF Leung *, SKW Tsui, WH Tam, M Ip, MH Wang

KEY MESSAGES

1. Of 166 Chinese infants, 71 (43%) developed eczema during infancy; 33 (46.5%) of these infants had persistent eczema by 12 months of age.
2. Atopy with locally important allergens was associated with infantile eczema. Infants with more severe eczema were more likely to have persistent disease by 12 months of age.
3. Eczema was associated with higher transepidermal water loss over antecubital fossae in early life.
4. Infants with eczema had lower alpha diversity over the right antecubital fossa at birth and 6 months, and over the left popliteal fossa at 3 months and 12 months.
5. The compositions of bacterial communities over the left antecubital fossa and the left popliteal fossa were less clustered in infants with eczema at 12 months.

Hong Kong Med J 2024;30(Suppl 3):S4-7
HMRF project number: 06170466

[1] TF Leung, [2] SKW Tsui, [3] WH Tam, [4] M Ip, [5] MH Wang

The Chinese University of Hong Kong, Hong Kong SAR, China
[1] *Department of Paediatrics*
[2] *School of Biomedical Sciences*
[3] *Department of Obstetrics and Gynaecology*
[4] *Department of Microbiology*
[5] *Jockey Club School of Public Health and Primary Care*

* Principal applicant and corresponding author: tfleung@cuhk.edu.hk

Introduction

Eczema is the commonest chronic skin disease in childhood; it can cause substantial family stress, as well as social and financial burdens. Microbes inside the human body are associated with many complex diseases. The skin harbours hundreds of bacterial species, and their composition depends on the microenvironment (eg, humidity and temperature). The skin microbiome is involved in training the immune system, enhancing innate barrier immunity and homoeostasis, and triggering inflammatory cell recruitment and cytokine release.

The skin microbiome that modulates eczema susceptibility is poorly studied, mainly because of the lower microbial biomass present on the skin. Next-generation sequencing enables better understanding of the skin microbiome. Perturbations of the skin microbiome are associated with inflammatory dermatological conditions such as eczema. Eczema flares are generally associated with a low-diversity skin microbiome and *Staphylococcus aureus* dominance.

Most studies on this topic have been cross-sectional and thus could not support causal inferences. In Irish infants, skin microbiome composition and diversity shift over time; infants who developed eczema had significantly different bacterial communities on the antecubital fossa at 2 months of age.[1]

Additionally, skin dryness might predict eczema development in Caucasian infants. Transepidermal water loss (TEWL) before 6 months of age is a biomarker for eczema by 12 months of age.[2] However, the association between eczema and increased TEWL remains poorly defined. This study aimed to identify the skin microbial signatures in Chinese infants who develop eczema during infancy.

Methods

This study included (1) a discovery cohort of 166 healthy Chinese term singleton infants delivered vaginally who were free of any clinically evident dermatitis within 4 weeks after birth and whose mothers were free of any significant pregnancy-related diseases and peripartum sepsis, and (2) a replication cohort comprising two independent populations of preschool and school-age Chinese children from paediatric clinics with and without eczema, as well as participants in the SMART Baby birth cohort who were followed up for 2 years.[3]

All recruited infants were assessed for eczema through home visits at 1 and 3 months and clinic visits at 6 and 12 months. Eczema was diagnosed using the Hanifin and Rajka criteria, and disease severity was assessed by SCORAD. At each visit, clinical staff collected flocked skin swabs by 1 minute of firm rubbing over areas of 5×5 cm on the left and right antecubital fossae, left popliteal fossa, and anterior chest between the nipples. Within 2 hours, these swabs were transported on ice to the laboratory and stored at -80°C until sequencing. Skin biophysical parameters, including TEWL and skin hydration (SH), were measured over the volar forearm. At 12 months, all infants underwent skin

prick tests for six locally important allergens with positive (histamine 10 mg/ml) and negative (diluent) controls to determine atopy status. Participants were diagnosed as having transient eczema if it resolved at or before the 12-month visit or as having persistent eczema if it remained active at the 12-month visit.

Genomic DNA was extracted with a low biomass protocol and subjected to polymerase chain reaction amplification targeting the V1-V3 region of the 16S rRNA gene using primer pair 27F/534R (27F:5'-AGAGTTTGATCCTGGCTCAG-3'; 534R: 5'-ATTACCGCGGCTGCTGG-3'). Library preparation and 16S rRNA-based sequencing were then performed. Demultiplexed raw sequencing data were obtained in FASTQ format. Because the sizes of the original DNA fragments were shorter than two read lengths, paired-end reads were merged for improved genome assembly. The sequencing data were imported into QIIME2; DADA2 was used for denoising and removal of sequencing errors. Taxonomies of amplicon sequence variants were assigned using the *phyloseq* R package for microbiome analysis. Alpha diversity (Shannon and Simpson index) and beta diversity (unweighted UniFrac distance) were assessed.

Associations between clinical factors and eczema outcomes were analysed using the Mann-Whitney *U* test for continuous variables and the Pearson Chi-squared test for categorical variables; significant associations were then analysed by logistic regression. Longitudinal analyses were performed by generalised estimating equations (GEEs). The significance level was set to 5% for all analyses. Regarding the skin microbiome, single-timepoint microbial differences in alpha diversity indices among eczema groups were compared using the Kruskal-Wallis rank sum test, whereas longitudinal differences were analysed by GEEs. Associations between beta diversity and eczema outcomes over time were analysed by permutational multivariate analysis of variance with the *adonis* function. Taxa that were differentially abundant over time and among eczema groups were identified by the analysis of compositions of microbiomes with bias correction method.

For the replication cohort, the skin microbiome over the left antecubital fossae was assessed by whole-genome shotgun sequencing and by 16S rRNA sequencing. The predictivity of any skin microbial signature identified in the discovery cohort was compared with signatures identified in the two populations of the replication cohort.

Results

In total, 81 male and 85 female Chinese infants were recruited between October 2019 and November 2020; 44% had a maternal history of allergy and 44% had a paternal history of allergy. At 12 months, 151 (91%) infants remained in the study; 71 (47%) had developed eczema by 12 months of age, and 38 of these infants had transient eczema. Maternal smoking was associated with transient eczema (21.1% vs 7.4%, P=0.030).

Atopy was observed in 34 (28.6%) of 119 infants at 12 months and was associated with eczema ever (adjusted odds ratio=3.05, P=0.012). All infants with transient eczema had mild eczema. Compared with the transient eczema group, the persistent eczema group had a higher SCORAD at 6 months (9.8 vs 17.4, P=0.047).

Compared with infants with no eczema, infants with eczema ever had higher TEWL over the left antecubital fossae at 3 months (11.8 vs 8.2 g/m^2/hour, P=0.003) and over the right antecubital fossae at 12 months (10.2 vs 13.4 g/m^2/hour, P=0.005) [Table].

TABLE. Associations of transepidermal water loss (TEWL) with eczema outcomes

Measurement site and timepoint	Median (interquartile range) TEWL, g/m^2/hour				P value			
	No eczema (EN)	Eczema ever (EE)	Transient eczema (ET)	Persistent eczema (EP)	EN vs EE	EN vs ET	EN vs EP	ET vs EP
LAF at baseline	6.2 (5.2-7.7)	6.3 (5.0-8.7)	5.8 (4.9-8.3)	6.9 (5.0-9.1)	0.726	0.636	0.270	0.368
RAF at baseline	6.4 (5.3-8.5)	7.4 (5.3-8.2)	7.5 (5.0-8.0)	7.0 (5.6-9.7)	0.793	0.814	0.451	0.504
LAF at 1 month	8.9 (6.3-12.0)	8.7 (5.3-10.1)	9.2 (4.8-10.1)	7.5 (5.4-10.4)	0.265	0.512	0.253	0.650
RAF at 1 month	8.3 (5.8-11.6)	9.2 (5.1-12.2)	11.0 (8.3-13.4)	5.1 (3.8-10.5)	0.767	0.082	0.102	0.014*
LAF at 3 months	8.2 (6.5-10.7)	11.8 (8.6-17.6)	9.7 (7.5-17.6)	12.5 (10.5-17.4)	0.003	0.132	0.001	0.264*
RAF at 3 months	8.8 (6.1-15.2)	10.1 (7.2-15.6)	8.3 (6.6-15.3)	12.0 (8.7-15.6)	0.302	0.768	0.203	0.373*
LAF at 6 months	7.8 (5.7-11.4)	9.0 (6.4-12.7)	7.4 (6.0-11.7)	10.1 (7.5-14.4)	0.237	0.893	0.086	0.186
RAF at 6 months	9.1 (7.1-13.2)	9.5 (7.9-14.4)	9.2 (8.0-12.6)	10.8 (7.7-17.1)	0.261	0.647	0.183	0.332
LAF at 12 months	8.8 (7.4-11.8)	10.4 (7.5-15.4)	10.2 (6.9-14.7)	10.4 (7.7-16.3)	0.179	0.537	0.121	0.473
RAF at 12 months	10.2 (8.8-13.4)	13.4 (9.8-18.8)	15.8 (9.9-20.2)	11.8 (9.7-15.1)	0.005	0.006	0.076	0.249

Abbreviations: LAF = left antecubital fossa, RAF = right antecubital fossa

* Sample size ≤30

Compared with the persistent eczema group, the transient eczema group had higher TEWL over the right antecubital fossae at 1 month (5.1 vs 11.0 g/m^2/hour, P=0.014).

The effects of time on all eczema outcomes were significant (P<0.001). SH over both left antecubital fossae (10.8 vs 40.5 arbitrary units [AU], P<0.001) and right antecubital fossae (11.9 vs 42.2 AU, P<0.001) were lower at baseline and became stable at 1 month. Similar results were observed for TEWL over both the left antecubital fossae (7.1 vs 10.9 AU, P<0.001) and the right antecubital fossae (7.1 vs 12.7 AU, P<0.001), regardless of eczema outcomes. SH and TEWL trajectories did not differ significantly among eczema groups.

For skin microbiome analysis, 2345 skin swabs were collected from 166 participants across five timepoints. After quality control, a total of 42 268 249 reads from 2305 skin microbiome samples were analysed (15 908 reads per sample). We observed significant longitudinal changes in both alpha and beta diversity indices of the skin microbiome at all sampling sites from birth to 12 months of age (P<0.001, Fig). Regarding inter-group differences in diversity indices, we detected a significant association between eczema ever and skin microbiome measured over the left antecubital fossae; the association was stronger when participants were stratified into eczema groups (persistent eczema, transient eczema, and no eczema by 12 months). At 12 months, beta diversity over the left antecubital fossae differed between infants with and without eczema (P=0.004), as well as between infants with persistent eczema and infants with transient eczema (P=0.001). For biodiversity over other body sites, alpha diversity indices measured over the right antecubital fossae at birth differed between infants with and without eczema (P=0.026-0.032). Similar findings were observed for alpha diversity indices over the left popliteal fossa at 3 months (P=0.020) and 12 months (P=0.007-0.013). Beta diversity measured over the right antecubital fossae at 3 months differed between infants with and without eczema (P=0.026), as well as among infants with persistent eczema, transient eczema, and no eczema (P=0.012).

GEE analyses revealed that the skin microbiome over the right antecubital fossae had significant single-timepoint effects on eczema ever (Shannon: B= -0.139, 95% confidence interval [CI]= -0.263 to -0.016, P=0.027; Simpson: B= -0.051, 95% CI= -0.101 to -0.002, P=0.041) and persistent eczema (Shannon: B= -0.093, 95% CI -0.176 to -0.009, P=0.029). No such association was detected for skin microbiome samples obtained at other body sites (results not

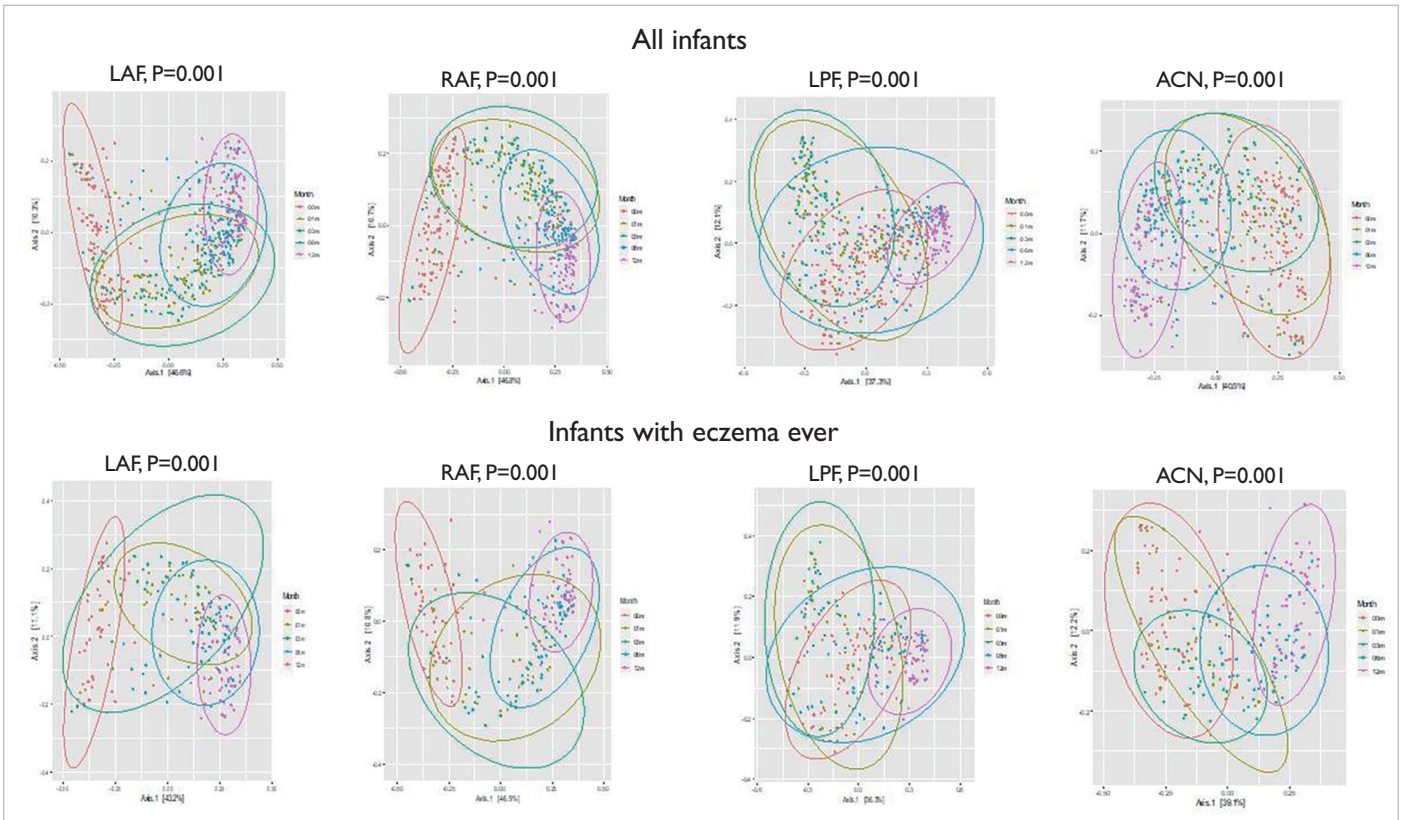

FIG. Principal coordinates analysis plots showing dynamic shifts in beta diversity indices over the left antecubital fossa (LAF), right antecubital fossa (RAF), left popliteal fossa (LPF), and anterior chest between the nipples (ACN) among all infants and infants with eczema.

shown). Additionally, GEE analyses did not show longitudinal effects of alpha diversity indices of the skin microbiome at any body site on the occurrence of eczema ever during infancy.

Multiple bacteria were associated with various eczema outcomes. For example, *Deinococcus* abundance over the right antecubital fossae at 1 month was significantly lower in infants with persistent eczema than in infants without eczema, whereas abundances of *Bergeyella* and *Chryseobacterium* over the right antecubital fossae at 3 months were lower in infants with persistent eczema than in infants with no eczema. However, these microbial signatures were not confirmed in the replication cohort. In the cross-sectional study, alpha diversity was lowest in lesional skin of infants with eczema (P<0.001) and lower in infants with atopic eczema at 12 months than in infants with non-atopic eczema (P<0.001).

Discussion

In the present study, eczema affected almost half of Chinese infants, although there was a high rate of remission by 12 months. Infantile eczema was associated with atopy only and not with other clinical, environmental, or biophysical factors. A family history of allergy was not reported to be a risk factor of eczema, probably owing to the small sample size.

The present study revealed postnatal alterations in biophysical parameters and suggested that the first month after birth is a vulnerable period owing to an immature skin barrier. Several studies from Japan and Ireland have supported our observation of an increase in TEWL within the first 1 to 2 months after birth. Patients with eczema exhibit dry and lichenified skin, and we found that TEWL at 3 months was associated with eczema. Our finding was consistent with that of other study.[4]

We found significantly lower alpha diversity among infants with eczema ever at several timepoints, suggestive of lower richness and evenness in the skin microbiome. This finding was consistent with that of a study of older children and adults.[5] Analyses of beta diversity suggested that bacterial community structures were altered in infantile eczema: infants with eczema ever exhibited significantly less clustered bacterial communities. This indicates that these infants harboured bacterial communities with greater phylogenetic distance among genera from different families.

Skin microbiome analyses in the replication cohort yielded discrepant findings among toddlers and preschool children with eczema. Alpha diversity was lower in older children with eczema but not in toddlers with eczema, compared with infants with no eczema. Furthermore, comparisons of the discovery and replication cohorts did not reveal any consistent microbial signature associated with eczema outcomes.

Conclusion

Atopy, biophysical parameters, and microbial diversity indices are predictive biomarkers for eczema outcomes in Chinese infants.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#06170466). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Chen Y, Yau JWK, Song Y, et al. Roles of early-life skin microbiota on natural course of infantile eczema. Arch Dis Child 2021;106(Suppl 1):A413-4.
2. Lee YL, Yu VSW, Chen Y, et al. Relationship between clinical factors and resolution of early-onset eczema in infancy: findings from a birth cohort in Hong Kong. Allergy 2023;78(Suppl 3):364-5.
3. Chen Y, Song Y, Chen Z, et al. Early-life skin microbial biomarkers for eczema phenotypes in Chinese toddlers. Pathogens 2023;12:697.

Acknowledgements

We thank the infants and their families for participating and for attending the follow-up visits.

References

1. Kennedy EA, Connolly J, Hourihane JO, et al. Skin microbiome before development of atopic dermatitis: early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. J Allergy Clin Immunol 2017;139:166-72.
2. Kelleher M, Dunn-Galvin A, Hourihane JO, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. J Allergy Clin Immunol 2015;135:930-5.e1.
3. Chen Y, Song Y, Chen Z, et al. Early-life skin microbial biomarkers for eczema phenotypes in Chinese toddlers. Pathogens 2023;12:697.
4. Alexander H, Brown S, Danby S, Flohr C. Research techniques made simple: transepidermal water loss measurement as a research tool. J Invest Dermatol 2018;138:2295-300.e1.
5. Paller AS, Kong HH, Seed P, et al. The microbiome in patients with atopic dermatitis. J Allergy Clin Immunol 2019;143:26-35.