

The role of skin and gut microbiota in the development of atopic eczema

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Summary

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Conventional culture-based studies have suggested that a reduction in microbial exposure in early life predisposes to atopic eczema and allergies. However, molecular microbiological methods have shown that conventional culture fails to grow around 80% of the bacterial flora. More recent work reviewed in this paper has employed next generation sequencing to study the influence of the gut and skin microbiota, both with regard to the risk of developing atopic eczema but also the role of pathogenic and commensal bacteria in established disease. Birth cohorts investigating the gastrointestinal tract reported reduced faecal microbiota diversity among those who later developed atopic eczema, using gel electrophoresis, real-time PCR or 16S ribosomal RNA gene pyrosequencing. However, the inverse association with reduced faecal bacterial diversity was not confirmed in cross-sectional studies among patients with established atopic eczema. Only two studies investigated the cutaneous microbiota in a longitudinal study design and both were unable to provide evidence that *Staphylococcus aureus* colonisation precedes the development of atopic eczema. Next generation sequencing has confirmed the cross-sectional association between atopic eczema and *S. aureus* colonisation. The two studies that used this approach have also shown that disease flares are associated with a significant fall in skin microbiota diversity and an increase in the relative abundance of both *S. aureus* and *epidermidis*. Interestingly, *S. aureus* elimination does not appear to be the main reason why atopic eczema improves after a flare and antimicrobial and anti-inflammatory therapy enhances bacterial diversity. Further, well-phenotyped birth cohorts that take key confounders, such as antibiotic exposure, into account are required.

What's already known about this topic?

- Culture-based studies have shown strong associations between cutaneous *Staphylococcus aureus* colonisation and established atopic eczema during and outside of the context of disease flares.
- Using the same approach, there is also evidence for an inverse relationship between gut bacterial diversity in early life and the later development of atopic eczema, in keeping with the 'biodiversity hypothesis'.
- However, bacterial culture misses around 80% of the bacteria detectable with next generation pyrosequencing.

What does this study add?

- 16S rRNA sequencing has shown that both *Staphylococcus aureus* and epidermidis proliferate whilst bacterial diversity drops at lesional sites when atopic eczema flares, but *S. aureus* elimination is not the main reason why atopic eczema gets better.
- Next generation sequencing studies have not found evidence that *S. aureus* colonisation triggers atopic eczema development, although the current body of literature is very small.
- In addition, there is further evidence that a reduced diversity of the faecal microbiota precedes the development of atopic eczema, an association that appears lost in established disease.

Eczema (syn. 'atopic eczema' or 'atopic dermatitis') affects at least one-fifth of the paediatric population in industrialised nations, often arises in early infancy and raises the risk of developing subsequent sensitisation, food allergy and asthma.¹ Pedigree studies of families carrying filaggrin loss-of-function mutations demonstrate a semi-dominant inheritance pattern for eczema.² Filaggrin loss-of-function variants impair keratinocyte differentiation and reduce the ability of the skin to retain water, leading to poor skin barrier function and dry skin.³ However, it is not known what instigates the cutaneous inflammation associated with eczema, although bacterial pathogens and changes in the diversity of the cutaneous microbiota may play a role. *Staphylococcus aureus* is, for instance, commonly found on the skin of eczema sufferers, especially with more severe disease. However, it remains uncertain whether bacterial dysbiosis (imbalance or reduced diversity of the microbial community) of the skin plays a causal role in the development of eczema and disease flares, or whether the observed expansion of *S. aureus* and reduction in bacterial diversity are primarily an epiphenomenon resulting from an impaired and inflamed skin barrier.

The 'hygiene hypothesis' was born out of the observation that the risk of hay fever and eczema is inversely associated with the number of siblings.⁴ At the time, David Strachan postulated that children living in larger families were protected from developing eczema and allergies by greater exposure to pathogens. Conventional bacterial culture-based work has suggested that commensal bacteria as well as pathogens may influence the development of eczema. For instance, faecal samples from Estonian and Swedish children with eczema or allergic sensitisation demonstrated a significantly higher prevalence of *S. aureus* with less frequent enterococci and bifidobacteria colonisation during infancy.⁵ More recently, studies using molecular methods such as real-time PCR on faecal samples collected at one month of age have shown that colonisation with *Escherichia coli* is associated with a greater risk of eczema at 2 years, and that *Clostridium difficile* colonisation in the gut is more common among children with eczema throughout the first 6 years of life (Dutch KOALA birth cohort).^{6,7}

With the advent of pyrosequencing technology, which is able to identify around 80% more bacteria in the gut than conventional culture-based methods, a much greater complexity and diversity of gut and skin microbiota has been demonstrated. Partly through this novel technology, the hygiene hypothesis has metamorphosed into the 'biodiversity hypothesis', which proposes that the diversity of the gut and skin microbiota are of greater importance than identifying individual bacterial species, and that this aids the development of a regulatory T cell, cytokine and complement network, which protects against autoimmune conditions, allergies and eczema.⁸

There is emerging evidence for intricate cross-talk between gut commensals and the gut associated immune system. For instance, commensal bacteria that colonise the gut mucosa from birth are essential for the seeding of immune cells and play an important role to ensure the gut microbiota remain stable and diverse.⁹ As for the skin, commensals are not required for the migration of immune cells into the skin (we have around 20 billion lymphocytes in our skin) but they control important immune factors, such as antimicrobial peptide (AMP) production and synthesis via keratinocytes. Animal models also suggest that inhibition of the C5a receptor leads to reduced cutaneous bacterial diversity and altered composition of the skin microbiota.⁹ Furthermore, skin commensals, such as *Staphylococcus epidermidis*, modulate local T cells through tuning of IL-1 production, and it is likely that microbial metabolites are recognised by antigen-presenting cells in the epidermis. Microbial metabolites might also diffuse into the dermis from sweat ducts and pilosebaceous gland units, further contributing to the cross-talk between commensals, potential pathogens and the skin immune-system. The question is how an altered skin or gut microbiota could lead to immunological skewing and subsequent eczema.¹⁰

We therefore conducted a systematic online literature search in Medline to identify and appraise the current evidence for the role of the skin and gut microbiota in the development and natural history of eczema. We included studies that looked at the risk of future eczema as well as research that studied the association between the skin and gut microbiota in established disease.

Table 1 Overview of prospective studies measuring diversity indices with later assessment of eczema or allergic sensitisation.

First author, year, journal	Cohort, country	Number of cases, controls	Clinical case definition	Microbiological technique used	Age at sampling	Main results
Abrahamsson, 2012, Journal of Allergy and Clinical Immunology	Probiotic trial, Sweden	20, 20	Eczema associated with allergic sensitisation until 2 years	V3-4 16S parallel sequencing	1 month	Reduced Shannon diversity amongst cases ($P < 0.004$)
Wang, 2008, Journal of Allergy and Clinical Immunology	Allergy Flora cohort, Sweden/Italy/UK	15, 20	Eczema associated with allergic sensitisation by 18 months	Terminal restriction fragment length polymorphism	7 days	Reduced number of TRFLP peaks amongst cases (7.0) vs. controls (9.5; $P = 0.03$)
Forno, 2008, Clinical & Molecular Allergy	Recruited from Brigham & Women's Hospital, Boston, USA	9, 21	Eczema at 6 months	Denaturing gradient gel electrophoresis	1 month and 4 months	Cases demonstrated a lower Shannon diversity index both at 1 ($P = 0.01$) and 4 months of age ($P = 0.02$).
Ismail, 2012, Pediatric Allergy and Immunology	Probiotic trial, Australia	Eczema; 33, 65 allergic sensitisation; 20, 62	Eczema (UK Working Party criteria) and allergic sensitisation	Terminal restriction fragment length polymorphism	7 days	Eczema cases demonstrated lower faecal microbial diversity versus healthy controls (13.1 mean peaks vs 15.5, $P = 0.003$ using restriction enzyme Alu1; 14.7 mean peaks vs 17.2, $P = 0.03$ using Sau96I), but no difference with respect to skin sensitisation
Bisgaard, 2011, Journal of Allergy and Clinical Immunology	Copenhagen Prospective Study on Asthma in Childhood (COPSAC), Denmark	Eczema; 127, 253 allergic sensitisation prevalence not reported	Hanifin and Rajka criteria for eczema at visits up to 6 yrs. Any allergic sensitisation measured by serum specific IgE	Denaturing gradient gel electrophoresis	1 month and 12 months	No relationship between faecal diversity and eczema. Diversity at one and twelve months was inversely related to allergic sensitization (serum specific IgE $P < 0.003$; skin prick test $P < 0.017$) at any of 0.5, 1.5, 4, and 6 years

Prospective studies relating early faecal microbiota characteristics to the development of eczema

The diversity or richness of infants' gut bacterial constituents has been reported in five birth cohorts, and each supports inverse relationships with eczema and/or allergic sensitisation (Table 1). Only one study utilised 16S ribosomal rRNA gene pyrosequencing of faecal DNA amongst cases and controls selected from larger cohorts. Abrahamsson *et al.* found that 20 children with eczema and allergic sensitisation selected at 18 months had a less diverse

faecal microbiota at 1 month of age compared to healthy controls.¹¹ The remaining four studies used gel based techniques to assess the diversity of the faecal microbiota, and three of these (the European collaborative Allergy Flora project, Australian and American studies) also reported reduced diversity amongst cases of eczema.^{12–14} Bisgaard *et al.* found a reduced diversity amongst faecal samples supplied from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) cohort at 1 month of age amongst children developing any allergic sensitisation up to 6 years of age, although there was no associations with eczema.¹⁵

Amongst studies conducted in infancy that looked for specific gut microbiota constituents, the Dutch KOALA birth cohort found a greater prevalence of *Escherichia coli* and *C. difficile* in those who later developed eczema.⁷ Infants carrying *Lactobacillus paracasei* were significantly less likely to develop eczema by 2 years. Abrahamsson *et al.* (mentioned above) sequenced faecal samples passed at 1 week, 1 month and 1 year of age from 20 cases with both eczema and sensitisation (diagnosed by either skin prick testing or serum specific-IgE) by 2 years, and compared these to controls who were participating in a probiotic trial in Sweden. They observed that the samples passed by cases with eczema had a lower diversity of total faecal microbiota at 1 month of age, and also yielded greater proportions of obligate anaerobes, such as the *Bacteroidetes* phylum and *Bacteroides* genera.¹¹ Similarly, eczema cases and controls selected from the placebo arm of a probiotic trial in Singapore showed a higher abundance of *Clostridium perfringens* by Fluorescence In Situ Hybridization combined with Flow Cytometry in those who developed eczema in the first 2 years of life.¹⁶

Thus, reduced gut microbial diversity in early life appears consistently associated with an increased risk of developing eczema, with the Dutch and Singaporean studies also reporting greater prevalence of *Clostridia* species amongst cases with eczema.

Cross-sectional studies assessing faecal microbiota characteristics amongst children with established eczema

Whilst faecal samples from young infants have demonstrated lesser diversity of faecal microbiota amongst infants who later develop eczema, the only cross-sectional study of faecal microbiota from patients with established eczema did not support this observation. This study enrolled next-generation sequencing, to examine the faecal samples from 90 children and adults with eczema from outpatient clinics in South Korea as well as 42 healthy controls. The study found no difference in overall gut microbiota diversity between the groups.¹⁷ However, greater abundance of *Faecalibacterium prausnitzii* and reduced short-chain fatty acids were detected amongst patients with eczema under 1 year of age. Metagenomic shotgun sequencing was consequently undertaken on a small subsample, which suggested greater functional responsiveness towards oxidative stress and handling of a major mucin component, N-acetylgalactosamine, amongst patients with eczema.¹⁷

It is intriguing that none of the studies described so far adjusted for dietary patterns or concomitant allergic disease. There is also currently no work to investigate whether microbiota at other anatomical sites, such as the oral cavity or respiratory tract, may influence the gut microbiota or play an additional role in the development of eczema. However, there is a small but growing body of work that has investigated the microbiota of the skin in healthy individuals and eczema sufferers. Interestingly, there is much higher bacterial diversity on human skin compared to the gut and oral cavity, probably because the skin

offers a number of ecological niches, from the skin surface down into the pilo-sebaceous gland units with varying skin pH, temperature, and sebum content.¹⁸ This may well be why there is great heterogeneity in the distribution of the skin microbiota depending on body site, in particular sebaceous, moist, and dry areas, with clear distribution patterns between these ecological niches, even between people.¹⁹ Furthermore, these microbial communities are stable over time.²⁰

Prospective studies relating cutaneous microbiota characteristics to the development of eczema

Prospective cohort studies have failed to convincingly demonstrate that the early emergence of *S. aureus* on the skin is associated with the instigation of eczema. The COPSAC cohort found no relationship between *S. aureus* culture from nasal or perineal sites at either 1 month or 1 year and the later development of eczema up to 3 years of age.²¹ Whilst another study reported that *S. aureus* growth from nasal swabs at 6 months was associated with an increased eczema risk during the second year of life, a large proportion of these children had already developed eczema by the time they were swabbed at 6 months.²²

Cross-sectional studies assessing skin microbiota characteristics amongst children with established eczema

Numerous culture-based studies support the clinical link between *S. aureus* burden and eczema severity. Greater skin barrier disruption, as measured by transepidermal water loss, is correlated with greater *S. aureus* colonisation.²³ Staphylococcal peptides and their superantigens have also been implicated in driving eczematous inflammation. For instance, patch testing adults with Staphylococcal enterotoxin B (SEB) induces an accumulation of T cells expressing SEB-reactive T-cell receptors, even in those without eczema.²⁴

More recently, sequencing of skin microbiota from children with eczema has demonstrated that *S. epidermidis* may also be involved in eczema pathogenesis. Eczema flares were not only associated with a significant fall in skin microbiota diversity but also a parallel increase in abundance of both *S. aureus* and *S. epidermidis*. In addition, topical anti-inflammatory and antimicrobial treatment in the run up to the flare was associated with reduced abundance in *S. aureus* and a restoration of diversity, suggesting that eczema treatment can prevent the change in bacterial diversity associated with a disease flare.²⁵ Interestingly, the same team examined skin microbiota from patients with monogenic immunodeficiency states that are associated with eczematous rashes, including STAT3-deficient hyper-IgE and Wiskott-Aldrich syndromes. Markers of eczema severity were positively correlated with a higher prevalence of *S. aureus* and *Corynebacterium* and there was also greater diversity of opportunistic fungi and less anatomical specificity in the context of immunodeficiency.

However, none of these studies have been able to compare subjects' skin microbiota with samples recovered before the inception of eczema, making it impossible to examine the sequence of events between changes in the skin microbiota and eczema onset as well as disease flares.

Where do we go from here?

Although eczema is associated with changes in the skin and gut microbiota, its inception and disease flares are unlikely to be driven by *S. aureus* alone. In contrast, antimicrobial therapy as part of topical eczema management appears to enhance bacterial diversity. Furthermore, the current evidence suggests that reduced microbial diversity before eczema develops is only seen in the gut microbiota rather than the skin.

This fits the observation that oral probiotics (dietary supplementation with lactobacilli and bifidobacteria) taken during the last trimester of pregnancy and in early life reduce the risk of eczema development.²⁶ A topical probiotic intervention has also been tried, using a 5% cream containing the spa water commensal *Vitreoscilla filiformis* among 75 6–70 year old patients with mild eczema. After 1 month, this led to a significant reduction in disease severity, itch and associated sleep loss, but there was no change in the abundance of Staphylococci, using conventional culture methods.²⁷ Animal studies subsequently suggested that the epicutaneous application of a lysate of *V. filiformis* suppresses cutaneous inflammation in mice through a toll-like-receptor 2 dependent upregulation in IL-10 production from dendritic cells, which also led to regulatory T cell induction. The targeted production of IL-10 through non-pathogenic bacteria has consequently been proposed as a promising therapeutic approach in eczema.²⁸

Nevertheless, the current literature is small and hampered by methodological diversity and has failed to take important confounding factors into account, such as infant diet, hygiene practices, and antibiotic exposure. Even the prospective studies used a case-control design and are therefore vulnerable to selection bias. To identify microbiota changes associated with the emergence of eczema, well-phenotyped birth cohorts are needed with long-term follow up. Rather than focusing on one body compartment alone, it will be important to study the skin, gut and perhaps even the oral cavity and respiratory tract together to allow a more holistic understanding of how our body microbiome interacts with the immune system and how it influences the development and natural history of eczema and allergic disease.

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