

ORIGINAL ARTICLE

WILEY

Understanding immunological origins of atopic dermatitis through multi-omic analysis

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Funding information

The support for this study was provided by a grant from the Gerber Foundation (#5295).

Editor: Carmen Riggioni

Abstract

Background: The pathophysiology of atopic dermatitis (AD) is multifactorial, impacted by individual medical, demographic, environmental, and immunologic factors. This study used multi-omic analyses to assess how host and microbial factors could contribute to infant AD development.

Methods: This longitudinal cohort study included 129 term infants, identified as AD ($n = 37$) or non-AD ($n = 92$) using the Infant Feeding Practices-II survey and review of medical records. Standardized surveys were used to assess medical and demographic traits (gestational age, sex, race, maternal AD, and atopy family history), and environmental exposures (delivery method, maternal tobacco use, pets, breastfeeding duration, and timing of solid food introduction). Saliva was collected at 6 months for multi-omic assessment of cytokines, microRNAs, mRNAs, and the microbiome. The contribution of each factor to AD status was assessed with logistic regression.

Results: Medical, demographic, and environmental factors did not differ between AD and non-AD infants. Five "omic" factors (IL-8/IL-6, miR-375-3p, miR-21-5p, bacterial diversity, and Proteobacteria) differed between groups ($p < .05$). The severity of AD was positively associated with levels of miR-375-3p ($R = .17$, $p = .049$) and Proteobacteria ($R = .22$, $p = .011$), and negatively associated with levels of miR-21-5p ($R = .20$, $p = .022$). Multi-omic features accounted for 17% of variance between groups, significantly improving an AD risk model employing medical, demographic, and environmental factors ($X^2 = 32.47$, $p = .006$).

Conclusion: Interactions between the microbiome and host signaling may predispose certain infants to AD by promoting a pro-inflammatory environment.

KEYWORDS

allergy, atopic dermatitis, atopy, biomarkers, cytokines, eczema, microbiome, oropharynx, saliva

1 | INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin condition impacting approximately 15% of children worldwide.¹ The incidence of AD has increased over the last 50 years, with approximately 60% of individuals

developing symptoms in the first 12 months of life.² Predisposition to AD may be influenced by medical and demographic traits, environmental factors, and underlying immunologic phenotypes.

Epidermal barrier breakdown is a key driver of AD.³ Causes of epidermal breakdown are multifactorial, resulting from genetic,

environmental, and immunological factors.³ Genetic predispositions in *Filaggrin* (*FLG*) increase risk of developing AD.^{3,4} Related disruptions in the skin barrier may allow allergen sensitization and colonization by pathogens that activate cytokine pathways implicated in AD.⁴ Production of these inflammatory components can be influenced by epitranscriptional factors, such as microribonucleic acids (miRNAs).⁵

Several studies have explored miRNA involvement in AD pathogenesis. miR-21-5p, miR-375-3p, and miR-146b-5p dysregulation has been identified in AD patients.^{6,7} For example, miR-375-3p has been revealed to regulate cytokine signaling by downregulating tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ) through the Yes-associated protein 1/lymphoepithelial Kazal-type-related inhibitor (YAP1/LEKTI) pathway.^{6,7} Additionally, there is evidence that miRNA expression may respond to microbial colonization.⁸

The cutaneous microbiome has been implicated in AD development.^{9–11} Inflamed skin has been associated with changes in the skin microbiome.¹⁰ The composition of bacteria, and their genomic content may perturb host inflammation, and result in AD.^{10,11} Certain permutations of bacterial phyla (Actinobacteria, Bacteroidetes, and Proteobacteria) within the respiratory and gastrointestinal tract have also been associated with the development of atopic conditions.¹²

Given the potential importance of host-microbe interactions in AD pathogenesis, the correlations between skin and oral microbes, and the ability to measure genetic, cytokine, and miRNA factors by sampling the oral epithelium, the current study harnessed infant saliva samples to interrogate multi-omic networks with potential relevance to AD pathogenesis.^{6–9,11–14}

Although various molecular factors have been implicated in AD pathophysiology, to our knowledge, few studies have employed a multi-omic approach to explore their interactions at the time of initial symptom onset. Such information could enhance our knowledge about the pathophysiologic mechanisms underlying this complex disease, leading to novel approaches for prevention and treatment. We hypothesized that interactions between specific microbes, miRNAs, messenger ribonucleic acids (mRNAs), and cytokines would modulate AD development. This hypothesis was tested using a longitudinal cohort study of breastfed infants from birth through 12 months.

2 | METHODS

2.1 | Participants

This prospective longitudinal cohort study involved a convenience sample of 221 infants. Eligible participants included breastfed, term infants. Exclusion criteria included maternal morbidities that could impact breastfeeding ability (e.g., cancer, drug addiction), plan for infant adoption, neonatal condition that could impact breastfeeding ability (e.g., cleft palate, prolonged NICU admission >7 days), non-English-speaking parent, or plan to pursue pediatric care outside our academic medical center. Enrollment of participants occurred within

Key Message

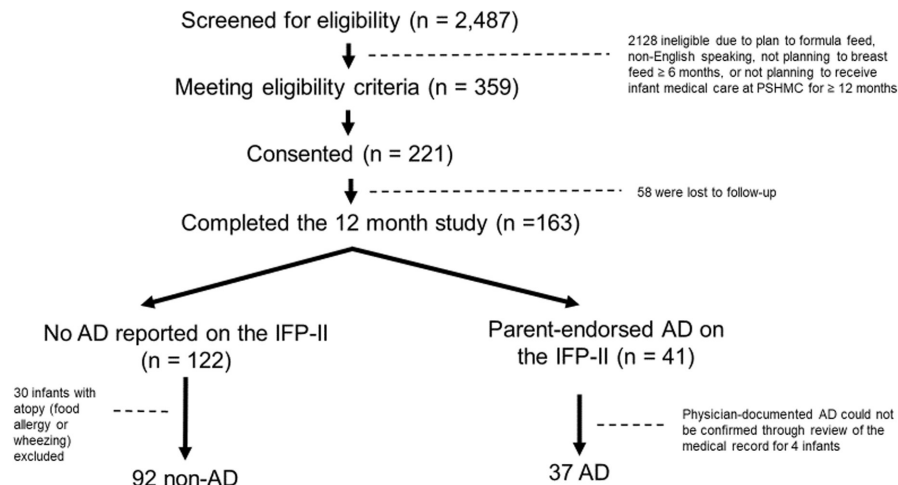
Atopic dermatitis is multifactorial, but the relative contributions of infant traits, environment, and underlying biology are poorly understood. This multi-omic study identified cytokines, microRNAs, and microbial factors associated with infant atopic dermatitis development. Levels of molecular factors contributed significantly to atopic dermatitis status, while controlling for the effects of medical, demographic, and environmental factors. Understanding how complex multi-omic networks contribute to atopic dermatitis development could enhance our knowledge about atopic dermatitis pathogenesis, and lead to the utilization and development of novel targeted therapies.

1 week of delivery at the newborn nursery or the outpatient pediatrics clinic. Of the 2487 infants screened for eligibility, 359 met criteria, 221 consented to participate, and 163 completed the 12-month study. Infants who completed the study were dichotomized into two groups (AD and non-AD) based on parent report of AD on the Infant Feeding Practices (IFP)-II survey,¹⁵ at 1, 4, 6, or 12 months. The presence or absence of physician-documented AD was confirmed for all participants through a review of the medical record. Non-AD infants for whom parents reported food allergies or wheezing were excluded, because these atopic conditions might result in similar perturbations in cytokines, miRNAs, or the microbiome.¹⁶ The final analysis included 92 infants without AD and 37 infants with AD (Figure 1). There was no difference in sex ($p = .28$), race ($p = .08$), ethnicity ($p = .36$), gestational age ($p = .49$), or family history of AD ($p = .55$) between the 129 included and the 92 excluded infants.

2.2 | Measures

During enrollment, electronic surveys were used to collect infant medical and demographic characteristics: gestational age (weeks), delivery method, biologic sex, and race (White, Black, Asian, Bi-Racial, Other). AD was assessed with the Scoring Atopic Dermatitis (SCORAD) by trained nurses at 1, 4, 6, and 12 months. The National Survey of Lead and Allergens in Housing (NSLAH)¹⁷ was administered at 1 month to assess the following AD-related factors: maternal tobacco use, maternal atopic dermatitis, pets in the home, use of allergy-prevention techniques, and family history of atopy in any first-degree relative. Duration of exclusive breastfeeding, timing of solid food introduction, food allergies, and wheezing were assessed with the IFP-II at 1, 4, 6, and 12 months. Introduction of the following food groups was examined: dairy, soy, sweet drinks, baby cereal, fruit, vegetables, shellfish or fish, peanut butter or nuts, and eggs.

FIGURE 1 Participant CONSORT diagram. The CONSORT diagram displays the number of participants who were screened (2487), eligible (359), consented (221), completed the 12-month study (163), and met atopic dermatitis (AD; $n = 37$) or non-AD ($n = 92$) criteria



2.3 | Sample collection and processing

Two non-fasting saliva samples were obtained from each infant at 6 months of age. One swab contained nucleic acid-stabilizing reagent for downstream RNA analysis (DNA Genotek). The second swab obtained for cytokine analysis contained no stabilizing reagents (Oasis Diagnostics). Both swabs were applied to the sublingual and parotid regions for 10–20 s, aliquoted, and stored at -80°C .

2.4 | Cytokine analysis

Next-generation enzyme-linked immunosorbent assays (ELISAs) were used to evaluate levels of four cytokines in infant saliva: C-X-C motif chemokine ligand (CXCL)-10, interleukin (IL)-18, IL-6, and IL-8. These analytes were selected based on their bioavailability in saliva, and their potential relevance to allergic conditions.^{18,19}

2.5 | RNA analysis

RNA was extracted from saliva samples using a miRNeasy Kit (Qiagen, Inc.). RNA quality was verified on an Agilent Bioanalyzer 2100 (Agilent). Using the Illumina TruSeq Small RNA Prep protocol and a NextSeq500 instrument (Illumina), RNA sequencing was completed at the SUNY Molecular Analysis Core. Human miRNA counts were quantified using miRBase, and messenger RNA (mRNA) counts were quantified using Ensemble v95. Through read quality score and total read count, veracity of RNA sequencing was verified for each sample. The RNA features with consistent detection (raw read counts ≥ 10 in $\geq 90\%$ of samples) in each category (miRNAs, mRNAs, bacteria) were median-normalized and mean-center-scaled prior to statistical analysis. The following features were selected a priori for comparison between AD and non-AD groups (based upon their identification in prior studies of atopy): (1) miRNAs—miR-21-5p, miR-375-3p, and miR-146b-5p; (2) mRNAs—*Filaggrin* (FLG), *Serine Peptidase Inhibitor, Kazal Type 5* (SPINK5), and *Toll-like Receptor 4* (TLR4); and (3) microbes—alpha diversity (Simpson's

index), Actinobacteria, Bacteroidetes, Proteobacteria, and Fusobacteria.^{5–7,10–12,20}

2.6 | Statistical analysis

Medical and demographic characteristics were compared between AD and non-AD groups using Welch's *t*-test or a chi-square test. Z-tests were used to compare the proportions of infants introduced to specific food groups at 4, 6, and 12 months. A Mann–Whitney *U* test was used to compare 6-month salivary levels of cytokines, miRNAs, mRNAs, and microbes between groups. Relationships between the cytokines, miRNAs, and microbial RNAs that differed between AD and non-AD groups were assessed with Spearman's rank correlations. Finally, hierarchical logistic regression was utilized to assess the contribution of saliva cytokines, miRNAs, mRNAs, and microbial RNAs to infant AD, relative to known risk factors. An initial model employing infant traits (gestational age, sex, maternal atopy, and family atopy history) was compared against a second model using environmental exposures (delivery mode, home allergy mitigation techniques, maternal tobacco use, pets, breastfeeding duration, and timing of solid food introduction), and a third model, adding salivary levels of cytokines, miRNAs, mRNAs, and microbes. The ability of each model to account for between-group variance (measured by McFadden's R^2) was reported, and significant gains across additive models ($p < .05$) were determined. Collinearity was evaluated with a variance inflation factor. The area under the curve (AUC), sensitivity, and specificity of the final model were reported.

3 | RESULTS

3.1 | Participants

The majority of infants were female (74/129; 57%), White race (98/129; 76%), and born via vaginal delivery (107/129; 82%) (Table 1). Approximately one-quarter had a maternal history of atopic dermatitis (37/129, 28%), and half had a family history of atopy in a

first-degree relative (65/129; 50%). Most were still breastfeeding at 6 months (110/129; 85%) and had been introduced to solid foods (mean age of solid food introduction: 5 months, IQR: 4–6). A higher proportion of infants with AD (21%) were consuming soy foods at 12 months than infants without AD (7%, $Z = 2.14$, $p = .032$). There was no difference between AD and non-AD groups in the proportion of infants consuming other food groups at 4, 6, or 12 months of age (Table S1). The majority of infants had pets in the home (80/124; 64%) but were not exposed to maternal smoking (104/106; 98%). Few families employed allergy mitigation techniques (16/124; 12%). There was no difference ($p > .05$) between AD ($n = 37$) and non-AD groups ($n = 92$) in medical, demographic, or environmental characteristics.

3.2 | AD characteristics

Across the 1, 4, 6, and 12 month time points, average severity of AD symptoms (assessed via SCORAD) was 16.9 (IQR: 8.0–20.4) for the AD group and 2.5 (IQR: 0.0–3.9) for the non-AD group ($p = .001$, $d = 1.69$). The average age of AD diagnosis was 5.4 (± 2.3) months. The majority (22/37, 59%) of infants with AD were prescribed topical steroids. Over one-third (15/37) provided a saliva sample prior to AD diagnosis.

3.3 | Saliva molecular profiles

None of the four cytokines (CXCL10, IL-8, IL-6, and IL-18) displayed significant differences ($p < .05$) between AD and non-AD groups (Table S2). However, the ratio of Th1 to Th2 cytokines (IL-8/IL-6) was higher ($d = 0.33$, $p = .041$) among infants with AD (Figure 2). Of the three miRNAs interrogated (miR-375-3p, miR-21-5p, and miR-146b-5p), two differed between groups. Levels of miR-375-3p were lower ($d = -0.50$, $p = .009$), and levels of miR-21-5p were higher among infants with AD ($d = 0.37$, $p = .007$). None of the three mRNAs (FLG, SPINK5, and TLR4) differed between AD and non-AD groups.

3.4 | Salivary microbial profiles

Alpha diversity of bacterial RNA expression (Simpson index) was higher among infants with AD ($d = 0.61$, $p = .001$) (Figure 2). Of the four bacterial phylum interrogated, only Proteobacteria displayed between-group differences ($d = 0.26$, $p = .014$). Proteobacteria RNA levels were higher among infants with AD.

3.5 | Relationships between AD-related molecular factors

The molecular factors with potential relationships to infant AD (IL-8/IL-6, miR-21-5p, miR-375-3p, Simpson index, and Proteobacteria)

TABLE 1 Participant characteristics

	All ($n = 129$), n (%)	AD ($n = 37$), n (%)	Non-AD ($n = 92$), n (%)
Infant sex, female	74 (57)	21 (56)	53 (57)
Gestational age (weeks), mean (IQR)	38.9 (38–40)	38.8 (38–40)	38.9 (38–40)
Infant race			
White	98 (76.0)	27 (72.9)	71 (77.1)
African American	7 (5.4)	3 (8.1)	4 (4.3)
Asian	5 (3.9)	4 (10.8)	1 (1.0)
Biracial	11 (8.5)	3 (8.1)	8 (8.6)
Other	8 (6.2)	0 (0)	8 (8.6)
Maternal AD	21 (16)	9 (24)	12 (13)
Atopy in 1st- degree relative	65 (50)	22 (59)	43 (46)
Vaginal delivery	107 (82)	30 (81)	77 (83)
Breastfed ≥6 months	110 (85)	32 (86)	78 (84)
Age of solid food introduction (months), mean (IQR)	5.5 (4–6)	5.2 (4–6)	5.6 (4–6)
Pets in home ($n = 124$)	80 (64)	22 (59)	58 (66)
Allergy mitigation	16 (12)	4 (10)	12 (13)
Maternal smoking ($n = 106$)	2 (1.9)	0 (0)	2 (2)
Sample collection time, mean (IQR)	11:40 (9:00–14:00)	11:30 (9:00–14:00)	11:50 (10:00–14:00)

Note: The table displays the number of participants with a given trait or environmental exposure and the total percentage. Data on pets in the home and maternal smoking were not available for all participants. For gestational age, age of solid food introduction, and time of saliva collection, mean values with interquartile range (IQR) are displayed.

were assessed for interrelatedness. Significant cross-category correlations were observed between miR-375-3p and Proteobacteria ($R = .21$, $p = .016$) (Figure 3). Severity of AD (measured as average SCORAD total score) was positively associated with levels of miR-375-3p ($R = .17$, $p = .049$) and Simpson's index ($R = .22$, $p = .011$), and negatively associated with levels of miR-21-5p ($R = .20$, $p = .022$).

3.6 | Modeling AD risk factors

A logistic regression model employing infant traits (gestational age, sex, maternal AD, and family atopy history) accounted for 3.4% of

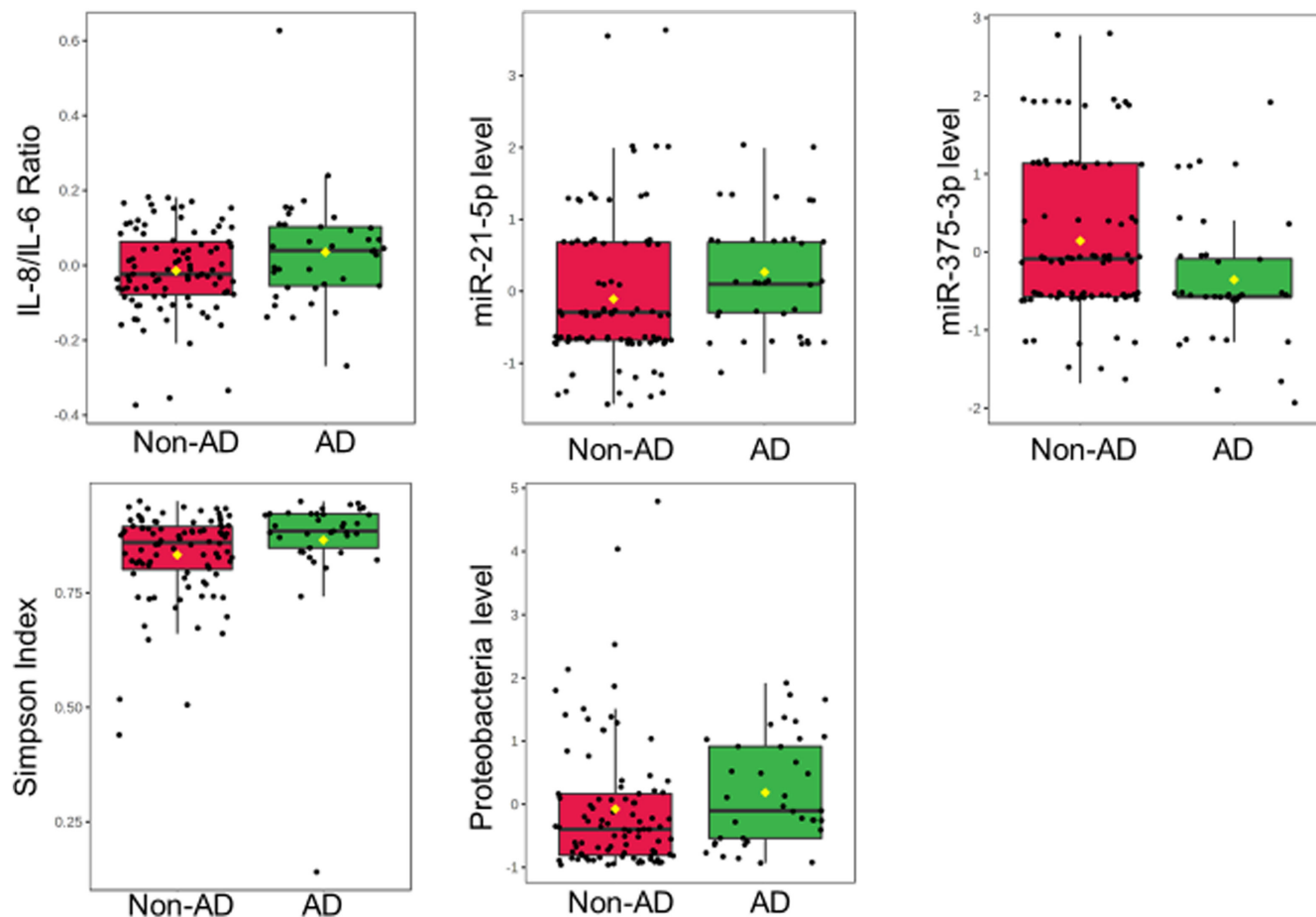


FIGURE 2 Salivary omic features display differences among infants with atopic dermatitis. The boxplots display normalized salivary quantities of omic features that differed between infants with atopic dermatitis (AD; $n = 37$) and infants without AD (non-AD; $n = 92$). Mann-Whitney U tests demonstrated higher levels of the Th1 and Th2 cytokines, interleukin-8 (IL-8), and interleukin-6 (IL-6) ($p = .041$, $d = 0.33$). Levels of miR-21-5p were higher in infants with AD ($p = .0079$, $d = 0.37$), whereas levels of miR-375-3p were lower ($p = .0094$, $d = -0.50$). Bacterial alpha diversity (measured via the Simpson index) was higher among infants with AD ($p = .004$, $d = 0.30$), and RNA levels of Proteobacteria were also higher ($p = .028$, $d = 0.26$)

the variance between AD and non-AD groups ($X^2 = 4.31$, $p = .36$). The addition of environmental exposures (delivery mode, maternal tobacco use, age of solid food introduction, breastfeeding duration, pets, or allergy-proofing) accounted for an additional 5.4% variance, but did not significantly improve the model ($X^2 = 11.07$, $p = .35$). A third model added salivary levels of five omic factors (IL-8/IL-6, miR-375-3p, miR-21-5p, Simpson index, and Proteobacteria). This model accounted for an additional 17.1% of variance and significantly improved differentiation between AD and non-AD infants ($X^2 = 32.47$, $p = .006$). The omic model identified infants with AD with 77% sensitivity and 69% specificity (AUC = 0.814) (Table S3).

4 | DISCUSSION

In this cohort of 129 infants, medical, demographic, and environmental factors did not significantly differ in those with AD. One cytokine ratio (IL-8/IL-6), two miRNAs (miR-375-3p and miR-21-5p), and one bacterial phylum (Proteobacteria) displayed differences among

infants with AD. miRNAs displayed the largest effect. Additionally, miRNAs and Proteobacteria levels correlated with AD severity. Five multi-omic factors (IL-8/IL-6 ratio, miR-375-3p, miR-21-5p, microbial alpha diversity, and Proteobacteria) contributed significantly to AD status.

The ability to understand factors involved in the molecular milieu underlying AD could enhance our knowledge about the pathophysiologic mechanisms underlying this multifactorial, complex disease, and aid the development of precision medicine approaches for AD treatment and prevention. Recent studies support a link between skin barrier dysfunction in AD and progression to subsequent allergic rhinitis and asthma, known as the “atopic march.”²¹ Early detection of infants at risk for this progression may provide a window to initiate personalized therapies and prevent additional atopic manifestations through antimicrobial interventions or novel biologic agents.

This study provides insights into the pathophysiology underlying AD and supports the role of several “omic” factors identified in the previous AD literature. Increased abundance of

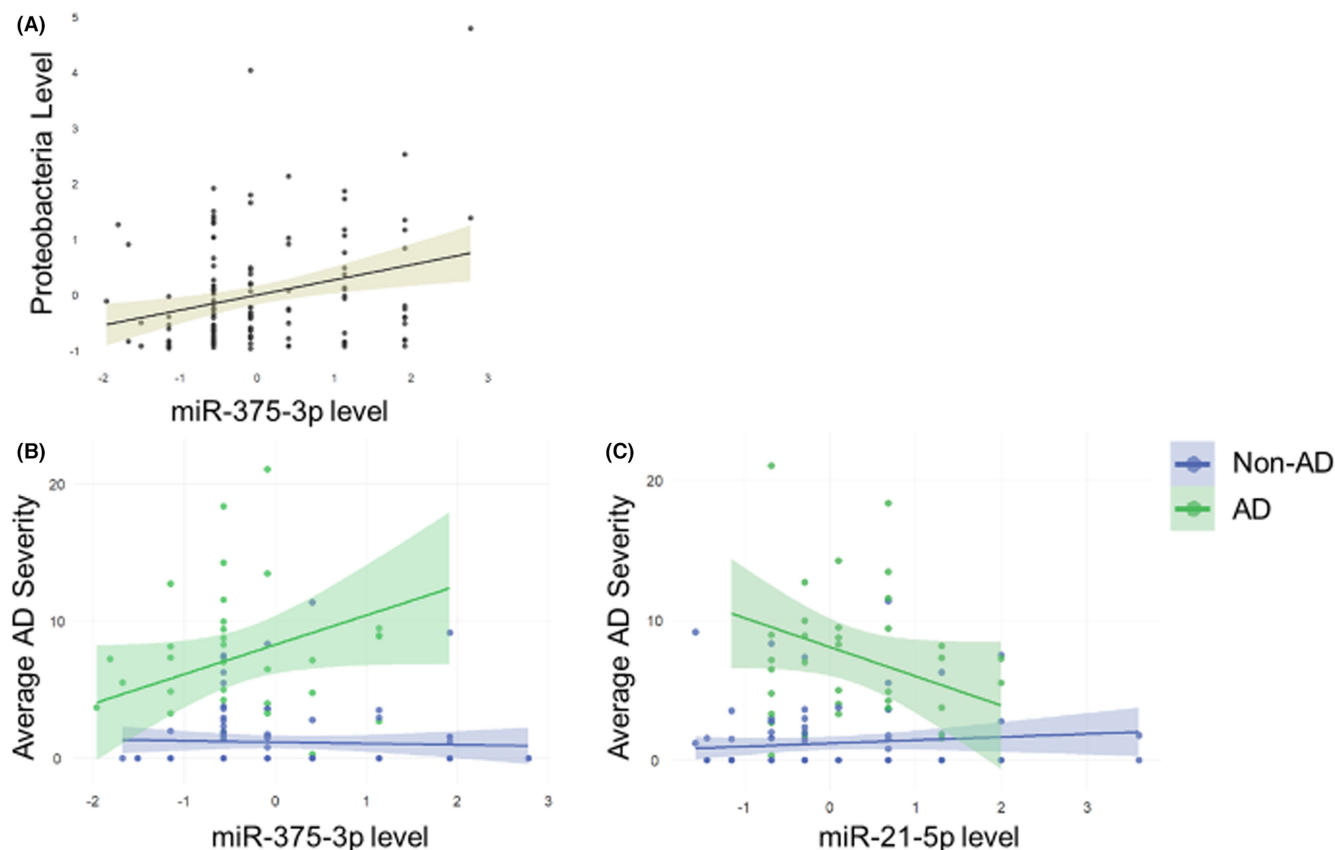


FIGURE 3 Salivary microRNAs display relationships with microbial levels and atopic dermatitis severity. Salivary levels of miR-375 were directly related to Proteobacteria levels in infant saliva (Spearman's $R = .21$, $p = .016$) (A). Levels of miR-375 were inversely related (Spearman's $R = -.17$, $p = .049$) to average severity of atopic dermatitis (AD), as measured with the Scoring Atopic Dermatitis (SCORAD) tool (B). In comparison, levels of miR-21-5p were directly related to average severity of AD (C). Separate linear trend lines are displayed for AD (green) and non-AD (blue) groups

Proteobacteria skin colonization has previously been associated with AD.²² Proteobacteria virulence factors such as membrane proteins and immunoglobulin proteases may mediate AD severity.²² Of the four bacterial phyla interrogated in this study, only Proteobacteria displayed differences in the AD and non-AD groups, and Proteobacteria levels directly correlated with AD severity. However, the current findings also support the importance of microbial diversity in AD. Alpha diversity was the most important predictor of AD status.

Perturbations in microbial colonization may lead to alterations in host inflammation. Elevated IL-8 has been detected in the stratum corneum of patients with AD,²³ whereas individuals with mutations in the IL-6 receptor have been noted to have severe AD.²⁴ This is consistent with our observation of an elevated IL-8/IL-6 ratio among infants with AD. We also found reductions in miR-375-3p among infants with AD. Reductions in miR-375 have been demonstrated in studies of allergic rhinitis,²⁵ and increases in miR-375 have been shown to protect against AD-associated inflammation.⁶

Based on these findings, we propose a conceptual framework for AD pathogenesis involving Proteobacteria perturbations, miRNA regulation, and perturbations in IL-8/IL-6 (Figure 4). miR-375 has

been found to reduce inflammation in allergic conditions through repression of the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway, and downstream effects on IL-6 signaling.²⁵ In contrast, elevations in miR-21 can lead to increases in JAK2/STAT3 signaling.²⁶ In fact, miR-375 and miR-21 may interact to regulate JAK2/STAT3 signaling.²⁷ Inhibition of the JAK2/STAT3 pathway has been shown to reduce AD-related inflammation in mice.²⁸ This may occur via the suppression of pro-inflammatory cytokines, such as IL-8.²⁹ Secretion of IL-8 in the upper airway can also be triggered by pathogenic Proteobacteria, such as *Haemophilus influenzae*.³⁰ It is also possible that perturbations in Proteobacteria levels lead to overall alterations in microbial diversity and modulations of miRNA signaling³¹—both miR-21 and miR-375 have been purported to respond to gut dysbiosis.³² We note that although miR-375-3p levels were lower in infants with AD, levels of miR-375-3p positively correlated with AD severity. A similar, but reverse, trend was noted for miR-21-5p. Therefore, we posit that in severe AD, cytokine increases may serve as a negative feedback loop for miRNA production within host epithelium.

There are several limitations of the current study. The primary site of AD pathology is the dermal and epidermal layers of the skin. Therefore, sampling the oral epithelium may have

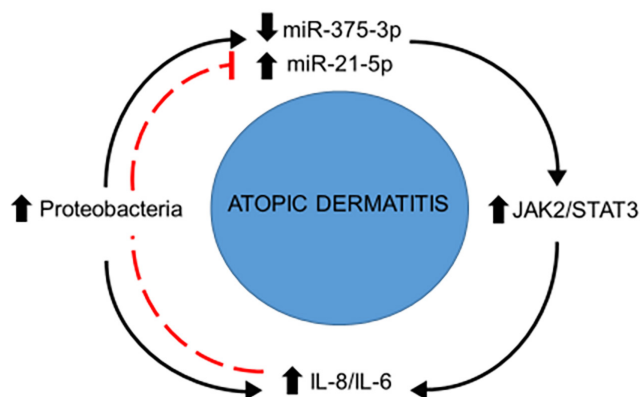


FIGURE 4 Conceptual model for microbe-microRNA interactions in atopic dermatitis. The results of this study, taken in context with the existing literature, support a conceptual framework for AD pathophysiology involving complex host-microbiome interactions. Elevated levels of pathogenic Proteobacteria (such as *Haemophilus influenzae*) or perturbations in microbial diversity may directly induce inflammatory cytokines such as interleukin-8 (IL-8). Alternatively, Proteobacteria may elicit host microRNA responses (such as increases in miR-21 or decreases in miR-375), which have been shown to stimulate JAK2/STAT3 signaling and impact the production of IL-6 and IL-8. In severe AD, chronic elevation of cytokines may serve as a negative feedback loop for miR-21 and miR-375 signaling (red lines)

limited applicability for AD pathogenesis. Previous studies have demonstrated that various gut microbial genera and species have influenced AD development through complex host-microbiome interactions. For example, *Escherichia coli* have been linked to higher risk of developing AD,³³ while one study demonstrated that non-AD patients tend to have increased *Rhizobium* abundance when compared to those with AD.³⁴ Our RNA-sequencing approach did not provide sufficient read depth to explore microbial groups at the genera and species level. This is largely a result of our focus on human microRNA, which dictated that RNA read counts first be aligned to the human genome.

The results of this study support the role of host-microbe interactions as an important contributor to AD development. Specifically, host miRNA signaling may mediate the inflammatory response to microbial colonization and predispose certain infants to AD. These findings should be validated in a larger study, directly sampling the site of epidermal pathology at the time of symptom onset.

AUTHOR CONTRIBUTIONS

Ramin Beheshti: Conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (equal); investigation (lead); methodology (lead); project administration (lead); writing – original draft (lead). **Daniel Mckeone:** Investigation (supporting); methodology (supporting); writing – review and editing (equal). **Scott Halstead:** Investigation (supporting); methodology (supporting); writing – review and editing (equal). **Steven D. Hicks:** Conceptualization (equal); data curation (equal); formal analysis

(equal); funding acquisition (lead); investigation (equal); methodology (equal); resources (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

The authors thank Alle Confair for participant enrollment and sample collection, and Desirae Chandran, and Karen Gentile and Kaitlyn Warren for sample processing.

CONFLICT OF INTEREST

SDH is a consultant and advisory board member for Quadrant Biosciences. The other authors have no financial relationships to disclose. The study does not discuss an investigative use of a commercial product.

ETHICS STATEMENT

This study was approved by the Institutional Review Board at the Penn State College of Medicine (STUDY00008657). Informed consent was provided by all participants.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pai.13817>.

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SUPPORTING INFORMATION

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How to cite this article: Beheshti R, Halstead S, McKeone D, Hicks SD. Understanding immunological origins of atopic dermatitis through multi-omic analysis. *Pediatr Allergy Immunol*. 2022;33:e13817. doi:10.1111/pai.13817