

Original Article

Events in Normal Skin Promote Early-Life Atopic Dermatitis—The MPAACH Cohort

Jocelyn M. Biagini Myers, PhD^{a,b}, Michael G. Sherenian, MD^{a,b}, Asel Baatyrbek Kyzy, BS^b, Rosario Alarcon, MS^b, Amen An, BS^b, Zachary Flege, BS^b, David Morgan, BS^b, Tammy Gonzalez, BS^c, Mariana L. Stevens, PhD^b, Hua He, MS^d, John W. Kroner, MS^b, Daniel Spagna, BS^b, Brittany Grashel, BS^b, Lisa J. Martin, PhD^{a,d}, Andrew B. Herr, PhD^{a,c,e}, and Gurjit K. Khurana Hershey, MD, PhD^{a,b} Cincinnati, Ohio

What is already known about this topic? Nonlesional skin in atopic dermatitis (AD) is abnormal, but the pathobiology of lesional and nonlesional skin and the definition of endotypes are poorly understood.

What does this article add to our knowledge? This study reveals that events in the nonlesional, not the lesional, skin promote the subsequent development of AD severity and cosensitization, which is a key risk factor for allergic comorbidities.

How does this study impact current management guidelines? Our study suggests that management of pediatric AD should include treatment of both lesional and nonlesional skin, because subclinical inflammation in normal-appearing areas may predispose to allergic comorbidities and more severe disease.

BACKGROUND: Nonlesional skin in atopic dermatitis (AD) is abnormal, but the pathobiology of lesional and nonlesional skin and the definition of endotypes are poorly understood.

OBJECTIVE: To define lesional and nonlesional endotypes of AD by building the first US-based early-life prospective cohort of children with AD, the Mechanisms of Progression from AD to Asthma in Children cohort.

METHODS: We assessed lesional and nonlesional skin transepidermal water loss, filaggrin (*FLG*) and alarmin (*S100A8*, *S100A9*) expression, staphylococcal colonization, and patterns of aeroallergen and food sensitization to define nonlesional and lesional phenotypes and endotypes.

RESULTS: Pathophysiologic changes were present in lesional and nonlesional skin and were associated with SCORing for

Atopic Dermatitis. Nonlesional skin had features characteristic of diseased skin including low *FLG* and high alarmin expression, and increased colonization with *Staphylococcus aureus*. In a multivariate model, nonlesional, but not lesional, *FLG* expression was associated with the development of cosensitization and moderate to severe AD. Lesional skin was characterized by further deficits in *FLG* expression ($P < .001$), but alarmin expression was the same as observed in nonlesional skin.

CONCLUSIONS: This study reveals that events in the nonlesional, not the lesional, skin promote the subsequent development of AD severity and cosensitization, which is a key risk factor for allergic comorbidities. Collectively, these data suggest the presence of a subclinical eczema endotype that may predispose to the development of allergic disease in the absence of overt eczema. This may represent a new definition of the atopic march that starts with skin barrier dysfunction rather than eczema. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: Atopic dermatitis; Atopic march; Children; Cohort study; *FLG* expression; Alarmin expression; *S aureus* colonization; Cosensitization

^aDepartment of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio

^bDivision of Asthma Research, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

^cDivision of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

^dDivision of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

^eDivision of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

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Corresponding author: Gurjit K. Khurana Hershey, MD, PhD, 3333 Burnet Ave, MLC 7037, Cincinnati, OH 45229. E-mail: gurjit.hershey@cchmc.org. 2213-2198

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INTRODUCTION

Atopic dermatitis (AD) is a common chronic, inflammatory skin disorder with a complex etiology and heterogeneous presentation affecting up to 20% of children worldwide.¹ AD has been highlighted as the first step in the “atopic march,” whereby AD typically predates the development of other allergic disorders. It has been estimated that one-third to half of patients with AD will develop asthma,² although the mechanisms that promote disease progression remain unclear. Atopic sensitization and food

Abbreviations used

AD- Atopic dermatitis
 FA- Food allergy
 FLG- Filaggrin
 IQR- Interquartile range
 MPAACH- Mechanisms of Progression of Atopic Dermatitis to
 Asthma in Children
 OR- Odds ratio
 SCORAD- SCORing Atopic Dermatitis
 SHS- Secondhand smoke
 SPT- Skin prick test
 TEWL- Transepidermal water loss

allergy (FA) have been reported to be major determinants of AD progression to respiratory allergy; however, recent data indicate that the mechanisms are far more complex.³ To evaluate the current status of knowledge in this area, the National Institute of Allergy and Infectious Diseases recently convened a workshop titled “Atopic dermatitis and the atopic march: Mechanisms and interventions.”¹ It concluded that only about 3% of children follow the complete course of what has been conventionally referred to as the atopic march, although early-life AD remains a major risk factor for the development of any atopic disease. It recommended that a new, large, prospective cohort study incorporating evaluations of skin, gut, airway, and peripheral blood and the use of multiparametric approaches is required to better define phenotypic/endotypic subgroups of AD and to predict AD outcomes and development of allergic comorbidities.

The Mechanisms of Progression of Atopic Dermatitis to Asthma in CHildren (MPAACH) cohort was designed to meet this need. MPAACH is the first US-based early-life cohort of children with AD. The goals of the cohort are to define AD phenotypes and endotypes, dissect the mechanisms that contribute to the progression of AD to other allergic disorders, and identify novel biomarkers that identify children at high risk for the development of asthma and wheezing phenotypes. There have been advances in AD research to identify AD biomarkers using tape strips, a minimally invasive method that samples the keratinocytes. However, most of these studies have been in adults with chronic AD,⁴⁻¹⁴ children with moderate to severe AD,^{15,16} or older children.^{17,18} MPAACH represents mild, moderate, and severe AD, which allows us to evaluate factors that contribute to the whole spectrum of AD severity over time.

MPAACH is uniquely designed to delineate immunologic, skin, biome, physiologic, genetic, genomic, epigenetic, and environmental factors that promote the development of allergic comorbidities in more than 550 infants and toddlers with AD. To enable mechanistic studies, extensive biospecimens are collected from both lesional and nonlesional skin. Unlike previous tape strip studies, our novel skin tape sampling methodology simultaneously samples the surface skin biome as well as the underlying human DNA and RNA, which can be used to characterize host responses to environmental cutaneous exposures. Herein, we focus on lesional and nonlesional skin transepidermal water loss (TEWL), host keratinocyte filaggrin (*FLG*) expression, skin staphylococcal colonization, and host keratinocyte *S100A8* and *S100A9* (alarmin) expression, as well as patterns of aeroallergen and food sensitization to define AD phenotypes and endotypes. A phenotype is any observable characteristic or trait of a disease without any implication of

mechanism, whereas an endotype is a subtype of a disease defined by distinct functional or pathobiological mechanisms. The purpose of this article was to identify phenotypes and endotypes of moderate to severe AD in the first 400 MPAACH children.

METHODS**Study design and inclusion/exclusion criteria**

MPAACH is a prospective early-life cohort of children with AD who will be followed for 5 years including annual visits (Figure 1) with extensive biospecimen collection (Table I).

Inclusion criteria were (1) age 1 to 2 years on enrollment; (2) gestation of greater than or equal to 36 weeks and (3) a diagnosis of AD (based on the Hanifin and Rajka Criteria for Atopic Dermatitis¹⁹), or the parent(s)/legal authorized representative indicates a positive response to each of the 3 questions from the Children's Eczema Questionnaire.²⁰ Exclusions include (1) a comorbid lung condition including cystic fibrosis, congenital anomaly, or bronchopulmonary dysplasia; (2) dependence on immunosuppression or oral steroids for a medical condition other than asthma; (3) condition that precludes sampling of the proposed biologic samples or completion of spirometry; and (4) a bleeding diathesis.

For the analyses presented herein, data were available for the first visit on the first 400 participants recruited into MPAACH from November 11, 2016, through September 5, 2018. Details on the recruitment strategy and outcomes, questionnaires administered, biologic specimens collected, planned assays, and comorbid condition and exposure definitions can be found in the Online Repository text and Figure E1 in this article's Online Repository at www.jaci-inpractice.org.

Data analysis

Descriptive statistics were used to characterize the study population. Frequency (percentage) was reported for categorical variables, while median (interquartile range [IQR]) was reported for continuous variables because some measures exhibited nonnormal distributions. Continuous variables (age, serum cotinine, IgE and vitamin D, TEWL, SCORing Atopic Dermatitis [SCORAD] score, and *FLG* expression) were assessed for normality through the Shapiro-Wilk test. The maximum value across the 2 tapes assayed for RNA expression were included in the final analysis for each gene. For ease of interpretation and display of the data, the gene expression levels were all multiplied by 1000. Details of the association testing can be found in this article's Online Repository text at www.jaci-inpractice.org.

RESULTS**Characteristics of MPAACH participants: Demographic characteristics, exposures, and comorbidities**

Among the first 400 MPAACH participants, 61.5% identified as black, 65.0% had public insurance, and 51.3% were males (Table II). The median age was 2.3 years. More than a third (34.5%) owned a dog and 11.8% owned a cat in the child's first year of life. Approximately half (49.5%) of the parent(s) reported exposures to secondhand smoke (SHS) and 8.5% reported exposure to vaping or e-cigarettes. The median serum cotinine was 0.37 ng/mL and was significantly higher in children whose parents reported SHS exposure (0.46 ± 4.22 vs 0.20 ± 1.51 ; $P = .006$). The median elemental carbon attributable to traffic

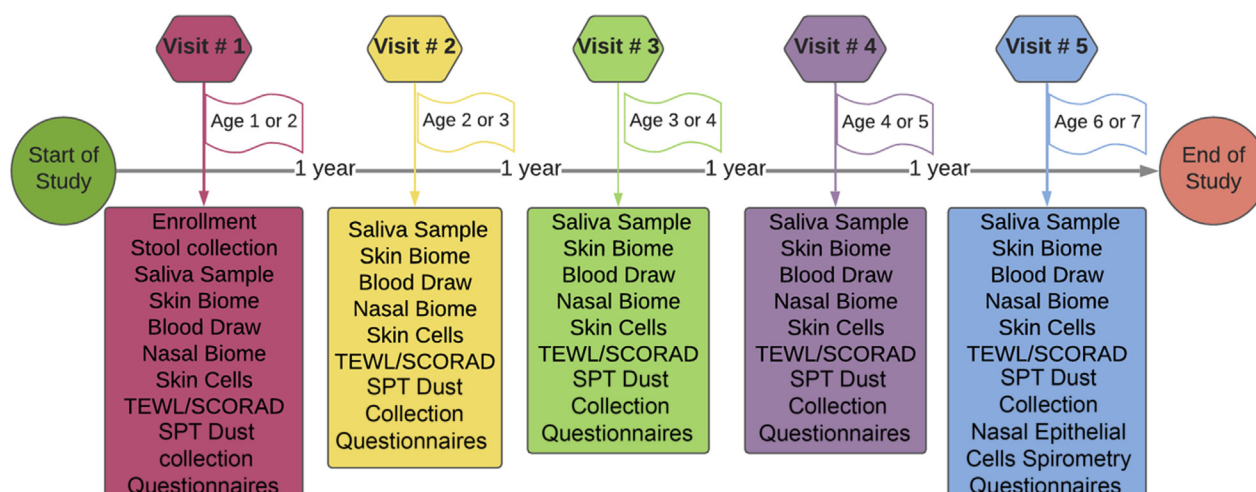


FIGURE 1. Overview of MPAACH study design, visit structure, and biospecimens collected.

TABLE I. Samples and specimens collected at each annual study visit from MPAACH participants

Source/assessment	Samples collected	Planned/completed assays
Saliva	DNA	Genotyping
Skin assessment	SCORAD	
	Lesional TEWL	
	Nonlesional TEWL	
Blood	PBMCs	Flow cytometry, cytometry by time of flight
	Serum	Vitamin D, cotinine, total IgE, specific IgE, cytokines, and chemokines
	Plasma	
	Whole blood	DNA and RNA
Sensitization assessment	11 aeroallergens	
	13 foods	
Keratinocytes	Lesional tape strip DNA	DNA methylation
	Nonlesional tape strip DNA	DNA methylation
	Lesional tape strip RNA	Gene expression
	Nonlesional tape strip RNA	Gene expression
Nasal samples	Biome swab	Microbiome analysis
	Nasal brushing DNA*	DNA methylation
	Nasal brushing RNA*	Gene expression
Gut	Stool	Microbiome analysis
Skin biome	Lesional contact plate—colonies	<i>S aureus</i> and <i>S epidermidis</i> presence and biofilm propensity (crystal violet assay)
	Nonlesional contact plate—colonies	<i>S aureus</i> and <i>S epidermidis</i> presence and biofilm propensity (crystal violet assay)
	Lesional tape strip—bacterial DNA	Microbiome analysis
	Nonlesional tape strip—bacterial DNA	Microbiome analysis
	Bedroom	Allergen levels, microbiome
	Primary activity room	Allergen levels, microbiome

*Sample collected at age 6- to 7-y study visit only.

level was $0.37 \mu\text{g}/\text{m}^3$. Parents reported a doctor's diagnosis of FA in 19.6% of the cohort. This may be underestimated because many of the children are younger than 2 years and have not yet been exposed to common food allergens such as egg and peanut. One-third (32.6%) reported at least 1 wheezing episode in the last 12 months, 24% reported recurrent wheeze, 17.1% reported wheeze without a cold, and 17.3% had allergic rhinitis.

Sensitization patterns and total IgE

Overall, 46.2% of the cohort was sensitized to at least 1 allergen. Almost 16% of the cohort was monosensitized (sensitized to only 1 allergen), 12.3% of the cohort was polysensitized (sensitized to ≥ 2 aeroallergens [33.3%] or ≥ 2 food allergens [66.7%] but not both), and 17.8% were cosensitized (sensitized to ≥ 1 aeroallergen and ≥ 1 food allergen; Figure 2, A). The most

TABLE II. Characteristics of the MPAACH cohort

Characteristic	N = 400
Demographic	
Black race	246 (61.5)
Public insurance	260 (65.0)
Male sex	205 (51.3)
Age (y), median (IQR)	2.3 (1.7-2.5)
Birth season	
Winter	109 (27.3)
Spring	125 (31.3)
Summer	87 (21.8)
Fall	79 (19.8)
Visit 1 season	
Winter	81 (20.3)
Spring	103 (25.8)
Summer	135 (33.8)
Fall	81 (20.3)
Environmental exposures	
Owned a dog in the first year of life	138 (34.5)
Currently live with a dog	102 (25.5)
Owned a cat in the first year of life	47 (11.8)
Currently live with a cat	40 (10.0)
Parental-reported SHS exposure (cigarettes/cigars)	198 (49.5)
Parental-reported exposure to vaping/E-cigarettes	34 (8.5)
Serum cotinine (ng/mL), median (IQR)*	0.37 (0.00-0.99)
ECAT ($\mu\text{g}/\text{m}^3$), median, (IQR)	0.37 (0.30-0.49)
AD Began age 0-3 mo	160 (40.2)
AD Severity	
SCORAD (score)	18.7 (11.4-29.8)
SCORAD (severity group)	
Mild (SCORAD score <25)	263 (65.9)
Moderate (SCORAD score ≥ 25)	118 (29.6)
Severe (SCORAD score ≥ 50)	18 (4.5)
Skin barrier function, median (IQR)	
Lesional skin	
TEWL ($\text{g}/\text{m}^2/\text{h}$)	11.7 (8.2-19.8)
Keratinocyte <i>FLG</i> expression†	0.92 (0.21-2.69)
Keratinocyte <i>S100A8</i> expression†	0.15 (0.04-0.36)
Keratinocyte <i>S100A9</i> expression†	0.25 (0.08-0.64)
Nonlesional skin	
TEWL ($\text{g}/\text{m}^2/\text{h}$)	9.4 (7.1-13.6)
Keratinocyte <i>FLG</i> expression†	1.61 (0.51-4.06)
Keratinocyte <i>S100A8</i> expression†	0.13 (0.04-0.40)
Keratinocyte <i>S100A9</i> expression†	0.23 (0.05-0.71)
Skin bacterial presence	
<i>S aureus</i>	106 (26.6)
<i>S epidermidis</i>	285 (71.4)
Nutritional status	
Serum vitamin D, median (IQR)*	30.0 (24.2-34.5)
Sensitization	
Total serum IgE, median (IQR)‡	37 (14-113)
SPT to aeroallergens	
Negative	262 (65.8)
Positive to 1 aeroallergen	66 (16.6)
Positive to 2+ aeroallergens	70 (17.6)

(continued)

TABLE II. (Continued)

SPT to food allergens	
Negative	279 (70.1)
Positive to 1 food allergen	46 (11.6)
Positive to 2+ food allergens	73 (18.3)
SPT Profile	
SPT Negative	214 (53.8)
Monosensitized	63 (15.9)
Aeroallergen only	21 (33.3)
Food allergen only	42 (66.7)
Polysensitized§	49 (12.3)
Aeroallergens only	23 (47.0)
Food allergens only	26 (53.1)
Cosensitized	71 (17.8)
Comorbid conditions	
Food allergy	78 (19.6)
Wheezing in the last 12 mo	130 (32.5)
Recurrent wheeze	96 (24.0)
Wheeze without a cold	68 (17.1)
Allergic rhinitis	69 (17.3)

ECAT, Elemental carbon attributable to traffic.

*n = 293.

†n = 354, normalized to 18S and multiplied by 1000 for ease of interpretation.

‡N = 284.

§Defined as SPT+ to ≥ 2 aeroallergens or ≥ 2 food allergens but not sensitized to food and aeroallergens.||Defined as SPT+ to ≥ 1 aeroallergen and ≥ 1 food allergen.

prevalent aeroallergen sensitizations were dog (13.3%) and trees (11.6%), whereas the most prevalent food allergens were egg (22.1%) and peanut (18.1%; Figure 2, B). Among those who were food negative, only 23.3% were sensitized to an aeroallergen. In contrast, 60.2% of the food-sensitized children were cosensitized to aeroallergen(s) ($P < .001$). The median total serum IgE was 37 IU/mL (Table II). Participants who were skin prick test (SPT) positive had higher total IgE levels (55 IU/mL) compared with SPT-negative children (26 IU/mL; $P < .0001$), and total serum IgE increased significantly with increasing number of positive SPT results ($r = 0.33$; $P < .0001$).

Factors related to clinical severity of AD

The median SCORAD of the cohort was 18.7. More than one-third of MPAACH children (34.1%) had moderate to severe AD, defined as a SCORAD score of 25 or more (Table II). Female participants had lower SCORAD scores ($P = .003$).

Children who were cosensitized to at least 1 aeroallergen and at least 1 food allergen had elevated SCORAD scores (median, 28.3 $\text{g}/\text{m}^2/\text{h}$) compared with children who were monosensitized or polysensitized (18.5 $\text{g}/\text{m}^2/\text{h}$) and those who were SPT negative (16.6 $\text{g}/\text{m}^2/\text{h}$; $P < .0001$). Compared with polysensitized children, cosensitized children had double the median total number of sensitizations (4 IQR 3-6 vs 2 IQR 2-3; $P < .0001$) and a higher median number of aeroallergen (2 IQR 1-3 vs 0 IQR 0-2; $P < .0001$) and food sensitizations (2 IQR 1-2 vs 2 IQR 0-2; $P = .0003$). To determine whether the increased SCORAD score observed in the cosensitized children was due to increased sensitization load, we performed multiple linear regression adjusting for the number of sensitizations. Even after adjustment for

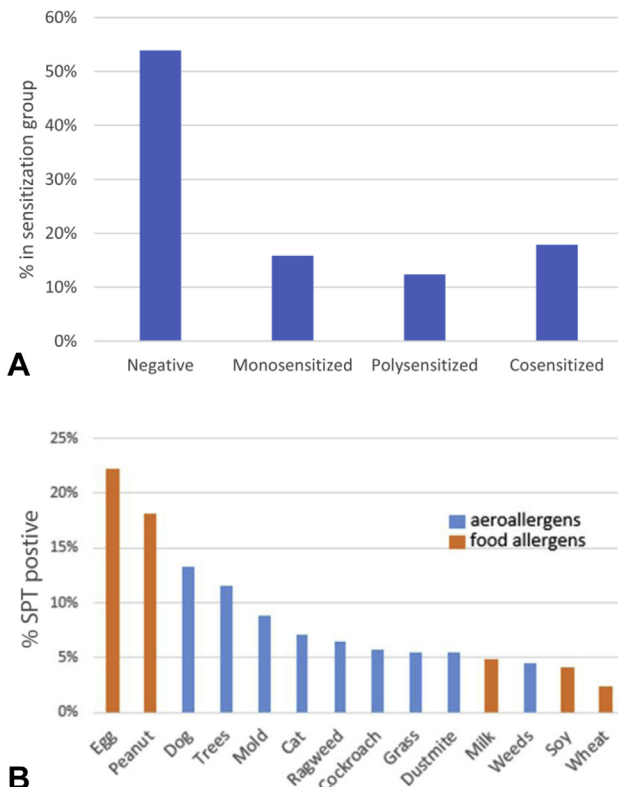


FIGURE 2. (A) Percentage of participants in MPAACH in each sensitization category. (B) Prevalence of sensitization to each individual aeroallergen and food allergen.

sensitization load, cosensitized children had significantly increased SCORAD score ($P = .026$) compared with polysensitized children, supporting that cosensitized children have increased AD severity independent of sensitization load.

Skin colonization with *S aureus* was significantly associated with AD severity, evident by an increase in SCORAD score ($P = .047$; see Figure E2, A, in this article's Online Repository at www.jaci-inpractice.org), but not with lesional or nonlesional TEWL (see Figure E2, C, and E). Colonization of the skin with *S epidermidis* was not associated with AD severity or TEWL (Fig E2, B, D, and F).

Children with higher SCORAD scores had increased serum cotinine levels ($r^2 = 0.18$; $P = .003$; Figure 3, A), even though there was no association between SCORAD score and parental-reported SHS exposure ($P = .87$). Thus, children with similar levels of parental-reported environmental exposure to SHS had higher biologic levels of serum cotinine if their AD was more severe, suggesting that nicotine is absorbed through the skin at significant levels, at least in children with AD. Children with allergic rhinitis also had significantly higher SCORAD scores (21.8 vs 18.4; $P = .02$). There was no association between SCORAD score and any wheezing in the last 12 months or elemental carbon attributable to traffic.

Molecular endotypes of AD

We next examined the pathophysiology of nonlesional and lesional skin and determined their relationships to severe AD to define endotypes. Median TEWL was higher for lesional skin

(11.7 g/m²/h) compared with nonlesional skin (9.4 g/m²/h; $P < .0001$; Table 1). Expression of *FLG* was significantly decreased in lesional skin compared with nonlesional skin (0.92 vs 1.61; $P < .0001$). There were no differences in *S100A8* or *S100A9* expression overall, but *S100A9* expression was nominally increased in the lesional skin of children with moderate to severe AD compared with nonlesional skin ($P = .077$).

Lesional skin. We determined associations of lesional TEWL, *FLG* expression, alarmin expression, and skin staphylococcal colonization with sensitization patterns and SCORAD score (Figure 3, A). Higher SCORAD score was associated with higher lesional TEWL ($P < .001$), aeroallergen ($P < .0001$), food allergen ($P < .0001$) and cosensitization ($P < .0001$), increased alarmin expression ($P < .0001$), and a marked decrease in lesional *FLG* expression ($P < .0001$). Lesional TEWL and lesional *FLG* expression were also inversely associated with each other ($P = .01$). SCORAD score was associated with an increase in *S100A8* ($P < .001$) and *S100A9* ($P < .001$) expression and a decrease in *FLG* expression ($P < .001$) in lesional skin. Increased TEWL and *FLG* expression were associated with increased *S100A8* ($P = .02$ and $P = .0003$, respectively) and *S100A9* expression levels ($P = .009$ and $P = .03$, respectively) in lesional skin.

In the lesional skin, both presence and load of food and aeroallergen sensitization were associated with decreased *FLG* expression and increased TEWL (all $P < .01$). TEWL was significantly increased in children who were cosensitized ($P = .0006$). Even after adjustment for sensitization load, cosensitized children had significantly increased lesional TEWL compared with polysensitized children. Children with FA had significantly higher (14.8 vs 11.1; $P = .002$) lesional TEWL and decreased lesional *FLG* expression (0.43 vs 1.07; $P = .001$).

Colonization with *S aureus* was associated with increased SCORAD score ($P = .047$; Figure 3, A) and decreased *FLG* expression in lesional skin (0.55 vs 1.09; $P = .001$; Figure 4). Staphylococcal presence (*S aureus* or *S epidermidis*) was not associated with lesional *S100A8* or *S100A9* expression. A higher level of serum cotinine was associated with a higher expression of *S100A8* in lesional skin ($r^2 = 0.16$; $P = .01$), suggesting that cutaneous SHS exposures trigger skin host defense responses. There was a borderline significant increase in *S100A9* expression as well ($r^2 = 0.12$; $P = .053$).

Taken together, these results illustrate that a molecular endotype of severe AD in the lesional skin is characterized by high lesional TEWL, low lesional *FLG* expression, high lesional *S100A8/A9* expression, *S aureus* colonization, cosensitization, and elevated serum cotinine.

Nonlesional skin. As in the lesional skin, higher SCORAD scores were associated with a decrease in nonlesional *FLG* expression ($P < .0001$; Figure 3, B) and increased nonlesional TEWL ($P = .001$), supporting that pathologic features characteristic of AD are present even in the nonlesional skin.

In contrast to lesional skin, presence and load of food sensitization, but not aeroallergen sensitization, was significantly associated with increased nonlesional TEWL ($P = .04$). Similar to our findings in lesional skin, children who were cosensitized had significantly decreased *FLG* expression in their nonlesional skin ($P = .0009$). In addition, children with FA had significantly higher nonlesional TEWL (11.8 vs 9.2; $P = .01$) and decreased

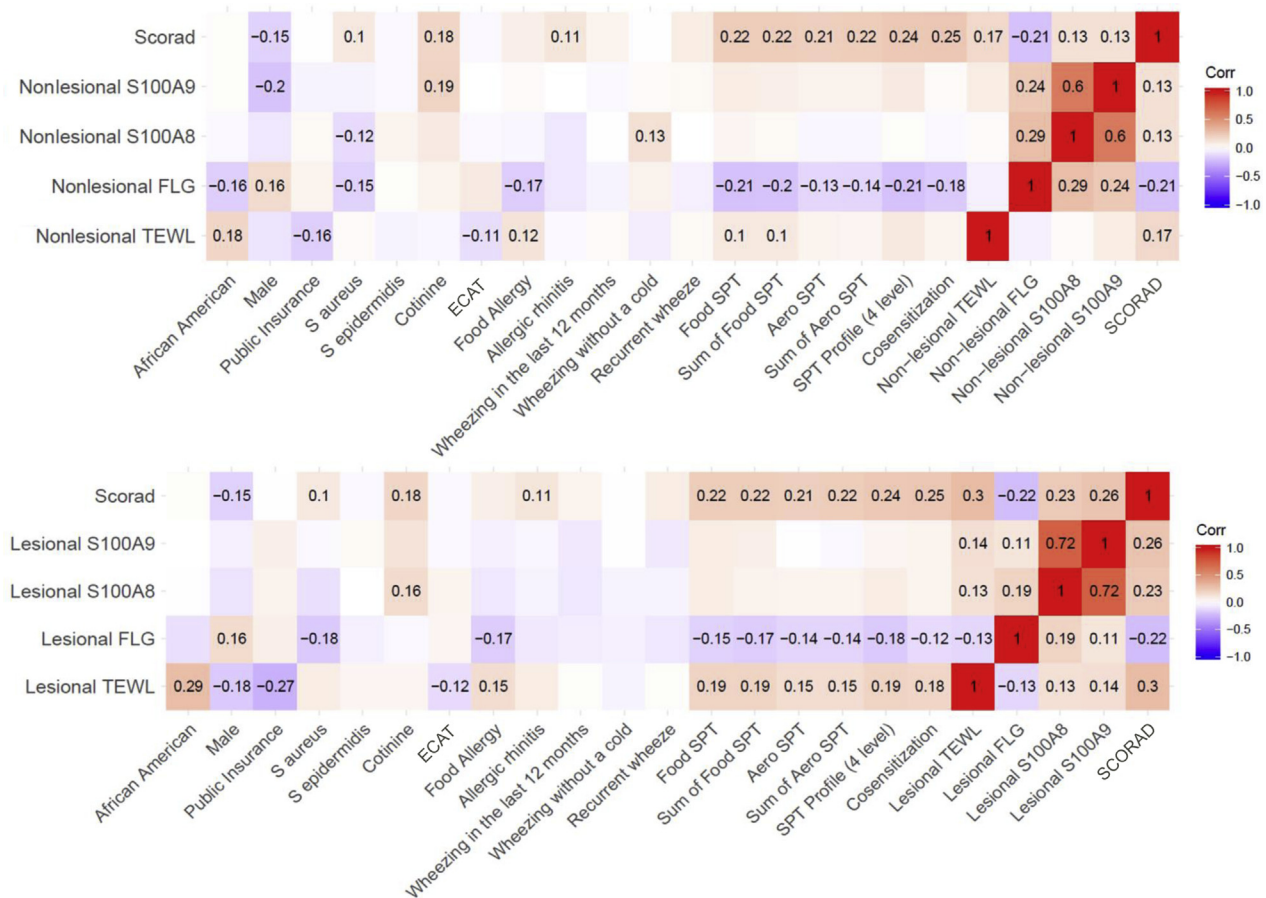


FIGURE 3. Associations of demographic characteristics, comorbidities, sensitization, and AD severity with factors in (A) nonlesional and (B) lesional skin in the MPAACH participants. Numbers shown are Spearman correlation coefficients if P is less than .05. ECAT, Elemental carbon attributable to traffic.

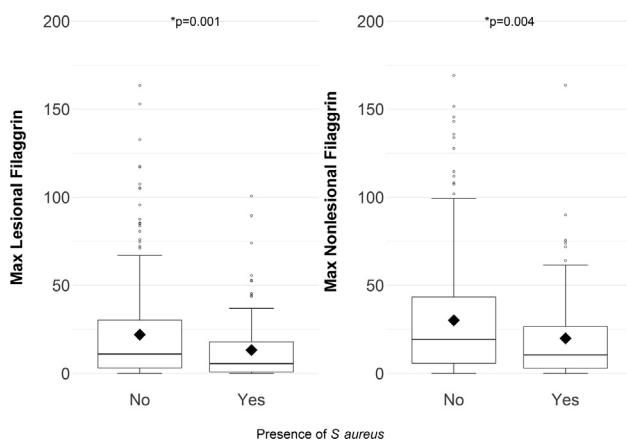


FIGURE 4. Association of the presence of *S aureus* colonization on the skin with nonlesional and lesional FLG gene expression.

nonlesional *FLG* expression (0.97 vs 1.77; $P = .002$). Both SCORAD score and nonlesional *FLG* expression were associated with increased *S100A8* ($P = .01$, $P < .0001$) and *S100A9* expression in nonlesional skin ($P = .02$, $P > .0001$). *S aureus*

colonization was associated with a significant decrease in *FLG* expression in nonlesional skin (1.05 vs 1.94; $P = .004$; Figure 4).

Collectively, these results illustrate that a molecular endotype of severe AD can be defined in nonlesional skin and it is characterized by high nonlesional TEWL, low nonlesional *FLG* expression, high nonlesional *S100A8* expression, *S aureus* colonization of nonlesional skin, polysensitization, food sensitization, and FA. These results confirm that even children without active AD have a global epithelial defect that is not limited to active or typically active lesional locations.

We next used multivariate logistic modeling to determine what combination of factors in the nonlesional skin drives cosensitization. Children with cosensitization were more likely to have decreased *FLG* expression in their nonlesional skin (odds ratio [OR], 0.78; 95% CI, 0.63-0.92; $P = .007$). In a similar model, a decrease in nonlesional *FLG* expression was associated with the presence of *S aureus* on the skin (OR, 0.86; 95% CI, 0.76-0.96; $P = .005$). These data demonstrate that the nonlesional (normal-appearing) skin in children with AD has skin barrier dysfunction as evidenced by decreased *FLG*. Furthermore, this visually normal skin may directly contribute to cosensitization and colonization with more persistent *S aureus* strains, and thus, promote the development of inflammation and clinically apparent AD.

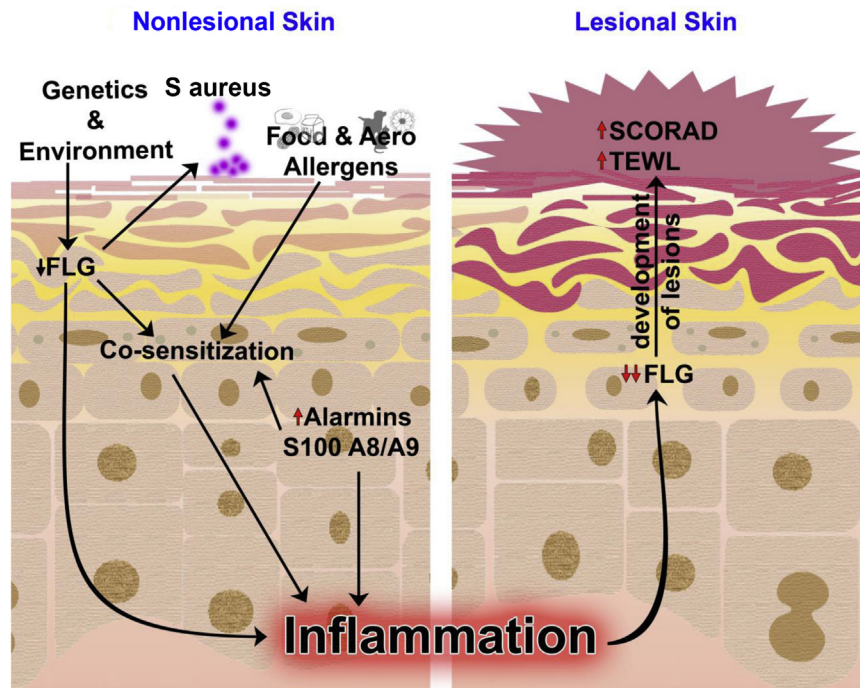


FIGURE 5. Hypothesized model of the factors that contribute to AD in nonlesional and lesional skin.

Multivariate model of moderate to severe AD

The presence of *S aureus*, sex, lesional and nonlesional TEWL and lesional and nonlesional *FLG*, and *S100A8* and *S100A9* expression were considered for entry into the model. The final model revealed that moderate to severe AD is characterized by a significant decrease in nonlesional *FLG* expression (OR, 0.89; 95% CI, 0.81-0.97; $P = .004$) and significant increases in lesional TEWL (OR, 1.03; 95% CI, 1.01-1.04; $P = .0002$) and lesional *S100A8* expression (OR, 1.25; 95% CI, 1.10-1.43; $P = .0003$). The results support that the expression level of *FLG* in nonlesional skin was significantly associated with cosensitization and the development of moderate to severe AD, whereas the expression of *FLG* in lesional skin fell out of the multivariate model, indicating that events in the nonlesional skin promote the subsequent development of lesions.

DISCUSSION

MPAACH is the first early-life prospective cohort of children with AD in the United States incorporating extensive evaluations of the skin, gut, airway, and peripheral blood, as well as the use of multiparameter approaches to define phenotypic and endotypic subgroups of AD. The rigorous phenotyping and comprehensive biospecimen collections make MPAACH uniquely poised to identify endotypes that predict AD outcomes including the development of other atopic conditions (FA, asthma, and allergic rhinitis) and enable mechanistic studies. In the first 400 MPAACH participants, we have observed distinct endotypes of AD in lesional and nonlesional skin that provide novel insights into disease pathophysiology (Figure 5). A common pathologic feature of both lesional and nonlesional skin in AD was decreased *FLG* expression. This supports that in patients with known AD,

barrier dysfunction is global beyond actively inflamed areas and that events in the nonlesional skin are integral to the development of atopic disorders. Furthermore, in our multivariate model, the expression level of *FLG* in nonlesional skin was associated with cosensitization and the development of moderate to severe AD, whereas the expression of *FLG* in the lesional skin was not, indicating that events in the nonlesional skin promote the subsequent development of lesions. Collectively, our data provide novel insights into disease pathophysiology, suggesting that the events taking place in the nonlesional skin may contribute to the development of active lesions by promoting inflammation, a feed-forward subsequent decrease in *FLG* expression, and increased skin permeability.

The decrease in *FLG* expression in nonlesional skin is likely driven by a combination of polygenetic and environmental factors. Strikingly, we found that nonlesional *FLG* expression was strongly associated with skin colonization by *S aureus* and cosensitization. Thus, although nonlesional skin may appear normal, it exhibits characteristics typical of diseased skin. Indeed, recent findings by Leung et al¹⁸ demonstrated that *FLG* content in the nonlesional skin of children with AD and FA was substantially lower than in nonatopic controls. Guttman-Yassky et al¹⁶ also reported that *FLG* expression was decreased in the lesional and nonlesional skin of children younger than 5 years compared with healthy controls. However, their study was restricted to children with moderate to severe eczema, which may explain why they did not see an association between SCORAD score and lesional or nonlesional *FLG* expression.¹⁶ Our findings provide additional evidence that this decrease in *FLG* is not simply an altered barrier but leads to skin dysbiosis and host alarmin expression by keratinocytes and likely contributes to AD symptoms and severity, as well as disease progression, that is, the development of allergic comorbidities. This nonlesional

endotype predicted increased AD severity and cosensitization, which are known risk factors for asthma and allergic rhinitis development.^{21,22} Thus, it is possible that biomarkers in the nonlesional skin in early life could predict subsequent allergic disease development. Indeed, biomarkers in nonlesional skin were strongly predictive of cosensitization, and cosensitization is a strong risk factor for asthma. We will be able to determine this in the MPAACH cohort as the children age.

MPAACH children had a subclinical form of eczema in their nonlesional skin. This raises the possibility that infants and toddlers without a diagnosis of eczema may actually have subclinical eczema with normal-appearing skin, but low skin *FLG* expression, skin dysbiosis, and increased skin alarmin expression. These physiologic changes may be sufficient to confer risk for the development of allergic disease without overt eczema. We previously identified 2 high-risk early-life groups who were at high risk for asthma development, 1 with early eczema and 1 without early eczema.²³ It is possible that some of the high-risk group without eczema actually had subclinical disease.

The sensitization rates we observed in MPAACH children were higher than those previously reported in the general population as well as in high-risk birth cohorts.^{24,25} Thus, the presence of eczema confers a higher risk for sensitization than parental atopy. Our data clearly demonstrate that cosensitization is associated with AD severity over and above polysensitization, and this was independent of the total number of sensitizations. Taken with our finding that children with higher SCORAD scores have barrier defects evidenced by high TEWL and low *FLG* expression, this suggests that both food and aeroallergen sensitization occur through the skin. Although it has been reported that children with AD may develop peanut and egg sensitizations through epidermal allergen exposure,^{26,27} our findings suggest cutaneous sensitization of not only food allergens but also aeroallergens.

Our data demonstrate that children with higher SCORAD scores ($r^2 = 0.17$; $P = .003$) had significantly increased levels of serum cotinine compared with children with lower SCORAD scores, despite equal levels of SHS exposure reported by the parent(s). This indicates that nicotine from SHS is being absorbed through the skin and that this absorption is directly correlated to the skin barrier dysfunction. Recent evidence supports that nicotine can be absorbed directly from the air at rates comparable to or higher than those observed via inhalation.^{28,29} Given our findings, it is likely that children with AD absorb nicotine and allergens in the air and on surfaces through the skin at higher rates, along with increased levels of many of the other toxic substances found in cigarette smoke and other exposures.

The MPAACH cohort has many strengths including its size (thus far we have recruited 557 participants), rigorous phenotyping of AD, comprehensive biospecimen collection, extensive biomarker determinations, and its longitudinal design. MPAACH encompasses a wide degree of variation in AD severity, evident by the wide range in SCORAD scores from 0.6 to 85.5 and is a diverse population with 61.5% subjects of black race. The first 250 participants enrolled were primarily between the ages of 2 and 3 years to evaluate the development of asthma at age 6 to 7 years within 5 years. However, we are now enrolling children as young as 2 to 6 months to evaluate the timing of

barrier dysfunction, sensitization, and colonization and how these factors contribute to the severity of AD and progression to allergic comorbidities.

CONCLUSION

MPAACH is the first early-life prospective cohort of children with AD in the United States. The results herein collectively suggest that events that occur in the nonlesional skin in AD contribute to the cosensitization and the development of skin inflammation and AD lesions (Figure 5). These findings are of high importance because very few children follow the traditional atopic march, emphasizing the need to better define AD disease phenotypes and endotypes that allow prediction of the natural history of AD.

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ONLINE REPOSITORY

RECRUITMENT SUMMARY

Beginning in November of 2017, eligible participants were invited to complete the first of 5 clinical examinations where questionnaires were administered and biologic specimens were collected. The participant's legally authorized representative (LAR)(s) provided parental permission; this study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Subjects were identified from the Cincinnati Children's Hospital Medical Center electronic medical record (EMR), during a Cincinnati Children's Hospital Medical Center clinical visit or from the general public through advertising.

Of the 2074 potential participants, 1625 (78.4%) were identified from the EMR at Cincinnati Children's Hospital Medical Center, 159 (7.7%) self-identified from public advertising in the community, 77 (3.7%) were identified during a clinical visit to the Cincinnati Children's Hospital Medical Center allergy clinic, and 213 (10.3%) were prescreened as ineligible (custody issues, comorbid disease) (see figure below). The rate of enrollment was highest among those who self-selected from the community (58.4%), followed by 24.7% identified from allergy clinics and 17.7% identified from the EMR.

Of those identified from the EMR, 27.9% could not be contacted by phone. The 1105 potential participants able to be contacted by phone, along with those identified from the community and the clinic ($n = 236$), resulted in 353 (26.3%) ineligible, 606 (45.2%) eligible, and 382 (28.5%) refused. The primary reasons for ineligibility were no diagnosis of AD or gestation period less than 36 weeks. The primary reasons for refusal were no interest in research or unwillingness to do the blood draw. Of the 606 who screened eligible, 400 were enrolled (72.0% from the EMR list, 23.3% self-identified from the community, and 4.8% identified from allergy clinic).

Potential participants identified from the EMR received up to 10 phone calls for screening and scheduling. A total of 6957 phone calls were placed to screen and schedule 588 participants (8.5%). Calls placed during weekday mornings and afternoons were more likely to result in a screened and scheduled participant (9.4% and 9.5%, respectively) than calls placed in the evening (7.2%) or on the weekend (6.3%, $P = .002$, data not shown).

QUESTIONNAIRES

All questionnaires are administered at each research visit unless indicated.

1. The MPAACH Research Visit Questionnaire was adapted from our validated New Visit Questionnaire.^{E1} This form collects information on skin symptoms such as timing of the onset of AD and the last flare, AD triggers, pruritis and sleep loss, AD medications, diet history including timing of introduction of solids, reactions to milk, egg, peanut, wheat, soy and tree nuts, food avoidance and FA, asthma, wheezing and respiratory symptoms, triggers, control and severity, environmental exposures including SHS, pets, mold home characteristics, daycare attendance, pets, cockroaches and mice, housing characteristics, daycare attendance, personal and family medical history, and birth characteristics.
2. The Demographic Data and Family Contact Form collects information on race and ethnicity of the child, mother and

father, socioeconomic indicators including income level, parental education level and age, and health insurance information, and requests the names and contact information of both parents as well as 2 relatives/friends not living with the child for retention purposes.

3. The validated Pediatric Allergic Disease Quality of Life Questionnaire^{E2} measures health-related quality of life that encompasses effects of allergic disease on a child's eyes, ears, nose, lungs, skin, emotions, and everyday activities (ages 6 y and up).
4. The validated Dermatitis Family Impact Questionnaire^{E3} measures the impact of childhood AD on family function.
5. The validated Infant's Dermatitis Quality of Life Index^{E4} measures the impact of AD on the infant (up to age 4 y).
6. The validated Asthma Control Test will be administered (with permissions from QualityMetric^{E5,E6}) to assess the level of asthma control (age 4 y and up).
7. The validated Patient-Reported Outcomes Measurement Information System inventories parental anxiety and depression.
8. The validated USDA 18-item core module food security scale measures the degree of food insecurity and hunger in the family.^{E7}

BIOLOGIC SAMPLE COLLECTIONS AND ASSAYS

The biologic sample collections and assessments performed at each of the study visits as well as the assays performed or planned for each sample type are outlined below and summarized in Table I. Lesional skin was defined by the Hanifin and Rajka diagnostic criteria for AD.^{E8} Nonlesional skin was defined as at least 10 cm from lesional sites, and nonlesional sites had no history of lesions at that site according to parental report.

1. SCORAD^{E9} is used at each visit to assess AD severity.
2. TEWL is determined at both lesional and nonlesional sites at each visit using the DermaLab TEWL probe (Cortex Technology, Hadsund, Denmark).
3. Keratinocyte sampling: Skin tape strips are taken from lesional and nonlesional skin at each visit. Briefly, skin cells of lesional and nonlesional sites are sampled by placing the tape strip on the skin, gently massaging the tape for 15 to 20 seconds, removing the tape, and storing it in ice-cold in BL buffer supplemented with 2% thio-glycerol (Promega, Madison, Wis). This process is repeated to provide a total of 11 tape strips for each sampled skin site. Collected skin tapes are flash frozen and stored at -80°C .
 - a. The first 3 tapes from each lesional or nonlesional site yielded the highest levels of microbial DNA and are stored for microbiome analysis by metagenomic shotgun sequencing.
 - b. Keratinocyte DNA and RNA are extracted from tapes 4-7 and 8-11, respectively. Complementary DNA is made with SuperScript IV VILO (ThermoFisher) and RT-quantitative PCR reactions are carried out with the following Taqman gene expression assays: *18S* (Hs03003631_g1), *FLG* (Hs00856927_g1), *S100A8* (Hs00374264_g1), and *S100A9* (Hs00610058_m1). *S100A8*, *S100A9*, and *FLG* expression levels were normalized to *18S* levels. The maximum gene expression value from either tape strip 8 or 9 from each subject is used to denote *S100A8*, *S100A9*, and *FLG* expression. Future Taqman gene expression arrays will include an expanded panel of genes implicated in AD.

4. Skin biome samples: Blood agar contact plates are used to collect host skin biome samples from lesional and non-lesional skin at each visit. Contact plates made from sheep blood agar (Hardy Diagnostics, Santa Maria, Calif) were placed on both nonlesional and lesional sites of MPAACH children for 30 seconds and then incubated for 48 hours at 37°C. Each morphologically distinct colony was further isolated on individual sheep blood agar plates (Remel, San Diego, Calif) and incubated for another 24 hours. *S aureus* and *S epidermidis* colonies were identified by culturing on Mannitol Salt agar (BD, Sparks, Md). We recognize that contact plates may undersample the skin biome; however, we will be able to validate the presence of the skin flora via metagenomic shotgun sequencing on the skin tapes as discussed above in section 3.
5. Skin biofilm strength: The strengths of the biofilms produced by the strains of *S aureus* and *S epidermidis* colonies cultured from the skin of the MPAACH participants were quantified through crystal violet assays.^{E10}
6. Nasal biome swab: A sample of the nasal biome is collected annually from the nasal passage using a HydraFlock 6" sterile standard flock nasal swab (Puritan Medical Products, Guilford, Maine).
7. Nasal epithelial cells: At visit 5, nasal epithelial cells sampling will be performed by using a CytoSoft Brush as previously described.^{E11} The sample is immediately placed on ice in 10%β-ME in RLT buffer (Qiagen AllPrep DNA/RNA Micro Kit, Hilden, Germany). Within 30 minutes of collection, the sample is flash frozen with dry ice and stored at -80°C. DNA and RNA will be extracted using the All-Prep DNA/RNA Micro Kit (Qiagen).
8. Gut biome: Stool samples are collected at the enrollment visit and banked at -80°C for microbiome analysis.
9. Allergic sensitization is assessed at each study visit by SPT to 11 aeroallergens and 13 foods. The aeroallergen panel includes mold mix 1 (*Alternaria tenuis*, *Hormodendrum cladosporioides*, *Helminthosporium interseminatum*, *Aspergillus fumigatus*, *Aspergillus Niger*, *Penicillium notatum*), mold mix 2 (*Rhizopus nigricans*, *Pullularia pullulans*, *Fusarium vasinfectum*, *Mucor racemosus*), ragweed (*Ambrosia trifida*, *Ambrosia artemisiifolia*), grass (Kentucky bluegrass, orchard, redtop, timothy, Sweet Vernon), tree mix 1 (pecan, maple BHR, oak RVW, American sycamore, black willow), tree mix 2 (white ash, birch mix [Red&White], black walnut, common cottonwood, and American elm), weeds (kochia, English plantain, lamb's quarters, marsh elder/poverty [BPT Mix], common cocklebur, and careless/pigweed [CR Mix]), mite mix (*D pteronyssinus* and *D farina*), cockroach (*P americana* and *B germanica*), and cat and dog (HollisterStier, Spokane, Wash). The food allergen panel includes cow's milk, egg white, egg yolk, soy, wheat, peanut, cashew, almond, walnut, pistachio, pecan, hazelnut, and brazil nut (Greer, Lenoir, NC). A positive SPT result is defined as a wheal measurement 3 mm or larger than that achieved with the diluent control.
10. Saliva is collected at each visit for DNA isolation using the OG-675 kit per manufacturer's instructions (DNA Genotek, Ottawa, Ontario, Canada).
11. Blood samples are collected at each visit and analyzed for the following:
 - a. PBMCs are analyzed by flow cytometry and cytometry by time of flight for immunophenotyping.
 - b. Serum
 - i. Serum levels of vitamin D and cotinine are quantified by ELISA.
 - ii. Specific IgE: If an MPAACH participant is SPT positive to 1 of the foods tested, then serum specific IgE level to that food is quantified via ImmunoCap on a Phadia 250 Immunoassay Analyzer (ThermoFisher Scientific, Waltham, Wash).
 - iii. Serum cytokines are quantified via a 21-Plex MILLIPLEX MAP Human High Sensitivity T Cell Panel (Millipore, Billerica, Mass) using a Luminex 100 machine (Bio-Rad Laboratories, Hercules, Calif).
 - c. A complete blood cell count with differential.
 - d. Whole blood DNA and RNA are isolated and stored.
 - e. Plasma is collected and banked.
12. Home dust: Parents are asked to wipe the top of the door frames of the child's bedroom and the primary activity room with a Swiffer (Procter and Gamble, Cincinnati, Ohio). The dust samples will be analyzed for microbiome analysis, dust allergen levels, and environmental relative moldiness index.^{E12}

COMORBID CONDITIONS

FA will be defined by either (1) a history of a reaction to the food and a positive SPT result or ImmunoCap to the food; (2) positive SPT result to the food and a positive oral food challenge to that food; or (3) LAR report of physician-diagnosed FA. Oral food challenges are conducted according to the PRACTALL recommendations.^{E13} The child is defined as allergic to the food if any of the following occurs: 2 or more organ system involvement, persistent objective symptoms, or 3 or more hives on 2 or more body parts. Asthma and wheeze phenotypes are defined as follows: Any wheeze is defined by LAR report of any wheeze in the last 12 months. Recurrent wheeze is defined as LAR report of 2 or more episodes of wheezing in the past 12 months. Wheezing without a cold is defined as LAR report of wheeze not associated with a cold or respiratory infection. Asthma will be defined by American Thoracic Society criteria based on objective measures of lung function and symptoms. Allergic rhinitis is defined as a combination of allergy symptoms and a positive SPT result to an aeroallergen.

Exposure Definitions

SHS is defined as present if any of 4 SHS-related questions previously published (maternal smoking, other household members smoking, total number of h/d around smoke >0, any SHS exposure riding in vehicles) were answered positively by the LAR. Serum cotinine was quantified by ELISA. Exposures to cats and dogs in the home are collected annually as well as during the first year of life. Exposure to traffic-related air pollution was calculated by estimating each child's exposure to elemental carbon attributable to traffic using a land-use regression model.^{E14}

DATA ANALYSIS-ASSOCIATION TESTING

To identify factors associated with clinical AD severity (SCORAD), we used nonparametric tests (Wilcoxon or Kruskal-Wallis rank sum test) to test distribution/median difference when the factors were categorical. Spearman correlations were used to examine the relationship between 2 continuous variables. Chi-

square tests were performed to test difference in proportions for moderate to severe AD when factors were categorical. When examining skin pathology, we first sought to determine whether there were differences in lesional and nonlesional skin with respect to TEWL, *FLG*, *S100A8*, and *S100A9*, using Wilcoxon rank sum test. A paired test was not used because not all participants had a lesional sample. To identify factors associated with lesional pathology, Wilcoxon or Kruskal-Wallis rank sum test or Spearman correlations were used as appropriate. Secondary analyses included *S100A8* and *S100A9*. Analyses of nonlesional mirrored the lesional skin so that we could determine whether lesional and nonlesional skin exhibited similar associations.

To access the joint effects on moderate/severe AD of all the significant factors, a multivariable logistic regression model was developed by stepwise selection using a *P* value cutoff of .05. The significant predictors included sex, lesional and nonlesional TEWL, lesional and nonlesional *FLG*, *S100A8* and *S100A9*, and presence of *S aureus* for model selection. Observations with outliers in a predictor tend to highly influence the parameter estimates of the model. Log transformations were performed on some variables (lesional and nonlesional *FLG*, *S100A8*, *S100A9*, and TEWL) to achieve a better fit to the data. The zeros in the expression variables were set to be 1/10 of the minimum of the corresponding variables. The *P* values and CI from the final models were reported.

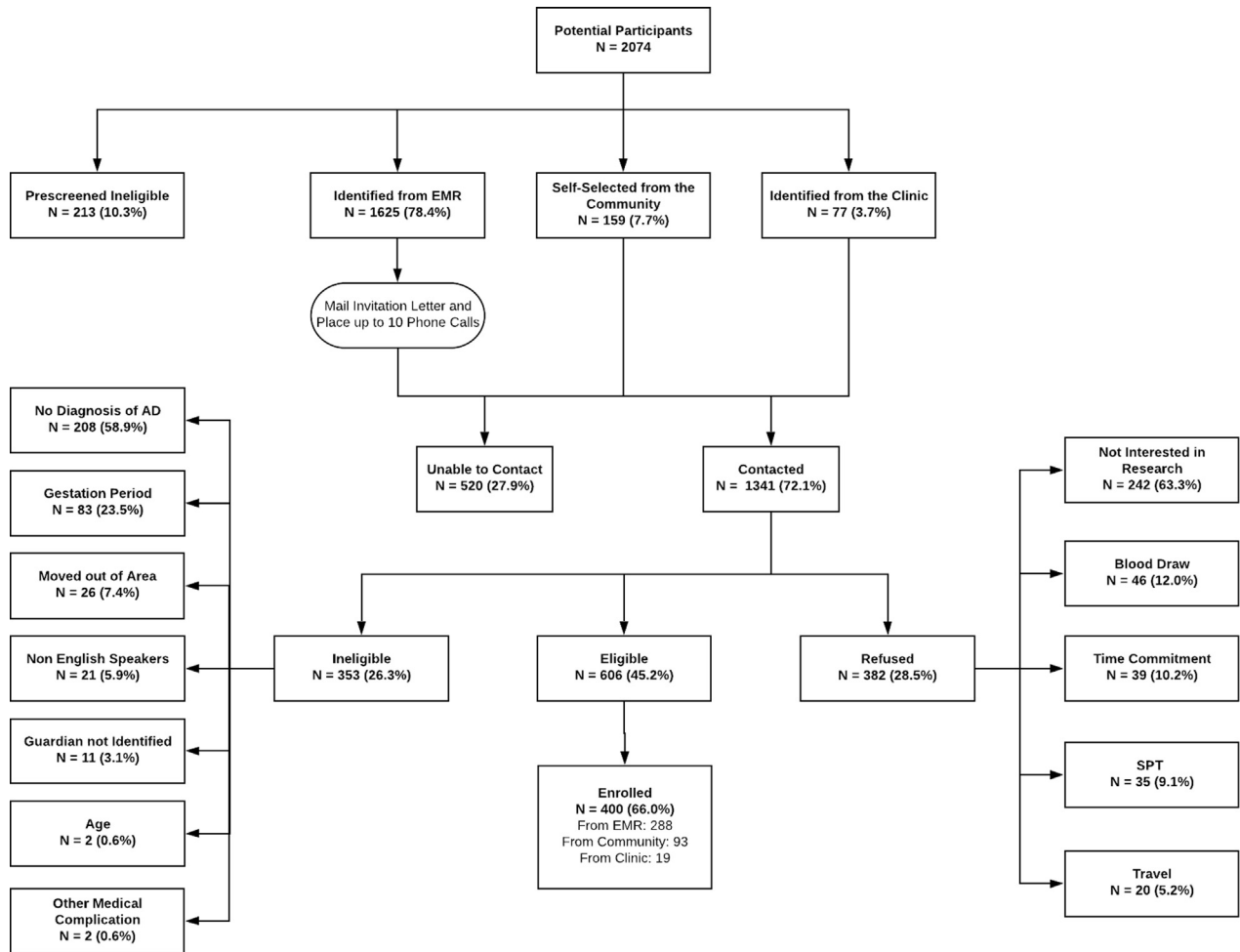


FIGURE E1. Flow diagram of recruitment methodology and outcomes.

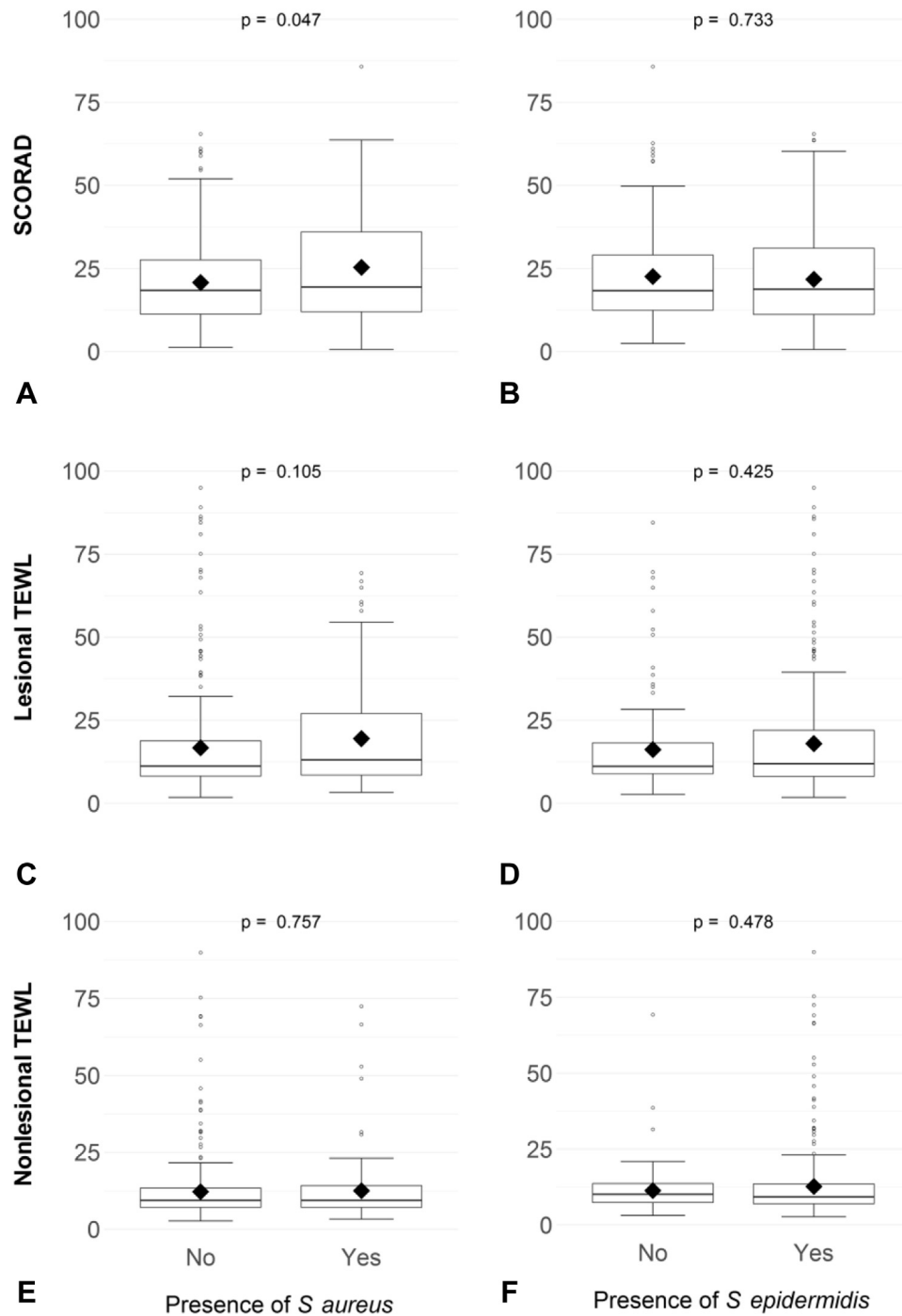


FIGURE E2. Skin staphylococcal colonization, AD severity, and skin barrier function in MPAACH participants. (A and B) SCORAD, (C and D) lesional TEWL, and (E and F) nonlesional TEWL.

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