Skin TARC/CCL17 increase precedes the development of childhood atopic dermatitis

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Background: It is unknown whether skin biomarkers collected in infancy can predict the onset of atopic dermatitis (AD) and be used in future prevention trials to identify children at risk.
Objectives: This study sought to examine whether skin biomarkers can predict AD during the first 2 years of life.
Methods: This study enrolled 300 term and 150 preterm children at birth and followed for AD until the age of 2 years. Skin tape strips were collected at 0 to 3 days and 2 months of age and analyzed for selected immune and barrier biomarkers. Hazard ratio (HR) with 95% confidence interval (CI) using Cox regression was calculated for the risk of AD.
Results: The 2-year prevalence of AD was 34.6% (99 of 286) and 21.2% (25 of 118) among term and preterm children, respectively. Skin biomarkers collected at birth did not predict AD. Elevated thymus- and activation-regulated chemokine/C-C motif chemokine ligand 17 -levels collected at 2 months of age increased the overall risk of AD (HR: 2.11; 95% CI: 1.36-3.26; $P = .0008$) and moderate-to-severe AD (HR: 4.97; 95% CI: 2.09-11.80; $P = .0003$). IL-8 and IL-18 predicted moderate-to-severe AD. Low filaggrin degradation product levels increased the risk of AD (HR: 2.04; 95% CI: 1.32-3.15; $P = .001$). Elevated biomarker levels at 2 months predicted AD at other skin sites and many months after collection.
Conclusions: This study showed that noninvasively collected skin biomarkers of barrier and immune pathways can precede the onset of AD. (J Allergy Clin Immunol 2023;151:1550-7.)

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Atopic dermatitis (AD) is a common chronic and recurrent pediatric inflammatory skin disease affecting 15% to 20% of children.[1] Most develop AD during the first 2 years of life, and nearly all cases begin before 5 years of age.[1,2]

The etiopathogenesis of AD involves a complex interplay between skin barrier and immune dysfunction.[1] Genetic factors contribute to development of AD, particularly mutations in the epidermal differentiation gene filaggrin *(FLG)*,[3] whereas genetic variants in immune regulation seem less important.[4]

So far, it is unknown whether skin biomarkers associated with immune and skin barrier alterations precede the onset of AD, in normal appearing skin of infants. In a recent small case-control study including 88 mature-born children, we showed that the relative amounts of sphingoid bases of different chain lengths and mean thymus- and activation-regulated chemokine/C-C motif chemokine ligand 17 (TARC/CCL17) levels differed between the 44 infants that developed AD in the first year and the 44 that did not.[5] However, in that study where statistical power was low, we were not able to examine whether skin biomarkers were associated with more severe forms of AD, whether skin biomarkers were associated with an increased risk of AD over time, whether skin tape strips collected from other anatomical skin areas than the hands could predict AD, and whether prediction also was possible in premature born children. Therefore, we here examined, in the full data set, whether selected skin biomarkers predicted the onset and severity of AD during the first 2 years of life. We used a prospective design including 450 children, of which 150 were born prematurely, and examined tape strips from dorsal aspects of the hands and the back.

Abbreviations used

AD:	Atopic dermatitis
aHR:	Adjusted hazard ratio
CI:	Confidence interval
CTACK:	Cutaneous T-cell attracting chemokine
EASI:	Eczema Area and Severity Index
HR:	Hazard ratio
IQR:	Interquartile range
SC:	Stratum corneum
TARC/CCL17:	Thymus and activation-regulated chemokine/C-C motif chemokine ligand 17
TEWL:	Transepidermal water loss
UCA:	Urocanic acid

METHODS

Study design and study population

The BABY (Barrier Dysfunction in Atopic Newborns Study) cohort is a Danish prospective birth cohort of 300 term and 150 preterm newborns (born weeks 24-37) designed to investigate whether AD can be predicted.[6] Newborns eligible for enrolment were full-term healthy singleton newborns with no antenatal use of steroids for fetal lung maturation and preterm newborns (gestational age below week 37+0) with no severe congenital abnormality or conditions affecting their life expectancy. Children were recruited from maternity and neonatal wards from August 8, 2017, until August 27, 2019. Term children were enrolled during the first 3 days of life and attended a follow-up visit at 2 and 12 months of age. Preterm children were enrolled during the first 31 days of life and attended a follow-up visit at 2 months of age corrected for their preterm birth. At 1.5 and 2 years of age, parents participated in a structured telephone interview. If a child developed any signs of AD until the age of 2 years, the child attended 1 additional in-person visit to verify a diagnosis of AD and assess the severity using the Eczema Area and Severity Index (EASI). An EASI score of 0.1 to 1.0 was defined as almost clear AD, 1.1 to 7.0 as mild AD, and >7.0 as moderate-to-severe AD.[7] Parents completed a structured questionnaire about family diseases when the child was 2 months old. Parental atopy was defined as self-reported physician-diagnosed AD, asthma, hay fever, and/or self-reported allergy to selected allergens (birch, grass, mugwort, horse, dog, cat, house dust mites, or molds).

The study was conducted in accordance with the Declaration of Helsinki and approved by the scientific Ethical Committee of the Capitol region (H-16042289 and H-16042294) and the local data protection agency (ID-no.: HGH-3017–040, I-suite no.:05578).

Assessment of skin biomarkers, TEWL, and FLG genotyping

Skin cells were collected by tape stripping of the stratum corneum (SC). Eight consecutive D-squame discs (22 mm in diameter; Cuderm, Dallas, Tex) were collected from a single skin area. A light standardized pressure was used after application of the discs. SC was collected from the dorsal aspect of the hand at 0 to 3 days and 2 months of age and from the skin between the shoulder blades at 2 months of age corrected for gestational age in, respectively, term and preterm children. These anatomical locations were chosen because the dorsal aspect of the hand is easily accessible and at the same time exposed to environmental stressors that may elicit AD areas seen in previous studies.[2,8] The skin area between the shoulder blades was the only anatomical location that was accessible in hospitalized preterm infants due to small size as well as tubes and patches covering other skin areas in most children.

Skin tapes 5 and 6 were analyzed for, respectively, FLG degradation products (natural moisturizing factor, histidine, pyrrolidone carboxylic acid, and trans and cis isomer urocanic acid [UCA] using liquid chromatography and selected cytokines and chemokines.[9,10] The selection of analyzed cytokines and chemokines was based on previous findings using the meso scale discovery–based platform and mainly included proinflammatory cytokines because cytokines from the adaptive immune system have proven difficult to measure, in part due to the very superficial layers of the epidermis that were examined.[11] Transepidermal water loss (TEWL) was measured 3 consecutive times on the central volar forearm using a portable, closed condenser-chamber device (AquaFlux model AF200; Biox Systems Ltd, London, United Kingdom) and the mean value was used for analyses.[12] This area was chosen because it is relatively easy to fixate the forearm without children becoming too upset. DNA collected via buccal swabs (Isohelix, Harrietsham, United Kingdom) was analyzed for 3 common *FLG* mutations (R501X, 2282del4, and R2447X) using TaqMan genotyping assay.[13]

Statistical analysis

Twins and triplets were excluded from the analyses. Biomarker levels were corrected for protein levels. Immune biomarkers where >50% of samples had undetectable levels were excluded (see Table E1 in this article's Online Repository at www.jacionline.org). Immune biomarkers and TEWL were dichotomized at a cutoff level of ≥75th percentile (defined as elevated levels), and skin barrier biomarkers were dichotomized at a cutoff level of ≤25th percentile (defined as low levels) (see Table E2 in this article's Online Repository at www.jacionline.org).[14] For baseline demographics, a mean with SD or a median with interquartile range (IQR) was calculated for, respectively, normally and non-normally distributed data. Hazard ratio (HR) with 95% confidence interval (CI) using Cox regression was calculated for the risk of AD and a significance level of $P < .05$ was used. Children with AD at the time of collecting tape strips were excluded in the analyses. Adjustment was made for parental atopy and *FLG* mutations in the term cohort but only parental atopy in the preterm cohort due to limited numbers of preterm children with an *FLG* mutation.

TABLE I. Baseline demographics

	Term children (n = 300)	Preterm children* (n = 126)
Sex, male	43 (129/300)	37 (47/126)
FLG gene mutation	9 (27/297)	4 (5/22)
Gestational age (wk)	39.6 (38.5-40.6)	33.4 (31.6-34.5)
Birth weight (g)	3544.9 ± 459.6	1943.4 ± 657.8
Birth length (cm)	51.9 ± 2.2	43.3 ± 4.9
Delivery method		
Vaginal	60 (179/300)	36 (45/125)
C-section, planned	27 (82/300)	4 (5/125)
C-section, acute	13 (39/300)	60 (75/125)
Parental atopy (self-reported)	63 (153/240)	73 (54/74)
Reason for preterm labor		
Preeclampsia	—	13 (17/126)
Intrauterine growth retardation	—	10 (13/126)
Maternal infection	—	2 (3/126)
Preterm labor	—	44 (56/126)
Other	—	44 (56/126)

Values are percentage (n_{cases}/n_{total}), median (IQR), or mean ± SD.
*Twin B and triplets B and C were excluded.

Parental history of atopy was defined as ≥1parent with a history of atopy. Due to missing data, imputations on parental atopy were made for 50 term and 32 preterm children. Differences in log-transformed biomarker levels according to time of onset of AD were tested by unpaired *t*-test for normally distributed data. No correction for multiple analyses was performed, because this was an exploratory study.

The ability for parental atopy, *FLG* mutation status, and skin biomarkers in discriminating children with later onset of AD from children without onset of AD was visualized by receiver-operating characteristic curves. Logistic regression fits were used to construct prediction scores combining biomarkers, where all data were standardized for stability.[15] The sensitivity and specificity were calculated. Quantitative comparisons were made using area under the curve , and highest accuracy was calculated as the F1 score:

$$F1 = 2 * \frac{recall * precision}{recall + precision},$$

Statistical analyses were performed using SAS Enterprise Guide version 7.1 (SAS Institute Inc, Cary, NC) and Python version 3.8 (for Windows) with statistical packages from SciPy. Figures were performed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, Calif).

RESULTS

Participant characteristics

We included 300 (43% male) term and 126 (37% male) preterm newborns (median gestational age: 33.4; IQR: 31.6-34.5) from the BABY cohort in the analyses (Table I). Respectively, 9% (27 of 297) and 4% (5 of 122) of term and preterm children were *FLG* mutation carriers, and 63% (153 of 240) and 73% (54 of 74) had a history of parental atopy. Among term and preterm children, 95% (286 of 300) and 93% (117 of 126) were followed until the age of 2 years.

AD in term and preterm children

The 2-year prevalence of AD was 35% (99 of 286) among term children, and 3% (9 of 286) developed AD before 2 months of age. The median age at onset of AD was 6 months (IQR: 3.0-11.0), median EASI score was 4.2 (IQR: 2.0-7.9), and 23% (23 of 99) had moderate-to-severe AD (see Table E3 in this article's Online Repository at www.jacionline.org). The risk of AD was increased in children with a history of a parental atopy (HR: 2.51; 95% CI: 1.49–4.23; $P = .0005$) and children with *FLG* mutations (HR: 2.81; 95% CI: 1.66–4.76; $P = .0001$). There was no association between children delivered by C-section and AD when compared to children delivered vaginally (HR: 0.94; 95% CI: 0.62-1.41).

The 2-year prevalence of AD was 21% (25 of 118) among preterm children, and 2% (2 of 118) developed AD before 2 months of age. The median age at AD onset was 8 months (IQR: 6.0-12.0), median EASI score was 1.6 (IQR: 1.1-3.1), and 8% (2 of 25) had moderate-to-severe AD (Table E3). The risk of AD was increased, but not significantly, in children with a history of a parental atopy (HR: 1.83; 95% CI: 0.53–6.34; $P = .3$), and children with *FLG* mutations (HR: 2.78; 95% CI: 0.65–11.82; $P = .2$). There was no association between children delivered by C-section and AD when compared to children delivered vaginally (HR: 0.86; 95% CI: 0.38-1.93).

Skin biomarkers at birth as predictors of AD in term children

Elevated IL-1α levels after birth increased the risk of AD within the first 2 years of life in crude analysis, but the association became borderline significant in adjusted analysis (adjusted hazard ratio [aHR]: 1.52; 95% CI: 1.00–2.33; $P = .05$) (see Table E4 in this article's Online Repository at www.jacionline.org). Low levels of FLG degradation products were not associated with AD (Table E4).

Skin biomarkers at 2 months of age as predictors of AD in term and preterm children

Elevated TARC/CCL17 levels at 2 months of age increased the risk of AD within the first 2 years of life in crude and adjusted analyses (aHR: 1.85; 95% CI: 1.18-2.89; $P = .007$) (Fig 1, Table II). Elevated TARC/CCL17 levels were more strongly associated with moderate-to-severe AD (aHR: 4.65; 95% CI: 1.91-11.31; $P = .0007$) (Figs 1 and 2). TARC/CCL17 levels were elevated both in children with AD onset before 6 months of age ($P = .0004$) and children with AD onset between 6 and 24 months of age ($P = .003$) when compared with children who did not develop AD (Fig 3). Changes in TARC/CCL17 levels during the first 2 months of life among, respectively, children who later develop AD and in children who did not develop AD are visualized in Fig E1 in this article's Online Repository (available at www.jacionline.org).

Elevated IL-8 levels and IL-18 levels increased the risk of moderate-to-severe AD in crude and adjusted analyses (aHR: 3.01; 95% CI: 1.24-7.31; $P = .02$ and aHR: 2.86; 95% CI: 1.17-6.98; $P = .02$, respectively) (Table III). Low UCA levels increased the risk of AD in crude and adjusted analyses (aHR: 1.68; 95% CI: 1.07-2.64; $P = .02$) (Table II). UCA levels were lower in children with AD onset before 6 months of age ($P = .001$) but not in children with AD onset between 6 and 24 months of age ($P = .1$) when compared with children who did not develop AD.

In preterm children, elevated TARC/CCL17 levels were borderline significantly associated with AD in adjusted analysis (aHR: 2.60; 95% CI: 0.98-6.85; $P = .05$). No other skin biomarkers were associated with elevated risk of AD among preterm children (Table II).

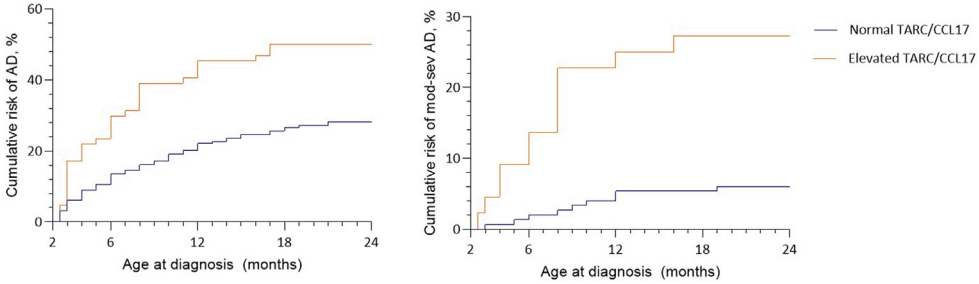

FIG 1. Cumulative risk of onset of AD, mild AD, and moderate-to-severe AD during the first 2 years of life for term children with normal versus elevated skin levels of TARC/CCL17 at 2 months of age.

TABLE II. Risk of developing AD during the first 2 years of life according to levels of skin barrier and immune biomarkers and TEWL measured in term and preterm children 2 months old*

	AD during the first 2 years of life							
	Term children				Preterm children†			
Mediator‡	HR (95% CI)§	*P* value	aHR (95% CI)‖	*P* value¶	HR (95% CI)#	*P* value	aHR (95% CI)**	*P* value††
Cytokines								
IL-1a	1.53 (0.99-2.40)	.06	1.19 (0.75-1.90)	.5	1.36 (0.48-3.87)	.6	1.58 (0.56-4.51)	.4
IL-1RA	1.49 (0.95-2.33)	.09	1.28 (0.81-2.03)	.3	1.22 (0.43-3.46)	.7	1.28 (0.45-3.64)	.6
IL-8	1.53 (0.97-2.41)	.07	1.36 (0.85-2.16)	.2	0.87 (0.28-2.67)	.8	1.03 (0.33-3.16)	1.0
CTACK	—	—	—	—	1.20 (0.42-3.41)	.7	1.43 (0.50-4.07)	.5
IL-1b	1.18 (0.75-1.89)	.5	0.83 (0.49-1.37)	.5	2.24 (0.85-5.80)	.1	2.60 (0.99-6.87)	.05
IL-18	1.46 (0.93-2.31)	.1	1.26 (0.80-2.01)	.3	1.03 (0.34-3.15)	1.0	1.06 (0.34-3.24)	.9
IL-31	0.77 (0.46-1.28)	.3	0.85 (0.50-1.44)	.5	1.34 (0.47-3.81)	.6	1.55 (0.54-4.14)	.4
IL-33	—	—	—	—	2.25 (0.86-5.91)	.1	2.15 (0.82-5.66)	.1
TSLP	—	—	—	—	0.58 (0.17-2.03)	.4	0.48 (0.14-1.68)	.3
TARC/CCL17	2.11 (1.36-3.26)	.0008‡‡	1.85 (1.18-2.89)	.007‡‡	2.23 (0.85-5.86)	.1	2.60 (0.98-6.85)	.05
IL-22	—	—	—	—	0.89 (0.29-2.73)	.8	0.93 (0.30-2.86)	.9
FLG degradation products								
NMF	1.76 (1.13-2.75)	.01‡‡	1.28 (0.77-2.13)	.3	1.33 (0.47-3.77)	.6	1.43 (0.50-4.07)	.5
HIS	1.05 (0.65-1.71)	.8	0.76 (0.45-1.29)	.3	1.79 (0.66-4.84)	.3	1.82 (0.67-4.93)	.2
PCA	1.54 (0.98-2.41)	.06	1.09 (0.66-1.80)	.7	1.33 (0.47-3.77)	.6	1.43 (0.50-4.07)	.5
UCA	2.04 (1.32-3.15)	.001‡‡	1.68 (1.07-2.64)	.02‡‡	1.78 (0.66-4.82)	.3	1.82 (0.67-4.91)	.2
TEWL	1.46 (0.92-2.29)	.11	1.29 (0.81-2.05)	.3	0.64 (0.21-1.95)	.4	0.53 (0.17-1.64)	.3

HIS, Histidine; *IL-1RA*, interleukin-1 receptor antagonist; *NMF*, natural moisturizing factor; *PCA*, pyrrolidone carboxylic acid.
*Cox regression HRs, aHRs, and 95% CIs.
†Preterm children were 2 months corrected for their due date.
‡All cytokines and TEWL were dichotomized at cutoff level: ≥75th percentile and all FLG degradation products were dichotomized at cutoff level ≤25th percentile.
§A total of 258 children were included (87 developed AD).
‖A total of 255 children were included (86 developed AD).
¶Adjusted for history of parental atopy and *FLG* gene mutation.
#A total of 98 children were included (17 developed AD).
**A total of 98 children were included (17 developed AD).
††Adjusted for history of parental atopy.
‡‡These values are statistically significant.

Diagnostic accuracy of skin biomarkers at 2 months of age in term children

FLG mutations, parental atopy, TARC/CCL17, and UCA could to some degree separate children who later developed AD from those who did not (see Fig E2 in this article's Online Repository at www.jacionline.org). The combination of all only slightly increased the ability to separate AD children from non-AD children compared to TARC/CCL17.

Transepidermal water loss

An elevated mean TEWL at birth or 2 months of age did not increase the risk of AD among term or preterm children (Table II).

DISCUSSION

Main findings

This large prospective birth cohort study is, to our knowledge, the first to show that noninvasively collected skin barrier and

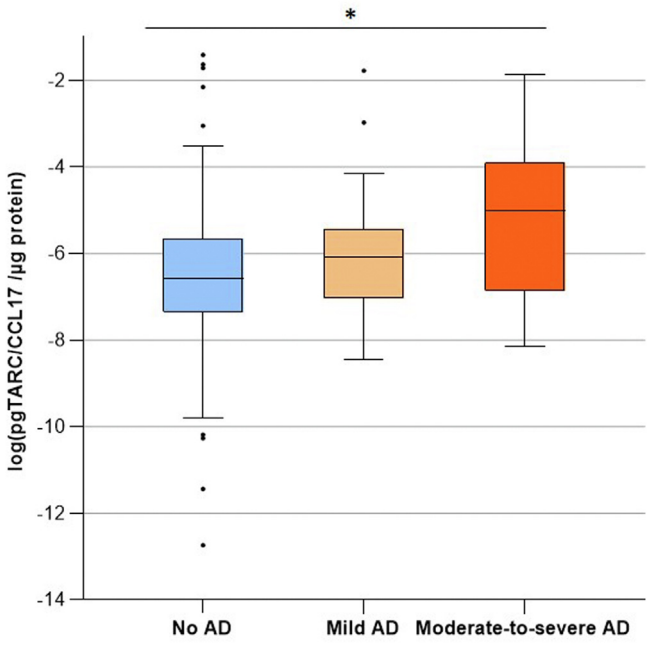

FIG 2. Boxplots of log-transformed levels of TARC/CCL17 and differences between these levels among 2-month-old term children that, respectively, do not develop AD and that do develop mild AD or moderate-to-severe AD. Only children with no development of AD at the time of collecting the SC samples were included in these analyses. *Significant difference $P < .05$ between no AD and moderate-to-severe AD.

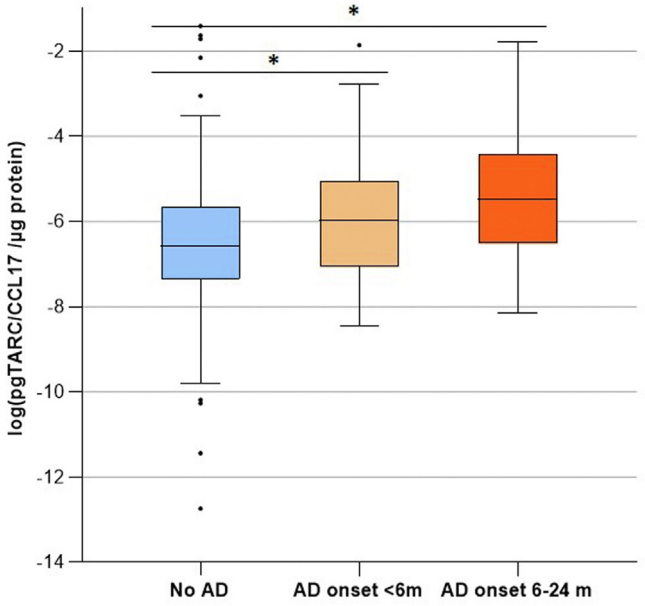

FIG 3. Boxplots of log-transformed levels of TARC/CCL17 and differences between these levels among 2-month-old term children that, respectively, do not develop AD and that do develop AD before 6 months of age or during the first 6 to 24 months of life. Only children with no development of AD at the time of collecting the SC samples were included in these analyses. *Significant difference $P < .05$ between no AD and, respectively, early and late onset of AD.

immune biomarkers can be used to predict subsequent onset of pediatric AD, and in particular more severe forms of AD. TARC/CCL17 and UCA levels predicted AD in adjusted analyses, whereas IL-8 and IL-18 only predicted more severe forms of AD. Importantly, AD occurring several months after collection, and at other skin sites, could be predicted, suggesting that skin changes preceding AD could be constitutional early in life.

Perspectives

There is an increasing interest in preventive strategies for AD. Two large randomized trials (BEEP [Barrier Enhancement for Eczema Prevention] and PreventADALL [Preventing Atopic Dermatitis and Allergies in Children]) found that emollient use (daily or >3 times/week) during the first year of life could not prevent AD.[16,17] A meta-analysis found probiotic supplementation during infancy decreased the risk of AD when compared to placebo (odds ratio: 0.66; 95% CI: 0.48-0.91; $P = .01$).[18] The randomized SOFTER (Softened Water for Eczema Prevention Pilot Trial) examined whether softening of domestic water supplies could reduce the risk of AD.[19] Recently, the STOP-AD (Short-term Topical Application to Prevent Atopic Dermatitis) trial showed that early intervention with emollients successfully reduced the incidence of AD.[20] Identification of predictive biomarkers of pediatric AD is important so that future preventive strategies can be targeted to high-risk infants.

In a small, nested case-control study of 88 children from the cohort described in this study, we recently showed that a reduction in certain lipids and an elevation of TARC/CCL17 at 2 months of age was associated with AD during the first year of life.[5] However, this study was limited by the matched study design, the short follow-up period, and the few children included. We were also not able to study a potential association with the severity of AD at onset and examine whether elevated TARC/CCL17 levels were associated with an increased risk of AD over time in a time-to event-analysis, that is, few months after collection as well as more than a year later, and whether skin biomarkers collected in other anatomical areas would show the same results. These questions were therefore examined in the current prospective birth cohort study of 450 children, where we identify that several immune biomarkers are elevated even long before AD becomes clinically manifest and that tape strips can be collected from other skin sites than the hands. We also support these findings by showing a severity-dependent association for some of the biomarkers, and we showed that low UCA levels predicted AD, in turn indicating that skin barrier dysfunction is also of importance.

TARC/CCL17, a mediator of type 2 inflammation, is central in the pathogenesis of AD, and represents the best biomarker in patients with established AD.[1,21] The major sources of TARC/CCL17 in AD skin include keratinocytes, vascular endothelial cells, T cells, and dendritic cells.[22] TARC/CCL17 binds to the C-C chemokine receptor type 4, which is expressed on T_H2 cells, thereby acting as a powerful T_H2 chemoattractant.[22] Studies have repeatedly found that serum TARC/CCL17 levels correlate with AD severity,[23-25] and in Japan, serum TARC/CCL17 has been a routine measurement since 2008.[26] We recently showed a significant correlation between increased AD severity and elevated TARC/CCL17, cutaneous T-cell attracting chemokine (CTACK), IL-8, and IL-18 levels in skin tape strips from 25 children with AD,[11] which further lend support to TARC/CCL17 as a key skin biomarker in pediatric AD.[11,27] One Japanese study of 70 children observed that umbilical cord serum TARC/CCL17 levels were higher in children who developed AD than those who did not

TABLE III. Risk of developing mild or moderate-to-severe AD during the first 2 years of life according to levels of skin barrier and immune biomarkers and TEWL measured at 2 months of age*

	Mild AD during the first 2 years of life				Mod-Sev AD during the first 2 years of life			
Mediator†	HR (95% CI)‡	*P* value	aHR (95% CI)§	*P* value‖	HR (95% CI)¶	*P* value	aHR (95% CI)#	*P* value‖
Cytokines								
IL-1a	2.00 (1.09-3.65)	.02**	1.63 (0.87-3.07)	.1	1.11 (0.41-3.04)	.8	0.70 (0.25-1.97)	.5
IL-1RA	0.88 (0.43-1.82)	.7	0.78 (0.37-1.66)	.5	2.79 (1.19-6.57)	.02**	1.75 (0.72-4.29)	.2
IL-8	1.30 (0.67-2.51)	.4	1.18 (0.59-2.35)	.6	2.96 (1.26-6.97)	.01**	3.01 (1.24-7.31)	.02**
IL-1b	0.64 (0.29-1.43)	.3	0.46 (0.20-1.07)	.07	2.61 (1.11-6.15)	.03**	1.47 (0.56-3.82)	.6
IL-18	0.70 (0.31-1.57)	.4	0.60 (0.27-1.37)	.2	4.08 (1.72-9.69)	.001**	2.86 (1.17-6.98)	.02**
IL-31	0.46 (0.20-1.10)	.08	0.55 (0.23-1.32)	.2	1.93 (0.81-4.57)	.1	2.19 (0.91-5.27)	.08
TARC/CCL17	1.10 (0.53-2.30)	.8	0.99 (0.46-2.14)	1.0	4.97 (2.09-11.80)	.0003**	4.65 (1.91-11.31)	.0007**
FLG degradation products								
NMF	1.54 (0.81-2.93)	.2	1.09 (0.51-2.32)	.8	1.93 (0.80-4.67)	.1	1.13 (0.38-3.35)	.8
HIS	1.11 (0.57-2.15)	.8	0.86 (0.42-1.75)	.7	1.36 (0.55-3.37)	.5	0.65 (0.18-2.36)	.5
PCA	1.31 (0.67-2.53)	.4	0.86 (0.40-1.88)	.7	1.82 (0.76-4.39)	.2	1.01 (0.34-2.94)	1.0
UCA	1.76 (0.93-3.30)	.08	1.45 (0.75-2.82)	.3	2.49 (1.05-5.91)	.04**	2.02 (0.83-4.90)	.1
TEWL	0.94 (0.46-1.95)	.9	0.79 (0.37-1.67)	.5	2.02 (0.84-4.88)	.12	1.38 (0.55-3.47)	.5

Mod-Sev, Moderate-to-severe.
*Cox regression HRs, aHRs, and 95% CIs.
†All cytokines and TEWL were dichotomized at cutoff level: ≥75th percentile and all FLG degradation products were dichotomized at cutoff level ≤25th percentile.
‡A total of 213 children were included (45 developed mild AD); children that developed almost clear or moderate-to-severe AD were excluded in this analysis.
§A total of 210 children were included (44 developed mild AD); children that developed almost clear or moderate-to-severe AD were excluded in this analysis.
‖Adjusted for history of parental atopy and *FLG* gene mutation.
¶A total of 189 children were included (21 developed moderate-to-severe AD); children that developed almost clear or mild AD were excluded in this analysis.
#A total of 187 children were included (21 developed moderate-to-severe AD); children that developed almost clear or mild AD were excluded in this analysis.
**These values are statistically significant.

($P < .001$),[28] irrespective of maternal history of AD or allergies. However, we observed no difference in skin TARC/CCL17 levels at birth in children with and without later AD, indicating that later time points should be used to measure skin TARC/CCL17 levels. In our study, elevated IL-8 and IL-18 levels increased the risk of more severe AD, whereas CTACK levels were below detection levels. CTACK and IL-18 were identified as promising biomarkers in AD.[21] While IL-8 is currently not recognized as a biomarker of AD, we recently found that IL-8 also was associated with AD severity in 2- to 15-year-old children with AD.[11] Our findings obviously need to be replicated in future cohorts. Interestingly, a birth cohort of 89 infants found that elevated skin levels of thymic stromal lymphopoietin (TSLP) increased the risk of AD during the first 2 years of life.[29] In our study, TSLP levels were below detection limit among term children, and there was no association with AD among preterm children. Interestingly, the levels of IL-1α at birth were increased in children that develop AD later in life while previous studies showed reduced IL-1α in AD skin.[27] IL-1α is present in large amount in the SC as a preformed pool and decreases when the skin barrier is damaged. On the other side, inflammation induces increased expression of a proinflammatory IL-1α in the epidermis and eventually a larger amount of IL-1α will diffuse from the epidermis into SC. The actual IL-1α concentration in the SC depends thus on 2 processes, and it seems that at birth the increased ingress of IL-1α from epidermis and/or still not built pool of IL-1α prevails.

Deficiency of FLG is a major contributor to the impaired skin barrier in AD.[3] In SC, FLG is degraded into free amino acids including UCA and pyrrolidone carboxylic acid.[3] Low UCA levels, collected at 2 months in term children increased the risk of AD. However, we found no association with AD onset after 6 months of age or moderate-to-severe AD. *FLG* mutations are the strongest genetic risk factor of AD and are associated with more severe AD and early onset of AD,[30] hence our results suggest that *FLG* mutations are more predictive of AD than FLG degradation products, natural moisturizing factor, in SC. This might, at least partly, be explained by environmental factors that could affect natural moisturizing factor levels, such as reduced ambient humidity or exposure to water and soaps.[31,32] It is unclear whether low UCA levels are rather associated with less atopic forms of AD. There was also no association with elevated levels of TEWL and an increased risk of AD. Therefore, our results emphasize that immune dysregulation may play a more predominant role prior to any visible signs of AD.

Preterm birth is associated with a decreased risk of AD,[33] but the underlying mechanisms are unclear. The 2-year prevalence of AD was higher among term children (35%) than preterm children (21%). This could partly be explained by a lower prevalence of *FLG* mutations observed among preterm children (term: 9% vs preterm: 4%), but possibly also a higher T_H1/T_H2 ratio in preterm children. No skin biomarkers predicted AD in preterm children, but elevated TARC/CCL17 levels were borderline significant in adjusted analysis and the magnitude of the HR doubled, suggesting that TARC/CCL17 is both a predictive biomarker of AD irrespective of preterm versus term status. Tape stripping was done in preterm children between the shoulder blades, which is a skin area rarely affected by AD unless AD is severe and has spread from other predilection sites. This indicates that skin biomarkers such as TARC/CCL17 appear to be elevated in skin areas outside of traditional AD sites, and that while the site of tissue collection may be important, perhaps all skin areas will show altered levels. Along this line, many term-born children with AD had their first clinical signs on the face and not the hands (59% of AD children in our study) where tapes were collected. Our data also indicate that elevated skin TARC/CCL17 levels do not only represent very early skin inflammation that can be measured, for example, weeks before AD becomes visible on the skin. We observed that

TARC/CCL17 levels were significantly elevated among children who developed AD after 6 months of age (Fig 3). Thus, while some TARC/CCL17 elevation may represent early AD lesions that are not yet clinically visible, TARC/CCL17 levels also predict cases that occur many months later.

AD is characterized by a T_H1/T_H2 imbalance, with increased T_H2 activity. In our study, T_H2 cytokines including IL-4, IL-5, and IL-13 were largely undetectable, whereas proinflammatory cytokines were more predominant. mRNA profiling of tape strips can identify a broader range of immune and skin barrier biomarkers in AD including T_H2 cytokines than our assay,[25,34] and it is possible that gene expression of these cytokines could be identified both at birth and 2 months of age. Though mRNA profiling has primarily been used to analyze skin biopsies in the past, it is important to use a minimally invasive method as tape stripping when identifying predictive skin biomarkers of AD, as more invasive methods are challenging or impossible to use in cohort studies of infants.[25] Future prospective studies should examine whether predictive skin biomarkers of AD can be measured using mRNA profiling.

Strength and limitations

The prospective design of the birth cohort, 450 children included, and the high retention rate (94.6% at 2 years) are major strengths. Several clinical visits during the follow-up period and clinical diagnosed AD minimize the risk of AD misclassification. Samples were collected from the dorsal aspects of the hand and between the shoulder blades, and we cannot exclude that other skin areas would be more representative. Only selected cytokines were measured. Due to the explorative nature of the study, and in accordance with authoritative recommendations, multiple analyses were conducted without correction for multiple testing increasing the risk of type II errors. Family predisposition to atopy was common, indicating participation bias limiting the generalizability of the results. We found a higher prevalence of AD (35%) than in the background population, which may be due to the high prevalence of a family history of atopy. Biomarkers of, respectively, preterm and term children were not compared due to differences in anatomical location of collection of tape strips and age (preterm children were corrected for their gestational age). Receiver-operating characteristic curves showed that while TARC/CCL17 levels can be used to predict onset of AD, the cutoff level we used led to both a high true-positive and false-positive rate and, therefore, more sensitive and specific markers are needed. As most T_H2 cytokines were undetectable, we are not able to conclude whether T_H2 cytokines are predictive of AD.

Conclusions

We identified for the first time that noninvasively collected skin biomarkers of barrier and immune pathways can precede the onset of AD. Elevated TARC/CCL17 levels, as well as elevated levels of other proinflammatory cytokines and low levels of barrier biomarkers, were associated with increased risk of AD.

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Clinical implications: Our findings lend support to the notion that future targeted trials to prevent AD can be designed, and this common disease can be prevented.

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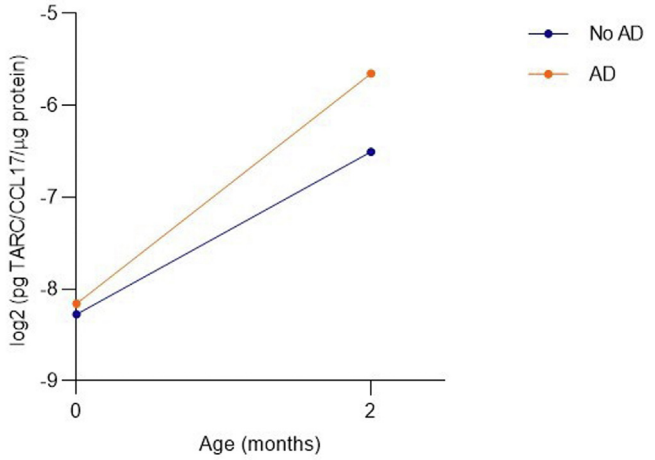

FIG E1. Changes in TARC/CCL17 levels during the first 2 months of life according to later AD status in term children. Children who had AD at time of collecting the tape strip were excluded from this analysis.

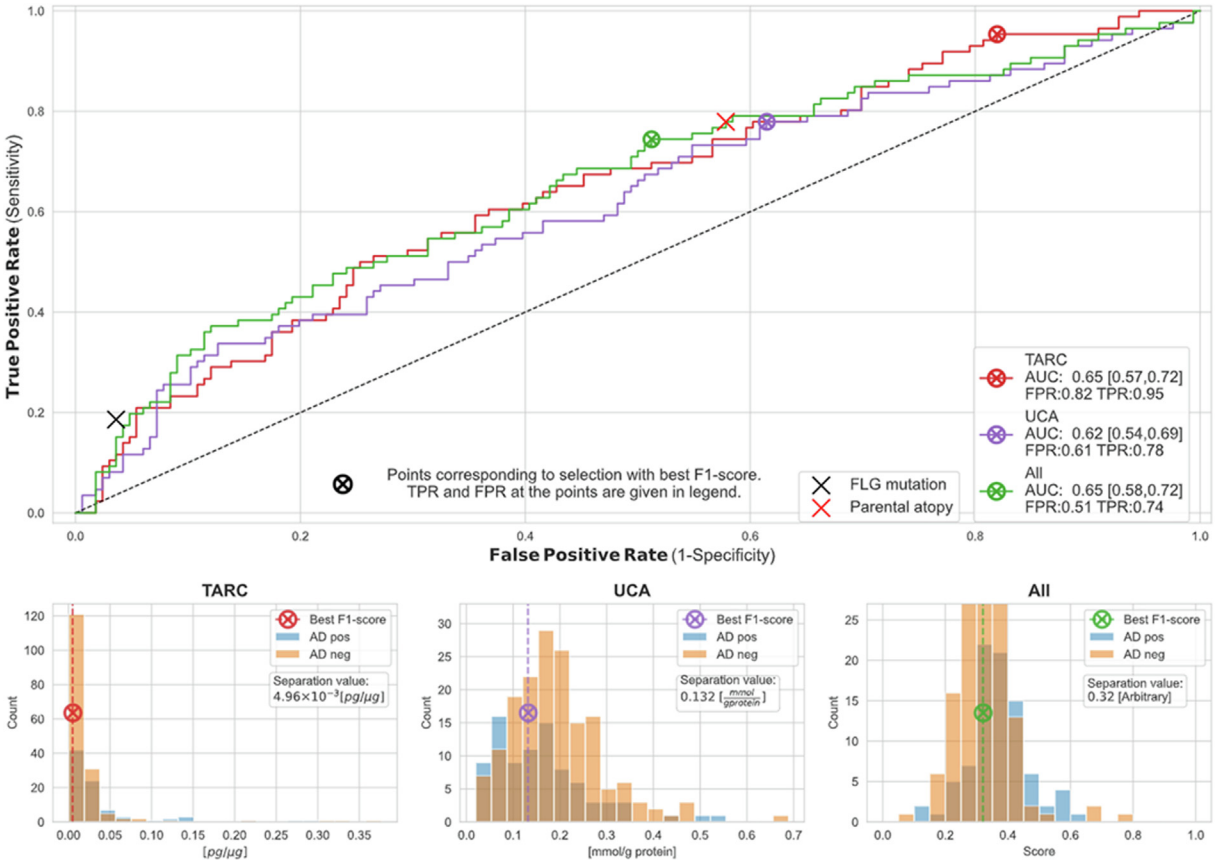

FIG E2. Receiver-operating characteristic curve showing the ability of individual TARC/CCL17 and UCA levels collected at 2 months of age, *FLG* gene mutation, and a history of parental atopy to predict onset of AD within the first 2 years of life in term children. *AUC*, Area under the curve; *FPR*, false-positive rate; *neg*, negative; *TPR*, true-positive rate; *pos*, positive.

TABLE E1. Proportion of cytokine levels below limit of detection (*LOD*) and below curve fit range (*CFR*)*

	Term children				Preterm children	
	Birth (n = 288)		2 months (n = 258)		2 months (n = 98)	
Cytokines	**Below LOD**	**Below CFR**	**Below LOD**	**Below CFR**	**Below LOD**	**Below CFR**
IL-1a	0	0	0	0	0	0
IL-1RA	0	0	0	0	0	0
IL-8	0	0	0	0	0	0
MDC	47	7	52	6	65	20
CTACK	20	5	38	14	41	0
IFNγ	52	2	66	4	59	0
Il-1b	0	0	0	0	0	0
IL-18	0	0	0	0	1	0
IL-4	49	12	64	9	76	11
IL-5	65	5	81	7	86	14
IL-10	72	3	88	5	91	6
IL-13	43	6	67	14	42	15
IL-31	7	0	18	1	2	0
IL-33	32	0	69	3	10	0
MIP-1b	47	13	61	28	71	27
TSLP	39	2	70	3	41	0
TARC/CCL17	33	0	17	2	26	2
MIP-3a	16	2	51	5	62	0
IL-22	26	2	52	9	37	0

Values are percentages.

MDC, Macrophage-derived chemokine; *MIP*, macrophage inflammatory protein.

*If >50% below LOD and CFR, it was excluded from the analyses.

TABLE E2. Cutoff levels of skin biomarkers and TEWL

	Term children		Preterm children
	Birth	2 months	2 months
Cytokines*			
IL-1a	≥19.1843470	≥26.8774982	≥2.029557930
IL-1RA	≥16.2637364	≥14.18392946	≥5.09805361
IL-8	≥0.2189756	≥0.1220018	≥0.1826278
CTACK	≥0.0298023	—	≥0.0911061
Il-1b	≥0.0071222	≥0.0333294	≥0.1615990
IL-18	≥0.0726531	≥0.1655326	≥0.2569772
IL-13	≥0.0937900	—	—
IL-31	≥0.2923642	≥0.5776269	≥0.7841208
IL-33	≥0.0077136	—	≥0.0319697
TSLP	≥0.0061844	—	≥0.0485573
TARC/CCL17	≥0.0057157	≥0.0252333	≥0.0389637
MIP-3a	≥0.1238155	—	—
IL-22	≥0.0035008	—	—
FLG degradation products†			
NMF	≤0.0985289	≤0.6121216	≤0.4064850
HIS	≤0.0175021	≤0.1364493	≤0.0753407
PCA	≤0.0449680	≤0.3468571	≤0.2353124
UCA	≤0.0296973	≤0.1129325	≤0.0824076
TEWL*	≥13.77	≥17.52	≥16.60

*Elevated levels defined as ≥75th percentile of all included samples.
†Decreased levels defined as ≤25th percentile of all included samples.

TABLE E3. Severity of AD according to the EASI among term and preterm children during the first 2 years of life

	Term children with AD (n = 99)	Preterm children with AD (n = 25)
Almost clear	6 (6/99)	12 (3/25)
Mild	51 (50/99)	44 (11/25)
Moderate-to-severe	23 (23/99)	8 (2/25)
Not assessed	20 (20/99)	36 (8/25)

Values are percentage (n_{cases}/n_{total}).

TABLE E4. Risk of developing AD during the first 2 years of life according to levels of skin barrier and immune biomarkers and TEWL measured at birth*

	AD during the first 2 years of life			
Mediator†	**HR (95% CI)‡**	***P* value**	**aHR (95% CI)§**	***P* value‖**
Cytokines				
IL-1a	1.75 (1.16-2.65)	.008¶	1.52 (1.00-2.33)	.05
IL-1RA	0.85 (0.53-1.37)	.5	0.89 (0.55-1.45)	.6
IL-8	0.90 (0.56-1.42)	.6	0.86 (0.54-1.38)	.5
CTACK	1.06 (0.68-1.66)	.8	1.06 (0.67-1.67)	.8
IL-1b	0.91 (0.57-1.44)	.7	0.87 (0.55-1.40)	.6
IL-18	1.10 (0.70-1.72)	.7	1.17 (0.75-1.84)	.5
IL-13	1.10 (0.70-1.72)	.7	1.03 (0.65-1.61)	.9
IL-31	0.79 (0.49-1.28)	.3	0.69 (0.43-1.13)	.1
IL-33	0.97 (0.61-1.53)	.9	0.98 (0.61-1.55)	.9
TSLP	1.03 (0.65-1.61)	.9	1.16 (0.73-1.83)	.5
TARC/CCL17	1.16 (0.75-1.81)	.5	1.13 (0.72-1.76)	.6
MIP-3a	1.36 (0.89-2.09)	.2	1.34 (0.87-2.06)	.2
IL-22	0.91 (0.57-1.45)	.7	0.83 (0.52-1.33)	.4
FLG degradation products				
NMF	1.18 (0.76-1.83)	.5	1.06 (0.68-1.65)	.8
HIS	1.02 (0.65-1.60)	.9	0.99 (0.63-1.57)	1.0
PCA	1.22 (0.79-1.89)	.4	1.11 (0.71-1.74)	.6
UCA	1.43 (0.94-2.19)	.1	1.32 (0.86-2.02)	.2
TEWL	0.87 (0.55-1.39)	.6	0.88 (0.55-1.40)	.6

*Cox regression HRs, aHRs, and 95% CIs.
†All cytokines and TEWL were dichotomized at cutoff level: ≥75th percentile and all FLG degradation products were dichotomized at cutoff level ≤25th percentile.
‡A total of 288 children were included (99 developed AD).
§A total of 285 children were included (98 developed AD).
‖Adjusted for history of parental AD and *FLG* gene mutation.
¶This value is statistically significant.