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Association between skin barrier development and early-onset atopic dermatitis: A longitudinal birth cohort study

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Background: A diagnosis of atopic dermatitis (AD) is common during infancy; however, it is unclear whether differential skin barrier development defines this period and signals disease onset in predisposed individuals.

Objective: We sought to study (NCT03143504) and assess the feasibility of remote skin testing from birth to monitor skin barrier maturation and model association with an AD diagnosis by age 12 months.

Methods: Biophysical testing and infrared spectroscopy were conducted at the maternity ward and family home. Tape stripping collected samples for desquamatory protease and natural moisturizing factor analysis. The 4 common European filaggrin risk alleles were screened.

Results: A total of 128 infants completed the study, with 20% developing mild disease. Significant changes in permeability barrier function, desquamatory protease activity, and molecular composition assessed spectroscopically were observed longitudinally, but only subtle evidence of differential skin barrier development was noted between infant subgroups. Common filaggrin risk alleles were strongly associated with early-onset disease and conferred a significant reduction in natural moisturizing factor and water content by age 4 weeks. Accounting for a family history of atopy, these parameters alongside a greater lipid/protein ratio and reduced chymotrypsin-like activity at birth were associated with AD. Measured in ambient conditions, transepidermal water loss did not signal disease risk at any stage.

Conclusions: Skin barrier dysfunction lacked an acquired modality but was considered proportional to cohort severity and suggests that a portfolio of tests used in a community setting has the potential to improve current AD risk evaluations from birth. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)

Key words: *Atopic dermatitis, remote skin testing, infant skin barrier, infrared spectroscopy*

Skin barrier breakdown is a significant component of atopic dermatitis (AD) pathogenesis. Although uninvolved skin appears healthy, underlying structural defects render it functionally inadequate and inflamed, which corresponds to disease severity.[1,2] A less-ordered lipid structure, protease hyperactivity, and low levels of natural moisturizing factor (NMF) within the stratum corneum (SC) are hallmarks of the barrier abnormality that associate with weakened permeability barrier function, signified by elevated transepidermal water loss (TEWL).[3-5]

With disease prevalence the greatest in younger children (aged ≤4 years) worldwide,[6] infants predisposed to AD are not born with clinical signs but have an increased risk of diagnosis before their first birthday.[7] Over this time period, the developing skin barrier is structurally and functionally immature, suggesting a fragility as it adapts to a terrestrial environment.[8,9] Considering the pathological evidence from established adult AD, skin barrier breakdown may therefore define a differential trajectory from birth that predates active disease. Modifying factors here may include climate, the home environment, and parental skin care practices, but their interaction with the maturing skin barrier is unclear.

In a medical era in which AD prevention is a key objective, pinpointing susceptible babies for early intervention is an unmet clinical need. One of the best indicators is familial atopic disease,[10] but when screening using this metric, it is estimated that about 40% of cases may be missed altogether.[11] Conversely, a risk of unnecessary treatment intervention exists, burdening new parents when time is scarce and mental health may be challenged.[12] Additional tools are therefore required to evaluate disease risk in a maternity or community setting. One possibility is TEWL measurement, but this may be unsuitable because of strict environmental requirements.[13]

To address these research questions, a longitudinal study was designed to monitor skin barrier development from birth and pilot noninvasive measures of lipid structure, protease activity, and NMF alongside TEWL for disease risk evaluation. Improving the early detection of at-risk infants may empower parents in future to take measured actions from birth to prevent or delay the possible emergence of AD in their baby.

METHODS

Study design

A longitudinal observational cohort study (clinicaltrials.gov reference no. NCT03143504) was performed to monitor infant skin development from birth to age 12 months. By capturing

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Abbreviations used
AD: Atopic dermatitis
ATR-FTIR: Attenuated total reflectance infrared spectroscopy
AUC: Area under the receiver-operating characteristic curve
C-L: Chymotrypsin-like
FLG: Filaggrin
NMF: Natural moisturizing factor
SC: Stratum corneum
VC: Vernix caseosa
3B2P: 3-bounce/2-pass

the incidence of AD, the association between subclinical barrier breakdown and disease risk by age 12 months could be preliminarily explored. The study was conducted by an experienced team of midwife and technical researchers trained in the instrumentation and clinical scoring, overseen by a senior dermatologist providing consultancy when required. Ethical permission was granted by the Preston North West National Health Service Research Ethics Committee (16/NW/0848), and informed parental consent was obtained. The reporting of this study conforms to strengthening the reporting of observational studies in epidemiology (STROBE) recommendations.[14]

Participants

Full-term, healthy, singleton neonates (≤72 hours old) and their mothers (≥18 years old) living within a 5-mile radius of the University of Sheffield were recruited at Jessop Wing Maternity Unit, Sheffield Teaching Hospital, United Kingdom, between April 2017 and December 2019. Table E1 (in the Online Repository available at www.jacionline.org) provides the full study eligibility criteria.

Sample size

A recruitment target of 180 neonates was considered appropriate to explore the feasibility of skin testing from birth, with 15% to 30% anticipated to develop AD by age 12 months (27-54 cases of disease). On the basis of a TEWL SD of 2.31 g/m^2/h,[15] a difference of 2 g/m^2/h at birth could be adequately detected between babies who do and do not develop AD (22 babies required for 80% power).

Skin assessments

Remote measurements were performed on the maternity ward (≤72 hours old) and the family home at 4 ± 2 weeks and 12 ± 1 months, with all follow-up visits completed by February 2020. The volar forearms, the right antecubital fossa, and the right thigh were the designated assessment sites. Measurements were performed at the test sites in the presence of eczema. The Neonatal Skin Condition Score[16] was calculated at birth, and a visual inspection of dryness and erythema was conducted at all time points.

Skin barrier function. A single TEWL reading was obtained in ambient conditions from each skin site using an AquaFlux AF200 closed chamber condensing device (Biox Systems Ltd, London, United Kingdom). A measurement was aborted if the infant was distressed.

Infrared spectroscopy. A portable 4300 handheld Fourier transform infrared (FTIR) spectrometer equipped with mercury cadmium telluride detector and 1-bounce/1-pass diamond attenuated total reflectance (ATR) accessory (Agilent Technologies, Santa Clara, Calif) collected absorption spectra in the mid infrared region from 32 scans at a resolution of 4 cm^{-1}. A single spectrum was obtained from each skin site and visually checked for quality. Because of inconsistent ATR-FTIR signal encountered at birth, a prototype 3-bounce/2-pass (3B2P) diamond ATR accessory was implemented by the recruitment of participant number 035. All subsequent babies were therefore assessed using the 3B2P accessory, with all previous participants (001-034) excluded from the spectroscopic end points at birth for consistency. For quantitative ATR-FTIR parameters, peak intensities related to total lipid (2850 cm^{-1} CH_2 stretching mode), sebum (1740 cm^{-1} C=O stretching mode), and water (1640 cm^{-1} H_2O deformation) were baseline-corrected and normalized to amide II at 1540 cm^{-1} to account for contact pressure.[17] Lipid structure was assessed by the position of the 2850 cm^{-1} CH_2 stretching vibration.[4] Negative peak intensities were excluded from the analysis. A commercial baby wipe was piloted for sebum removal before an additional ATR-FTIR measurement being taken.

Tape stripping. Three serial 14-mm D squame discs (CuDerm, Dallas, Tex) were pooled for *ex vivo* desquamatory protease analysis (left forearm) and NMF quantification (left forearm and right antecubital fossa).

Desquamatory protease activity

Caseinolytic and chymotrypsin-like (C-L) activities were assayed using EnzCheck (Invitrogen, Paisley, United Kingdom) and MeOSuc-Arg-Pro-Tyr-AMC (Peptide Protein Research Ltd, Southampton, United Kingdom) substrates. Specific activity (nU/μg) was calculated using SC mass estimated by densitometry.

Measuring NMF abundance *in vivo by* ATR-FTIR

As previously described,[18] a real-time spectroscopic measure of NMF was developed by calibrating infrared absorption across the fingerprint spectral region (1090-1653 cm^{-1}) using a single composite quantitative measure of NMF obtained by *ex vivo* laboratory analysis. Three serial tape strips were extracted and pooled, with pyrrolidone carboxylic acid and urocanic acid analyzed by a high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) combined with free amino acid quantification by *o*-phthalaldehyde derivatization. Sampling sites in a proportion of infants at age 4 weeks and 12 months were randomly allocated to model calibration and validation sets. Spectra were normalized to amide II (1540 cm^{-1}) before modeling. The final chemometric model was validated using a within-cohort subset of infants, independent to the model build.

Filaggrin genotyping

Saliva was collected at age 12 months and genomic DNA extracted using the Oragene OG-250 sampling device and PrepIT L2P kit (DNA Genotek, Ottawa, Ontario, Canada). The 4 common European filaggrin (*FLG*) risk alleles (R501X, 2282del4, R2447X, and S3247X) were screened by Taqman

(Thermo Fisher Scientific, Waltham, Mass) or Sanger sequencing (Azenta Life Sciences, Takeley, United Kingdom) using established probe and primer sets.[19]

Outcome measures

Primary outcomes were the change in skin barrier function, molecular composition, and desquamatory protease activity from birth to age 4 weeks and 12 months. Secondary outcomes included (1) the incidence of AD diagnosed by a general practitioner in primary care (remote diagnosis, parent-reported to study team by 12 months) or a study investigator using the UK Working Party criteria at the 12-month visit[20]; (2) parental-reported skin rashes; (3) the frequency of FLG risk alleles; and (4) the association of skin barrier function, molecular composition, and desquamatory protease activity measured at birth and age 4 weeks with an AD diagnosis by age 12 months. In the presence of clinical signs, the Eczema Area and Severity Index score was conducted by a trained researcher at the home visits as a measure of disease severity. Completion of the 12-month visit was the study end point. Additional outcomes on infant skin care practices and parental satisfaction captured by diary, questionnaire, and semi-structured interviews will be reported in subsequent articles.

Statistical analysis

Study data were captured using FileMaker Pro (Claris, London, United Kingdom) and collated in Excel. Normality was checked using the Q-Q plot, and parameters were log-transformed when appropriate. For primary outcomes, a matched, mixed-model, 1-way ANOVA or nonparametric equivalent compared means over time. For secondary outcomes associated with AD diagnosis, (1) a 2-way ANOVA compared parameter means over time between infants with and without disease; (2) a matched, mixed-model ANCOVA investigated the relationship between TEWL, temperature, and time; and (3) multiple logistic regression using the log-likelihood ratio test ($P \leq .05$) for stepwise selection modeled parameters at birth and age 4 weeks (including *FLG* status), controlling for familial atopy, sex, and gestation period. Withdrawn participants were excluded from AD risk analysis. Statistical tests were performed using GraphPad Prism 9 (GraphPad, San Diego, Calif) or IBM SPSS statistics (version 27; IBM, Armonk, NY). Panorama Pro (LabCognition, Cologne, Germany) was used for spectroscopic chemometric analysis.

RESULTS

A total of 689 eligible families were screened. Of the 180 neonates recruited (mean age, 33.61 ± 17.66 hours at first assessment), 128 completed the 12-month home visit (Fig 1). Two babies were older than 72 hours at the first assessment but were included in the analysis. Following informed consent, 52 participants withdrew because of screening failure following enrollment (n = 3); retraction of parental consent (n = 9); or loss to follow-up after 3 failed attempts to schedule a home visit (n = 40). Mean age at the 4-week and 12-month assessment was 33.43 ± 6.68 days and 12.02 ± 0.75 months, respectively. Twenty-five infants (20%) developed AD by age 12 months (Table I) confirmed by a general practitioner (n = 19) or investigator-diagnosed (n = 6). The proportion of infants carrying at least 1 common *FLG* variant allele was 13% (13 of 99), with a higher prevalence in the AD group (35%) compared with no disease (8%). Familial atopy was high (79%), suggesting infants highly predisposed to AD development. None of the children developed AD by age 4 weeks. At age 12 months, the mean whole-body Eczema Area and Severity Index score was 1.5 (18 of 25 infants with clinical signs), indicative of mild disease well controlled by the study end point. The infant group with AD reported a greater proportion of skin rashes (Table I) and general skin complaints (see Table E2 in this article's Online Repository at www.jacionline.org). Overall, parents were satisfied with the skin tests performed when asked in the 12-month exit questionnaire (see Table E3 in this article's Online Repository at www.jacionline.org).

Skin barrier development from birth

As a biomarker of permeability barrier function, TEWL proved highly variable at all developmental time points (Fig 2, *A*), which likely reflected the wide range of ambient temperatures encountered at assessment (Fig 2, *B*). For example, at birth, a far greater cohort SD (8.89 g/m^2/h) was noted compared with climate-controlled conditions.[15] Accordingly, temperature (but not humidity) correlated with TEWL across all study time points ($r = 0.29$; $P \leq .001$; data not shown). As expected, higher temperatures were encountered at the maternity ward at birth compared with the family home at ages 4 weeks and 12 months. Accounting for this covariate using a matched, mixed-model ANCOVA confirmed a significant effect of time ($P \leq .001$) on mean TEWL (log-transformed) from birth to age 12 months, indicating a weakening infant permeability barrier over this period (Fig 2, *C*).

When comparing averaged skin surface ATR-FTIR spectra collected over the course of the study, clear differences in absorption related to SC lipid abundance and conformational ordering (2850 cm^{-1}), sebum (1740 cm^{-1}), and water / NMF (1653-1090 cm^{-1}) existed between age groups that warranted further investigation (see Fig E2 in this article's Online Repository at www.jacionline.org). By developing and validating a real-time *in vivo* measurement of NMF, a reduction was observed at the skin surface in infants carrying *FLG* risk alleles at age 4 weeks (see Fig E3 in this article's Online Repository at www.jacionline.org). After collecting baseline ATR-FTIR spectra, cleansing the skin by commercial baby wipe to remove sebum proved unsuccessful, and therefore the postwipe measurements were not pursued further in the cohort analysis (data not shown).

A summary of skin development over the first year of life is presented in Fig 3. Like TEWL, several biomarkers changed significantly from birth. Caseinolytic protease activity increased 5-fold by age 4 weeks, suggesting a rapid rise in desquamation and cell turnover (Fig 3, *A*). Supportive of this was the 1.4-fold rise in C-L protease activity (Fig 3, *B*). An increase in ATR-FTIR–modeled NMF abundance from birth was accompanied by a greater peak ratio (1640/1540 cm^{-1}) at age 4 weeks, indicative of higher water content (Fig 3, *C* and *D*). SC lipids and sebum (peak 2850/1540 cm^{-1} and 1740/1540 cm^{-1}) decreased from birth alongside an improvement in lipid structure denoted by the shift to a more orthorhombic phase (lower peak 2850 cm^{-1} center of gravity).[21] Although minimal vernix caseosa (VC) presence was noted across the cohort, these observations were presumably due to greater sebaceous lipid deposition at birth (Fig 3, *E-G*). There was little change in investigator-observed skin dryness from birth, but an inverse relationship with spectroscopically determined water content was noted across all time points (Fig 3,

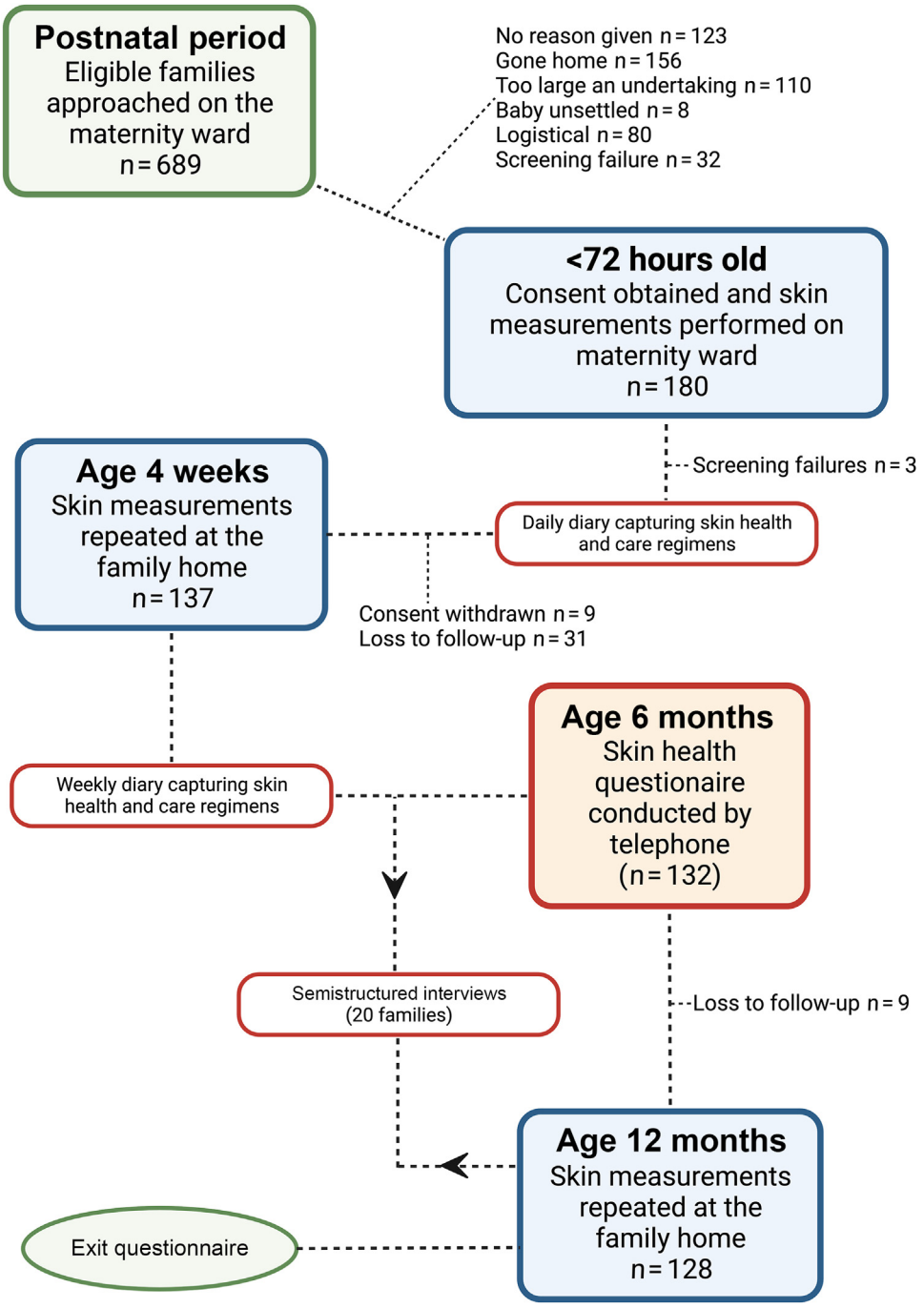

FIG 1. Participant pathway.

H). Cohort stratification by season of birth did not reveal any significant differences in skin barrier development throughout the first 4 weeks of life (data not shown). Between age 4 weeks and 12 months, there were further reductions in NMF, water, and lipid abundance in conjunction with increased TEWL.

Subtle differential skin barrier development between infants with and without disease

For TEWL and ATR-FTIR parameters, similar trends were observed across anatomical sites between infants with and without AD (see Fig E4 in this article's Online Repository at www.jacionline.org). Consequently, the mean of these body sites was calculated (1 measurement parameter per infant, per time point), allowing greater precision to address the secondary end points of AD risk. Overall, few differences in early skin barrier maturation were observed between infants with and without AD; however, subtle patterns existed before onset of clinical disease (Fig 4). For example, C-L protease activity at birth was on average 40% lower in infants who developed AD (Fig 4, *B*). Although no difference in visual dryness was apparent by age 4 weeks (Table I), spectroscopically modeled NMF and water content were on average 15% and

TABLE I. Cohort characteristics stratified by AD diagnosis

Characteristics	No AD	Confirmed AD	Unknown (W/LTFU)	Total
No. of infants, n	103	25	49	177
Sex: male, n (%)	51 (50)	10 (40)	17 (35)	78 (44)
Birth weight (g)	3480 ± 408 (2330-4380)	3477 ± 480 (2620-4540)	3487 ± 517 (2560-4890)	3482 ± 447 (2330-4890)
Gestation (d)	280 ± 9 (260-299)	282 ± 9 (262-290)	280 ± 9 (263-295)	280 ± 9 (260-299)
Mode of delivery, n (% cesarean section)	42 (41)	9 (36)	23 (47)	74 (42)
Season of birth, n (%)				
Autumn	32 (31)	11 (44)	20 (41)	63 (36)
Winter	13 (13)	6 (24)	6 (12)	25 (14)
Spring	26 (25)	3 (12)	10 (20)	39 (22)
Summer	32 (31)	5 (20)	13 (27)	50 (28)
Age at assessment				
Birth (h)	33.38 ± 16.84 (9-88)	39.72 ± 21.57 (11-95)	30.85 ± 16.72 (9-71)	33.61 ± 17.66 (9-95)
4 wk (d)	33.16 ± 7.11 (25-64)	34.52 ± 4.54 (28-45)	—	33.43 ± 6.68 (25-64)
12 mo	12.01 ± 0.76 (11-15)	12.04 ± 0.73 (11-14)	—	12.02 ± 0.75 (11-15)
NSCS	3.46 ± 0.72 (3-7)	3.44 ± 0.51 (3-4)	3.57 ± 0.58 (3-5)	3.49 ± 0.66 (3-7)
Parent-reported rash,* n (%)	34 (33)	16 (70)	—	50 (28)
Mean dryness score				
Birth	1.25 ± 0.41 (1-3)	1.24 ± 0.39 (1-2)	1.49 ± 0.56 (1-3)	1.31 ± 0.46 (1-3)
4 wk	1.18 ± 0.35 (1-3)	1.22 ± 0.39 (1-2)	—	1.19 ± 0.36 (1-3)
12 mo	1.08 ± 0.24 (1-2)	1.02 ± 0.07 (1-2)	—	1.07 ± 0.22 (1-2)
Baby's ethnicity, n (%)				
Asian	4 (4)	2 (8)	4 (8)	10 (6)
Black	5 (5)	—	1 (2)	6 (3)
Other	3 (3)	1 (4)	—	4 (2)
Mixed	10 (10)	2 (8)	3 (6)	15 (8)
White	81 (79)	20 (80)	41 (84)	142 (80)
Family atopy† (%)				
AD	40 (39)	14 (56)	21 (43)	75 (42)
Asthma	33 (32)	7 (28)	19 (39)	59 (33)
Allergic rhinitis	58 (56)	18 (72)	32 (65)	108 (61)
Any atopy	76 (74)	22 (88)	42 (86)	140 (79)
FLG variants,‡ n (%)	6/79 (8)	7/20 (35)	—	13/99 (13)
EASI score§	—	1.5 ± 1.5 (0.2-5.8)	—	—

Data are presented as mean ± SD (range). NSCS range, 3-9. Dryness (mean of forearm, antecubital fossa, and thigh) scored visually by 3-point scale (1 = no dryness; 2 = dry skin; 3 = very dry skin).

EASI, Eczema Area and Severity Index; *LTFU*, lost to follow-up; *NSCS*, Neonatal Skin Condition Score; *W*, withdrawn.

*One or more episodes of a parent-reported erythema throughout the study period.

†At least 1 first-degree relative with atopic disease.

‡Babies carrying at least 1 common risk allele.

§Averaged whole-body assessment of active disease at 12 mo (n = 18).

7% lower, respectively, in infants who developed disease, indicating a dryer skin surface at this developmental point (Fig 4, *C* and *D*). The strong inherited NMF defect evident at this time point likely influenced this finding (Fig E3). Using a matched, mixed-model ANOVA with the Fisher least significant difference (LSD) post-test, a significant effect of infant subgroup on ATR-FTIR lipid peak 2850/1540 cm^{-1} ($P = .0371$) was highlighted (Fig 4, *F*). Here, surface lipids at birth were 27% greater (relative to protein) in infants who developed disease, a trend corroborated by lipid esters (Fig 4, *F* and *G*). Although mean TEWL was elevated at birth in the infant AD group, no significant differential permeability barrier function was noted, representing comparable skin barrier health throughout the study (Fig 2, *D*). Correlation analysis was performed on all parameters tested but yielded no significant associations.

Factors associated with disease risk by age 12 months

To explore parameters associated with an AD diagnosis by age 12 months, logistic regression modeling was used controlling for sex and gestation period (Table II). Modeling first-degree atopy alone was not associated with an AD diagnosis (area under the receiver-operating characteristic curve [AUC], 0.61 ns; data not shown) nor was any form of atopy (AD, asthma, or hay fever) when modeled in turn (first-degree relative; data not shown). By comparison, the forward selection of independent variables using a log-likelihood ratio threshold ($P \leq .05$) revealed 5 parameters of additive value to family atopy for early disease risk evaluation (models 1-5) indicated by a significant AUC. A greater ATR-FTIR peak 2850/1540 cm^{-1} (birth), reduced C-L protease activity (birth), low NMF (4 weeks), and a drier skin surface (4 weeks) were early signals of disease development. Those carrying at least 1 common *FLG* variant allele (model 5) were 6 times more likely to develop AD by age 12 months (AUC, 0.73). A second round of forward selection combined sequentially each significant parameter. Modeling *FLG* status with C-L protease activity (model 6; AUC, 0.78) correctly classified 30% and 99% of cases with and without AD, respectively, at a 50% cutoff. In contrast, TEWL was not associated with AD at any time point.

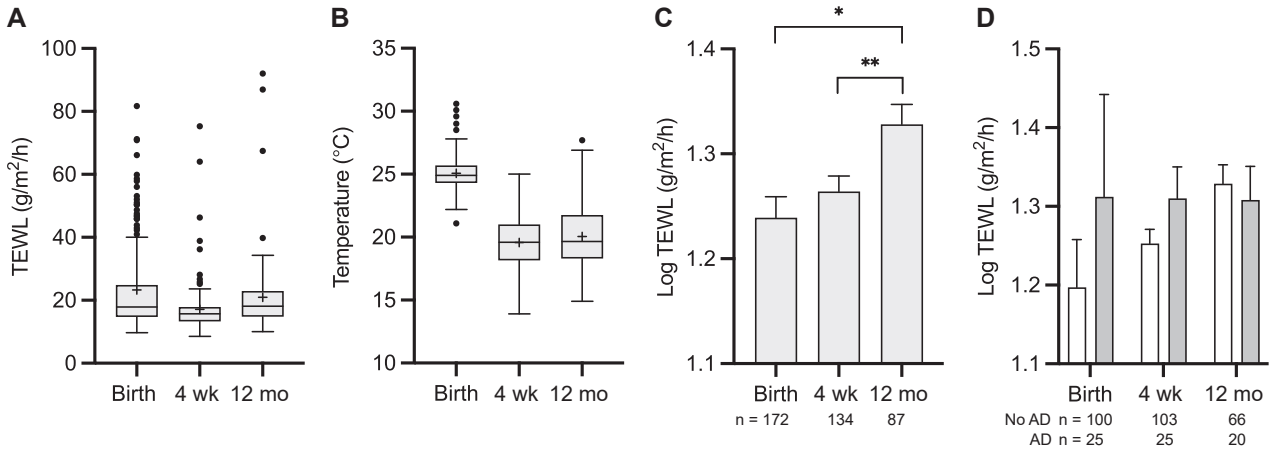

FIG 2. Development of skin barrier function from birth measured in ambient conditions. **A**, A wide range of raw TEWL was observed in accordance with **B**, the highly variable ambient conditions encountered at assessment. **C**, Controlling for temperature, a significant weakening of skin barrier function was observed from birth to age 12 months, albeit **D**, no significant difference in TEWL was noted between infants who did (*darker shading*) and did not develop AD. A matched, mixed-model ANCOVA reported a significant effect of time ($P = .002$) and temperature ($P < .001$) but not AD group on log-transformed TEWL, with asterisks denoting the result of a Bonferroni post-test to compare groups (*$P = .011$, **$P = .006$). The mean of a single TEWL measurement collected from the right forearm, right thigh, and right antecubital fossa is presented as comparable trends were observed across anatomical sites over time (see Fig E1 in this article's Online Repository at www.jacionline.org).

DISCUSSION

From birth, a significant period of skin barrier maturation[8,9] coincides with an increased risk of developing AD.[7] By performing a portfolio of tests remotely in a longitudinal birth cohort, this exploratory study identified subtle signals that predated AD onset. A primary *FLG* defect strongly conferred early disease with reduced NMF and a drier skin surface observed by age 4 weeks spectroscopically, highlighting potential as a rapid, bedside genotyping tool compared with laboratory analysis. In conjunction with reduced C-L protease activity and increased surface lipids at birth, assessment of these parameters in a community setting proved feasible and was of additive value for early AD risk evaluation when accounting for a family history of atopy, a metric currently used to identify high-risk infants for prophylactic intervention.[22]

This study reports that developmentally, skin barrier maturation continues till at least the conclusion of the first year. From birth the SC rapidly acidifies and hydrates.[23,24] Here, using ATR-FTIR peak 1640 cm^{-1} attributed to the bending mode of water,[25] a significantly more hydrated skin surface was reported at age 4 weeks. This is in agreement with Raman spectroscopy measuring greater water content throughout the entire depth of the infant SC compared with adults.[8] Underlying this evolution toward hydrophilicity may be changing NMF abundance, from low levels at birth increasing with postnatal age.[26,27] A similar trend was observed for C-L desquamatory activity—attributable to KLK-7[28]—that can be modulated through changes in biochemical environment.[29] As a biomarker of skin health, TEWL increased from birth over this early period, although there is uncertainty in this finding because of the extreme range of ambient conditions encountered by the study. Nevertheless, the observed trend in TEWL is corroborated by a larger infant study,[30] with further weakening of permeability barrier function noted in our cohort beyond more steady-state conditions at age 4 weeks, supporting an extended period of optimization from birth.[8] Although minimal VC was noted by this study, bulk surface lipids and lipid esters measured spectroscopically were elevated at birth. With VC comprising triglycerides, wax esters, and squalene of sebaceous origin, any remnants would increase lipid/protein ratio and disorder lipid organization (increased proportion of branched chain fatty acids) beyond the background SC signal.[31] The gradual decline in lipid esters reflects the change in sebum at the neonate skin surface from birth.[32,33]

A secondary outcome measure of this study was disease incidence, allowing the relationship between early skin barrier dysfunction and AD diagnosis to be retrospectively assessed. Although a small cohort, the rate of AD was consistent with that in 2 similar UK cohorts,[22,34] albeit lacking more active disease cases, which may reflect a bias toward the more common early-onset/resolving prognosis.[35] However, a 2% and 14% rate of moderate to severe AD reported in infants suggests that the early-onset trajectory, although prevalent, is predominantly mild.[22,34]

This study reinforces the association between common European *FLG* variants and early AD development.[35,36] Here, a functional consequence was reduced NMF abundance at age 4 weeks representative of *FLG* genotype, an observation supported by Raman microspectroscopy (AUC, 0.79-0.83) performed around birth.[37] Considering the constraint of ATR-FTIR measurements to the skin surface, where NMF levels are low following birth, it is plausible that around age 4 weeks is the optimum time to differentiate *FLG* endotypes using this technique. We saw little indication of an acquired NMF defect, representative of a cohort devoid of overt inflammation.[5,38]

There is evidence that weakened permeability barrier function predates early-onset AD.[39,40] Although a similar trend here was observed at birth, no significant difference in TEWL was noted between infant subgroups throughout the study. With a clear TEWL abnormality observed in more severe infant disease cases by age 12 months,[5] this likely reflects an absence of inflammation. A further contributory factor could

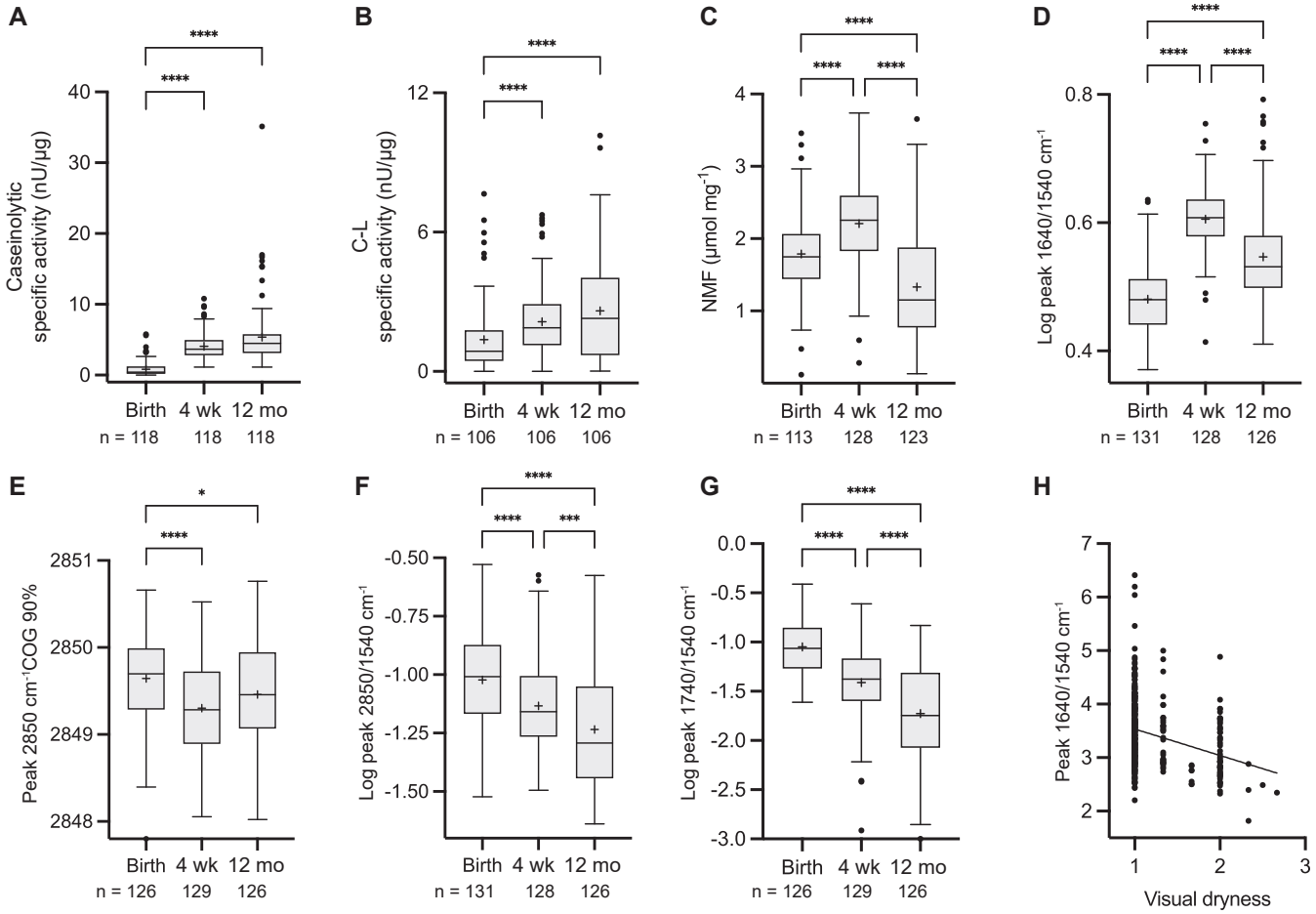

FIG 3. Skin barrier development from birth. **A-G**, Mean change in surface caseinolytic (Fig 3, *A*) and C-L protease activity (Fig 3, *B*), *in vivo* ATR-FTIR–modeled NMF (Fig 3, *C*), water content (Fig 3, *D*), lipid structure (Fig 3, *E*), total lipids (Fig 3, *F*), and lipid esters (Fig 3, *G*) from birth to ages 4 weeks and 12 months. Protease measurements were obtained from 1 repeat on the left forearm only. For spectroscopic parameters, the mean of a single measurement from both forearms, right antecubital fossa, and right thigh was taken as comparable patterns were observed across anatomical sites over time (see Fig E1). Infants with a minimum of 2 successful ATR-FTIR spectra were included for analysis. A Friedman test, with asterisks denoting a significant Dunn multiple comparison test, was used for Fig 3, *A* and *B*, only. For all other parts, a matched, mixed-model ANOVA was performed, with asterisks denoting a significant Tukey post-test to compare time points (*$P \leq .05$, **$P \leq .01$, ****$P \leq .0001$). Log transformation was used where indicated. **H**, A significant association was noted between investigator-observed dryness and spectroscopically determined water content across all time points using the Spearman rank test ($r = -0.29$; ***$P < .001$).

also be the omission of TEWL measurements from the cheek or face, a common site of symptomatic onset.[39,40] Nor was TEWL performed in environmentally controlled conditions in which the passage of water through the SC is the sole rate-limiting factor of vapor flux following an appropriate period of acclimatization.[41] The lack of precision we observed suggests that a group of 25 babies with AD was underpowered to obtain a reliable cohort estimate of TEWL when measured in unstandardized conditions.

The study produced some novel results. One skin parameter associated with disease that modeled independently to the pronounced inherited *FLG* defect was reduced KLK7 activity at birth. This contrasts to the elevated expression and activity of desquamatory proteases as a hallmark of established AD.[28,42] Relevant to this finding is increased serine protease inhibitor (Serpin) A12 abundance within the VC of infants who develop AD by age 24 months.[43] Expressed in the adult epidermis and an inhibitor of KLK7, Serpin A12 activity may explain the initial reduction in C-L proteolytic activity observed in the infants with AD, when the VC is retained.[44,45]

A strength of this project was its pragmatic design, balancing scientific objectives alongside consideration of the families involved over the longer-term study duration. To maximize participant retention, portable equipment was used, enabling remote data collection at the bedside and family home, skin tests were restricted to the surface layers, and follow-up ceased at 12 months to capture most AD cases over a minimum time frame. Even under these circumstances, 23% of the families recruited either withdrew or were lost to follow-up by age 4 weeks, a comparable rate to that in a similar study conducted by our group,[15] suggesting that more flexible assessment points are required in future work.

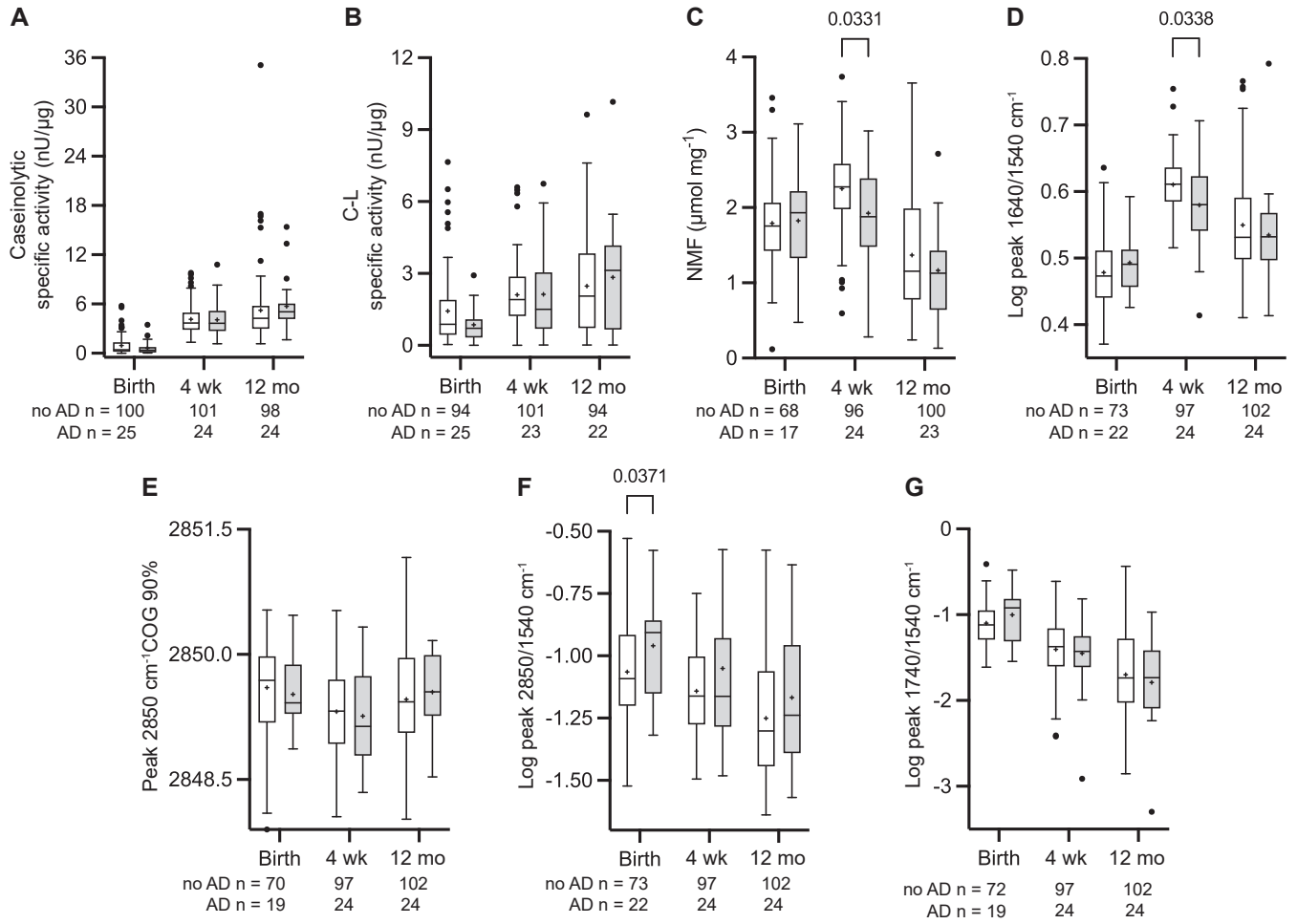

FIG 4. Subtle differential skin barrier development between infants with and without disease. **A-G**, Mean surface caseinolytic specific activity (Fig 4, *A*), C-L protease activity (Fig 4, *B*), *in vivo* ATR-FTIR–modeled NMF (Fig 4, *C*), water content (Fig 4, *D*), lipid structure (Fig 4, *E*), total lipid (Fig 4, *F*), and lipid esters (Fig 4, *G*) in infants who developed AD by age 12 months (*darker shading*) compared with those that did not. Protease measurements were obtained from 1 repeat on the left forearm only. For all skin parameters determined spectroscopically, the mean of a single measurement from both forearms, right antecubital fossa, and right thigh was taken as comparable patterns were observed between anatomical sites across time (see Fig E4). Infants with a minimum of 2 successful ATR-FTIR spectra were included for analysis. A matched, mixed-model ANOVA reported an overall significant effect of infant subgroup (AD vs no AD) on ATR-FTIR lipid peak 2850/1540 cm^{-1} only (Fig 4, *F*). *P* values denote the result of an exploratory uncorrected Fisher least significant diffference (LSD) post-test. Log transformations were used where indicated. Statistical analyses were performed on log-transformed data for Fig 4, *A* and *B*.

The study was limited by design to primarily focus on the feasibility of the remote methodology tested. A single instance of general practitioner–confirmed AD was used as a study end point —albeit diagnosis in early childhood is challenging—when multiple clinical observations are required throughout infancy to ensure established criteria of disease are fulfilled.[46] For example, at age 12 months, only 28% of infants with current eczematous lesions received a UK Working Party diagnosis because of the uncertainty of itch.[46] Further study limitations include the employment of early, fixed assessment points at a time of significant adaptation to terrestrial life and the decision to combine anatomical sites in the longitudinal analysis. Both may have contributed to clearer evidence of skin barrier breakdown being missed at a later developmental stage. Nor was a hypothesized SC lipid ordering defect assessed spectroscopically because of the contamination of sebum.[4] Recent evidence has associated early infant AD development with altered SC lipid composition, characterized by higher ratios of shorter chain sphingosine bases and lower phytosphingosine levels, but the subsequent effect on skin barrier function remains unclear.[47] Finally, a limitation of the observational design is unintended sources of confounding. Here, for instance, most participating families were of White ethnicity with a history of atopic disease, limiting the applicability of our findings to lighter skin phototypes at a high risk of AD.

It can be reasoned that the subtle skin barrier dysfunction noted, indicative of the inherited *FLG* defect, was proportional to the mild AD observed that lacked the anticipated acquired defect in TEWL, lipid structure, and NMF.[5,38,48] Larger and more definitive trials should therefore incorporate a greater proportion of

TABLE II. Forward selection modeling of parameters associated with AD by 12 mo

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
No AD/AD, n	73/22	94/25	96/24	97/24	79/20	74/20
Parameter	2850/1540 cm^{-1}*	C-L activity*	NMF†	1640/1540 cm^{-1}†	*FLG* variant‡	*FLG* variant‡
	1.7 (1.1-2.9) .04	0.6 (0.3-0.9) .05	0.4 (0.2-0.9) .03	0.2 (0.1-0.7) .01	6.0 (1.6-23.2) .008	6.2 (1.6-27.6) .01
						C-L activity*
						0.6 (0.3-1.0) .07
Sex	0.6 (0.2-1.6) .30	0.7 (0.3-1.7) .39	0.7 (0.2-1.7) .39	0.8 (0.3-2.2) .70	1.0 (0.3-3.3) .93	1.1 (0.3-3.5) .93
Gestation	1.0 (1.0-1.1) .41	1.0 (1.0-1.1) .34	1.0 (1.0-1.1) .72	1.0 (1.0-1.1) .52	1.0 (1.0-1.1) .22	1.0 (1.0-1.1) .17
Family atopy	2.0 (0.6-9.5) .32	2.9 (0.9-13.2) .12	1.9 (0.6-9.0) .33	2.1 (0.6-9.8) .28	5.3 (0.90-104) .13	8.0 (1.3-166) .07
AUC	0.68 ($P \leq .01$)	0.69 ($P \leq .01$)	0.67 ($P \leq .05$)	0.69 ($P \leq .01$)	0.73 ($P \leq .01$)	0.78 ($P \leq .01$)

Mean of spectroscopic parameters (models 1-4) taken from a minimum of 2 anatomical sites. Odds ratio (95% CI) and *P* values are given.
*Birth.
†Age 4 weeks; family atopy: at least 1 first-degree relative with atopic disease.
‡Babies carrying at least 1 common risk allele.

more active disease cases to place the current data set into context, using a more robust AD diagnosis to explore further skin barrier risk signals independently of the established inherited *FLG* defect to improve the current disease modeling. Early detection and intervention of at-risk babies remains an unmet clinical need, with the more severe, persistent cases predisposing to further atopic comorbidities in childhood.[34,35,49]

DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Key messages

- **Biophysical skin testing at birth was well received by the participating families with subtle skin barrier dysfunction accompanying the mild disease observed in this study.**
- **An inherited loss of FLG conferred a reduction in NMF at age 4 weeks determined by real-time infrared spectroscopy at the "bedside" that was associated with AD onset.**
- **Reduced C-L activity combined with inherited *FLG* loss provided the strongest indication of disease (AUC, 0.78), highlighting a novel mechanism for further exploration.**

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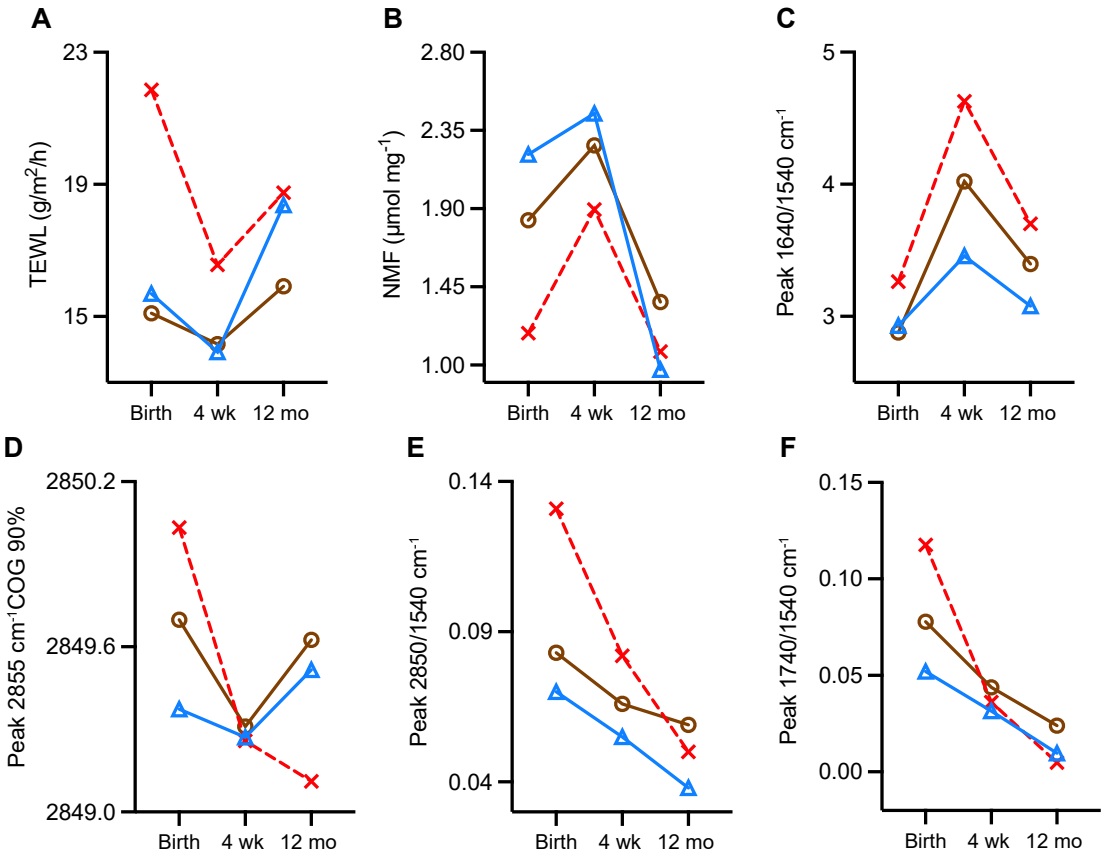

FIG E1. Different anatomical sites show comparable skin surface development from birth. **A-F**, Median change over time in TEWL (Fig E1, *A*), *in vivo* modeled NMF (Fig E1, *B*), water content (Fig E1, *C*), lipid structure (Fig E1, *D*), total lipid (Fig E1, *E*), and lipid esters (Fig E1, *F*) at the forearm (*brown circle*), right antecubital fossa (*red cross*), and right thigh (*blue triangle*). A single TEWL or ATR-FTIR measurement from each site was collected per infant. Forearm shows the average of the right and left sides (ATR-FTIR only).

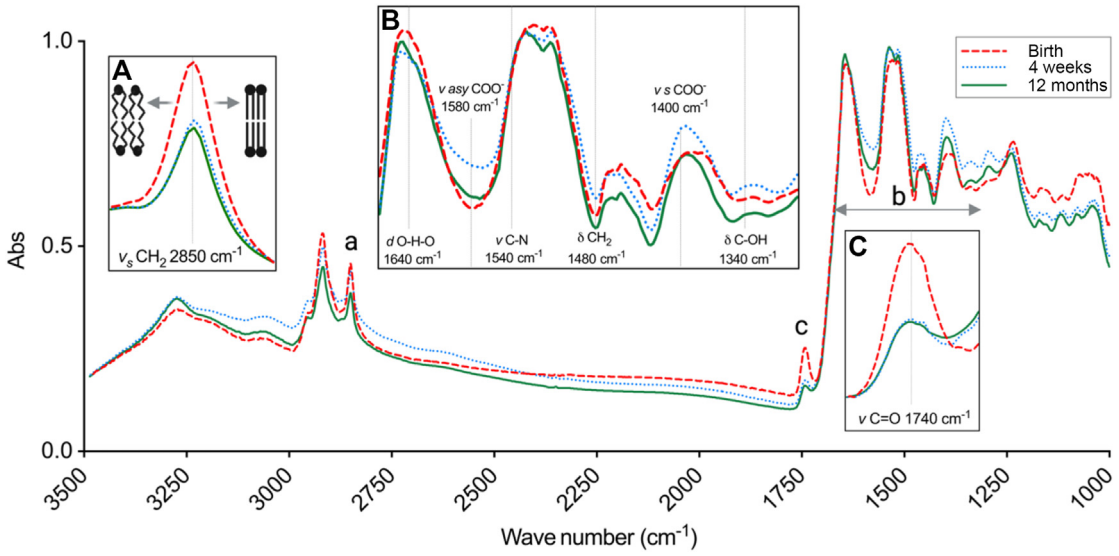

FIG E2. Mean ATR-FTIR spectra collected at the skin surface show clear differences in absorption between developmental time points at birth (*red dashed line*), 4 weeks (*blue dotted line*), and 12 months (*green solid line*). **A**, SC lipid abundance and confirmational ordering measured by 2850 cm^{-1} peak intensity and location (CH_2 symmetric stretch). Lower wave numbers are associated with a more orthorhombic (ordered) lipid phase.[E1] **B**, Water[E2] (O-H-O deformation at 1640 cm^{-1}), amide II (v C-N at 1540 cm^{-1}), and NMF abundance measured from multiple signals related to amino acids and their derivatives throughout the fingerprint spectral region.[E3] **C**, Sebum abundance measured by 1740 cm^{-1} peak intensity[E4] (lipid ester C=O stretch). The mean of both forearms, the right antecubital fossa, and right thigh is presented.

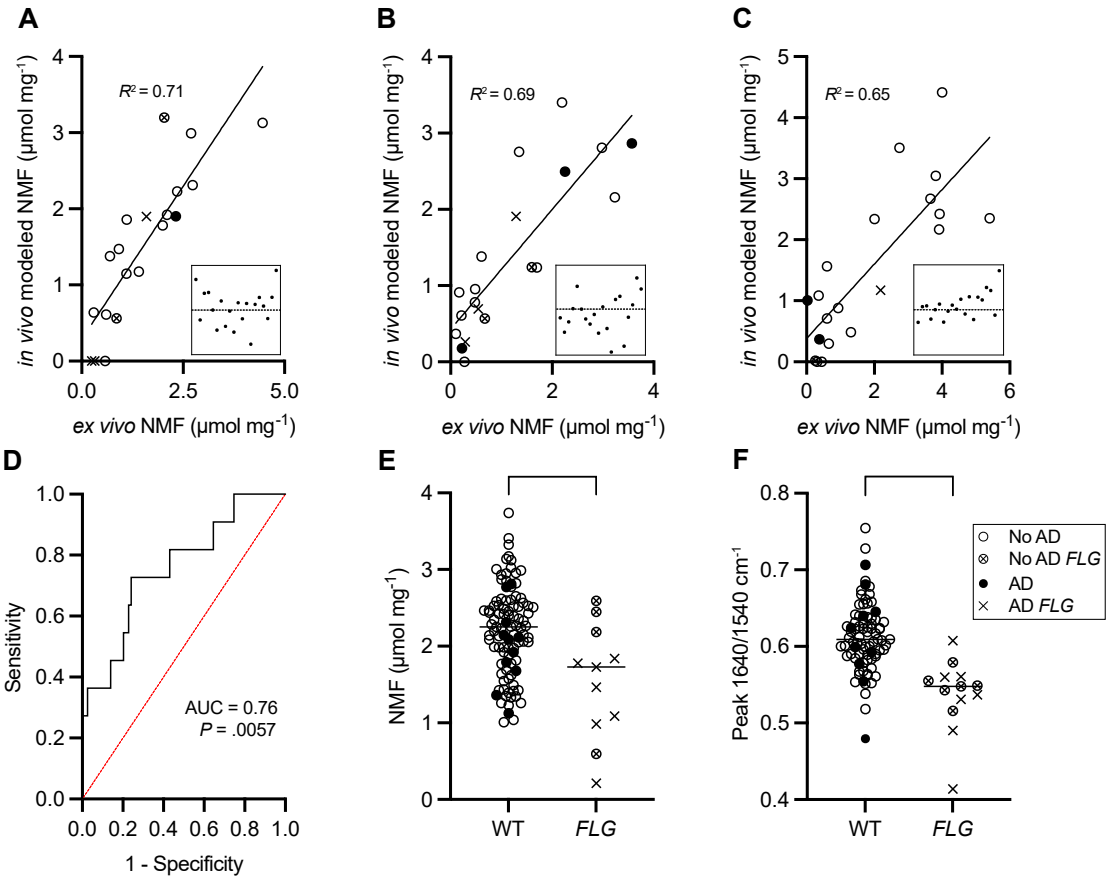

FIG E3. *In vivo* modeling of NMF at ages 4 weeks and 12 months by infrared spectroscopy. A single skin surface ATR-FTIR spectrum collected *in vivo* at 4 weeks (forearm) and 12 months (antecubital fossa) was calibrated using a composite *ex vivo* measure of NMF (discs 1-3) by partial least-squares regression. **A** and **B**, Plot of *in vivo* modeled vs *ex vivo* quantified NMF (the sum of free amino acids, pyrrolidone carboxylic acid, and urocanic acid) in a subset of infants for model calibration (Fig E3, *A*) and model validation sampling sites (Fig E3, *B*) (n = 20). **C**, The NMF model was further validated in a within-cohort subset of infants independent to the model build (n = 21). **D**, In the wider STAR cohort (n = 110), *in vivo* modeled NMF at age 4 weeks was moderately predictive of the *FLG* genotype ($P = .0057$). **E** and **F**, On average, infants carrying at least 1 common *FLG* variant allele had significantly reduced levels of *in vivo* modeled NMF (mean difference, 0.67 µmol mg^{-1}; 95% CI, 0.29-1.04) (Fig E3, *E*) and water content at the skin surface (Fig E3, *F*) by age 4 weeks. R^2 is the coefficient of determination. Asterisks denote the result of an unpaired student *t* test (***$P < .001$). Residual plots inset (Fig E3, *A-C*).

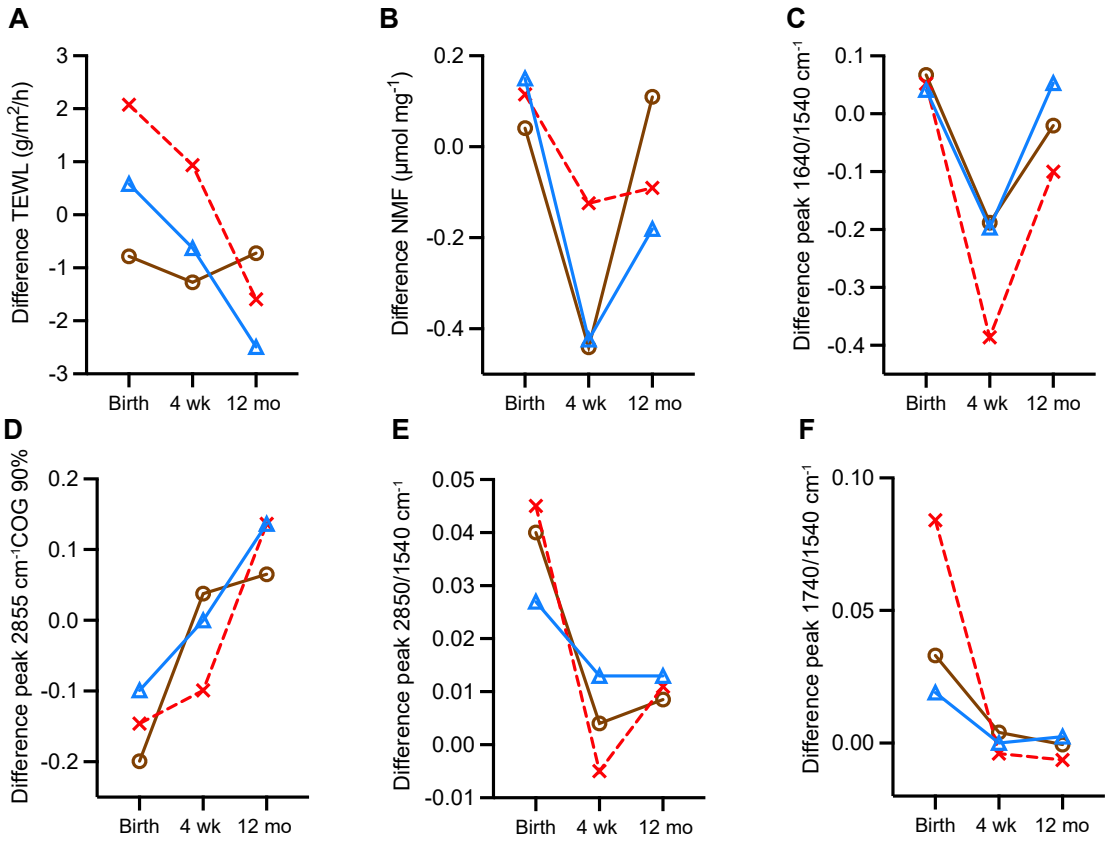

FIG E4. Similar developmental trajectories across anatomical sites between infant subgroups. **A-F**, Median change (AD minus no AD) over time in TEWL (Fig E4, *A*),*in vivo* modeled NMF (Fig E4, *B*), water content (Fig E4, *C*), lipid structure (Fig E4, *D*), total lipid (Fig E4, *E*), and lipid esters (Fig E4, *F*) at the forearm (*brown circle*), right antecubital fossa (*red cross*), and right thigh (*blue triangle*). A single TEWL or ATR-FTIR measurement from each anatomical site was collected per infant. Forearm shows the average of the right and left sides (ATR-FTIR only).

TABLE E1. Study eligibility criteria

Inclusion criteria	Exclusion criteria
Baby	
Healthy, full-term, ≤72 h old	Admission to neonatal unit
	Major congenital malformations or limb defects, illness, social issues, or logistical reasons that will prevent comfortable participation by the family
	Currently participating in another clinical study
	The baby is to be adopted
Mother	
Age ≥ 18 y	Unable to give informed consent
Singleton pregnancy booked in to give birth at the maternity unit	*Carrying* a baby with known chromosomal abnormality or syndromic diagnosis
Lives within a 5-mile radius of the University of Sheffield	

TABLE E2. Study adverse events

	Total no. of events (%)	
Adverse event type	**No AD**	**AD**
General disorders	3 (2)	4 (16)
Skin and subcutaneous tissue disorder	63 (28)	30 (80)
Infections and infestations	121 (46)	19 (48)
Immune system disorders	8 (5)	4 (16)
Gastrointestinal disorders	38 (20)	8 (20)
Respiratory, thoracic, and mediastinal disorders	10 (6)	1 (4)
Familial and genetic disorders	14 (8)	1 (4)
Neoplasms benign, malignant, and unspecified	3 (2)	0
Reproductive system and breast disorders	2 (1)	0
Pregnancy, puerperium, and perinatal conditions	8 (5)	0
Injury, poisoning, and procedural complications	2 (1)	0
Eye disorders	1 (1)	0
Uncoded	3 (1)	2 (4)
Total	276	69

TABLE E3. Parental satisfaction with skin testing in the 12-mo exit questionnaire

Question	Extremely satisfied	Fairly satisfied	Neutral	Somewhat dissatisfied	Extremely dissatisfied
How do you feel about the *TEWL* machine used to assess your baby's skin?	56 (53)	29 (27)	12 (11)	9 (9)	0
How do you feel about the *spectrometer* used to assess your baby's skin?	64 (60)	30 (28)	7 (7)	5 (5)	0
How do you feel about us *tape-stripping* your baby's skin?	84 (79)	17 (16)	4 (4)	1 (1)	0

Percentages are given in parentheses.