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ORIGINAL ARTICLE

Electrical impedance spectroscopy detects skin barrier dysfunction in childhood atopic dermatitis

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Abstract

Background: Skin barrier dysfunction is associated with the development of atopic dermatitis (AD), however methods to assess skin barrier function are limited. We investigated the use of electrical impedance spectroscopy (EIS) to detect skin barrier dysfunction in children with AD of the CARE (Childhood AlleRgy, nutrition, and Environment) cohort.

Methods: EIS measurements taken at multiple time points from 4 months to 3-year-old children, who developed AD ($n = 66$) and those who did not ($n = 49$) were investigated. Using only the EIS measurement and the AD status, we developed a machine learning algorithm that produces a score (EIS/AD score) which reflects the probability that a given measurement is from a child with active AD. We investigated the diagnostic ability of this score and its association with clinical characteristics and age.

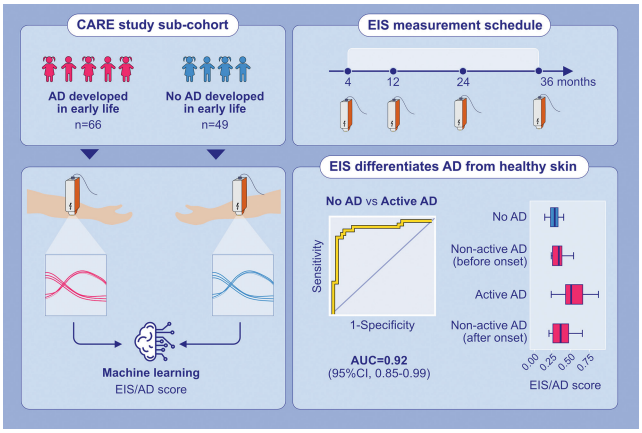
Results: Based on the EIS/AD score, the EIS algorithm was able to clearly discriminate between healthy skin and clinically unaffected skin of children with active AD (area under the curve 0.92, 95% CI 0.85–0.99). It was also able to detect a difference between healthy skin and AD skin when the child did not have active AD. There was no clear association between the EIS/AD score and the severity of AD or sensitisation to the tested allergens. The performance of the algorithm was not affected by age.

Conclusions: This study shows that EIS can detect skin barrier dysfunction and differentiate skin of children with AD from healthy skin and suggests that EIS may have the ability to predict future AD development.

KEYWORDS
atopic dermatitis, electrical impedance spectroscopy, epithelial barrier, paediatric, skin barrier assessment

Abbreviations: AD, atopic dermatitis; AUC, area under the curve; CARE, Childhood AlleRgy, nutrition and Environment; CI, confidence interval; EIS, electrical impedance spectroscopy.

The members of CK-CARE Study Group are provided in Appendix



GRAPHICAL ABSTRACT

This study investigates the use of electrical impedance spectroscopy to detect skin barrier dysfunction in children with AD of the CARE cohort. Based on the EIS/AD score, EIS algorithm is able to clearly differentiate healthy skin and clinically unaffected skin of 4 months to 3-year-old children with active AD. EIS/AD score is also different between healthy skin, skin of children with non-active AD (before onset or after onset of AD but without active symptoms) and active AD.

1 | INTRODUCTION

Approximately 10%–20% of children in developed countries suffer from atopic dermatitis (AD), a chronic inflammatory skin disease that is characterized by recurrent pruritic eczematous lesions.[1] While the majority of children outgrow the disease, those with severe disease or early sensitisation are at increased risk of persistent disease and of developing other allergic comorbidities.[2–4]

Skin barrier dysfunction is suggested to be one of the important steps in the development of AD.[5–7] Components of the skin barrier that are affected in AD include the lack of epidermal protein Filaggrin (FLG) and barrier lipids in the stratum corneum, and deficiency of tight junction proteins of the stratum granulosum.[6] This may cause penetration of allergens, pathogens and irritants, that lead to a complex interaction with microbial dysbiosis, and immune dysregulation, resulting in chronic inflammation and cutaneous remodelling.[5–7] Children with AD are reported to have increased risk of food allergy, asthma, and allergic rhinitis as described by atopic march.[8] Additionally, FLG gene mutations are shown to increase the risk of dysfunctional barrier, food allergy and asthma, highlighting the role of allergen exposure through barrier defective skin as one of the routes of sensitisation and the development of other allergic diseases.[9,10] Abnormalities in the skin barrier are also found in clinically unaffected skin of patients with AD,[1] and liberal and frequent use of emollients to repair the epidermal barrier is recommended as a basic treatment for AD.[11] In addition, inhibition of IL-4/IL-13 signaling improves AD by downregulation of inflammation, which also restores skin barrier function in patients with moderate-to-severe AD.[12] Current knowledge on the role of the skin barrier in AD suggest that skin barrier assessment may be useful in early diagnosis, management of disease, and possibly identifying those with high risk of developing AD.

Transepidermal water loss (TEWL) measurement is one of the few available methods to assess skin barrier function in vivo.[13] Previous studies have shown that TEWL is increased in both clinically affected and unaffected skin sites of AD patients and shows a positive correlation with the severity of disease.[14–16] Using TEWL as an assessment of skin barrier function, however, is limited to research settings, partly because of its sensitivity to environmental factors such as humidity, temperature and airflow, thus requiring a controlled test condition and the acclimatization of the subjects for 20–30 min prior to measurement.[13] TEWL is also affected by factors such as sex, ethnicity, anatomical site of measurement, and thus the interpretation of the measurements is not straightforward.[17]

Electrical impedance spectroscopy (EIS) has been utilized to identify histopathological alterations that occur in skin diseases by detecting the change in skin electrical conductivity, which is determined by factors such as the compactness and structure of cells, lipid, and water content.[18,19] The use of EIS as a tool to detect skin barrier dysfunction has been reported in mice models[20] and recently, in adult patients with AD using the Nevisense® device.[21] This device measures bio-impedance of the skin at a broad range of frequencies at several depths and has already been validated as a tool to assist the differential diagnosis of skin cancer and benign skin lesions.[22–24] EIS measurement using Nevisense® can be completed in a few minutes and appears to be unaffected by environmental factors.

To this date, the use of EIS as a tool to detect skin barrier dysfunction has not been tested among infants or young age children, who have different skin physiology as well as clinical and pathophysiological characteristics of AD compared to adults.[25–28] This is also the age group that would benefit most from this noninvasive, quick, and easy method. Here, we aimed to assess whether EIS can be used to identify skin barrier dysfunction or the changes in skin in children who developed AD in the first 3 years of life using data from the CARE (Childhood AlleRgy, nutrition, and Environment) birth cohort study. To achieve this, we first assessed whether EIS differs between healthy skin and skin from children with AD when they had skin lesions (active AD). We further investigated if EIS differentiates

healthy, active AD and non-active AD skin, and the association between EIS and clinical characteristics.

2 | METHODS

2.1 | Study population

CARE study is a prospective birth cohort study conducted in St. Gallen, Switzerland, that has been ongoing since 2016.[29,30] The overarching goal of this larger study is to investigate the association between early life exposures and the development of allergic diseases including AD. Healthy newborns are recruited at the Cantonal Hospital of St. Gallen and followed up by physical examinations (at 4 months and 1, 2 and 3 years old) and questionnaires (at birth, 4 months, 1, 1.5, 2 and 3 years old) to collect information on their symptoms of allergic diseases, as well as demographic, and environmental data. At each physical examination, the presence or absence of AD lesions and the severity of AD using the SCORAD (SCOring AD) index[31] are assessed by the study doctor. Skin prick tests (SPTs) are conducted at the 1-, 2- and 3-year-old examinations for the following allergens: wheat, egg, peanut, cow's milk, house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), timothy grass pollen, birch pollen and cat hair (ALK-Abelló Schweiz). A SPT with a mean wheal diameter of 2 mm or larger was considered as positive. The study has been approved by the Ethics Commission Ostschweiz (EKOS).

2.2 | Definitions

AD status of each child was assessed using the information from both the physical examination and questionnaires at 4 months, 1, 2, and 3 years old (only for those who completed follow up by January 2022). Children with AD were defined as those with at least one time point where AD was diagnosed by the study doctor at the physical examination. EIS measurements taken from children with AD were classified as:

A Active AD measurements: taken when the child had active symptoms.
B Non-active AD measurements

(i) Before onset measurements: taken before the child developed AD.
(ii) After AD measurements: taken after the onset of AD but when the child did not have active symptoms.

The onset of disease was determined by the earliest occurrence of either AD diagnosed by the physical examination or the report of symptoms (itchy rash accompanied by scratching at specific locations) or of a doctor's diagnosis of AD in the questionnaire.

Children with no AD were defined as those not having any AD diagnosed at the physical examination, nor having any AD symptoms or diagnosis reported in the questionnaires at all follow ups to 2 years old (and 3 years old if completed). EIS measurements taken from these children were used as control measurements for the development of the EIS/AD score. Children who only had report of symptoms or a doctor's diagnosis of AD in the questionnaire but did not have AD diagnosed by the physical examination at any time point were not included in this analysis.

2.3 | EIS measurements

At each physical examination, EIS measurements were taken using a Nevisense® device (SciBase), which can measure electrical impedance at different frequencies between 1 kHz and 2.5 MHz.[21] Data from 11 frequencies between 1 kHz and 1.0 MHz were used. Each EIS measurement contains 220 data points, where one data point corresponds to the impedance level at a certain depth and frequency. Three measurements (triplets) from each child were taken on the ventral forearm, where there were no apparent skin lesions. The ventral forearm was first cleaned by wiping the skin five times from the elbow to wrist using a pad containing saline. Before placing the electrode on the skin to conduct each measurement, the measurement site was moistened by placing a saline pad on for 30 sec and then dried using a dry cotton pad. Each measurement was completed within a few seconds and without causing any pain.

Since EIS measurements have been started in April 2019 and the CARE study was initiated in 2016, some children did not have EIS measurements from earlier time points. Additionally, some children did not complete all measurements up to 2 years but were included in the analysis since AD was diagnosed earlier or in at least one of the physical examinations. All children with at least one EIS measurement completed and had clear AD status were included in this analysis.

2.4 | Development of EIS/AD score and EIS/age score

A machine learning algorithm was trained to differentiate control measurements (no AD) from active AD measurements, as defined above. Only the EIS measurements with the AD status, and no other information of the child or the measurement (such as age or sex), were used as input for the algorithm development. All 220 data points from each EIS measurement were used as numerical inputs. Children with active AD and control measurements were randomly divided into two groups, one for the cross-validation (CV) set, which is for training, and the other for testing (Figure 1). The model pipeline consisted of standardization, a Principal Component Analysis (PCA) and a Support Vector Classifier (SVC).[32] The optimization process is explained in the supplementary material in detail. The CV groups were alternated (one

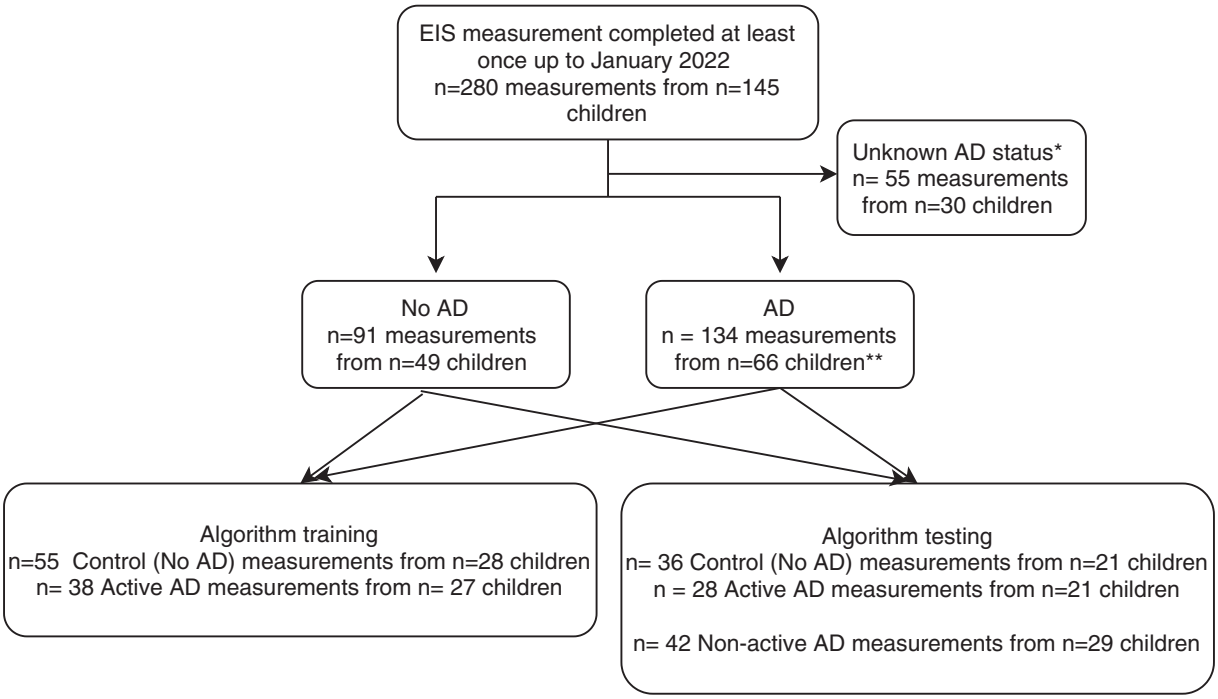


FIGURE 1 Overview of the study population and EIS measurements for the EIS/AD score. Control measurements: measurements taken from children without AD (No AD group). Active AD measurements: measurements taken from children with AD when they had symptoms. Non-active AD measurements: measurements taken from children with AD when they did not have symptoms. Among the $n=42$ non-active measurements, $n=13$ were taken before onset of AD, $n=28$ after the onset, and there was $n=1$ measurement unclassified because the AD onset for this child was unclear. The total number of measurements are larger than the total number of children in each box, since each child had one or more measurements taken at different time points. *Incomplete follow up to determine AD status. **The numbers of measurements do not add up to the total for measurements taken from children with AD since $n=26$ non-active AD measurements from the children that were used in the algorithm training dataset were not used for the analysis.

for validation, three for training) and evaluated for each parameter in a specified range with an area under the curve (AUC) score. The parameters that maximized the mean AUC for all four cross validation groups were chosen. The final pipeline was then trained on the CV set and evaluated on the test set (control and active AD measurements). The generated score (EIS/AD score) for each measurement ranged from 0 to 1, which reflected the probability of the measurement being an active AD measurement. All triplets taken at each visit were used for algorithm development and the median scores of the triplets were used for testing and further statistical analysis.

A separate machine learning algorithm to differentiate the measurements taken at 4 months and 3 years old was developed to assess the effect of age on EIS using similar methods. The generated score (EIS/age score) represented the probability that a given measurement was taken at 3 years old. For this algorithm, only children without AD were included. Training was done using 4 month and 3-year-old measurements, then tested on all age groups.

2.5 | Statistical analysis

Demographic and clinical characteristics of non-AD and AD group were compared using chi-squared test or Fisher's exact tests.

The AUC with associated 95% CI to quantify the performance of the machine learning algorithm was based on the DeLong method. Before onset measurements and after AD measurements were combined as non-active AD measurements for statistical tests, due to the sample size and the assumption that there may be subclinical skin barrier dysfunction in both situations. To investigate whether the EIS/AD score can be used to predict AD status (control, non-active AD, and active AD), cumulative link models were employed. To account for dependencies within data (due to repeated measurements on some children), models were fitted to 1000 permutated datasets generated from the original data, each with only one randomly chosen observation per child. Median values of the model parameter estimates are reported.

The correlation between EIS/AD scores and SCORAD was assessed by Pearson's correlation coefficient, using all active AD measurements. Comparison of EIS/AD scores by each AD status within each age group were conducted by ANOVA with posthoc test using Benjamini-Hochberg adjustment for multiple comparisons. EIS/AD scores by sensitisation status, demographic and early life factors within each AD status were compared by *t*-test with Benjamini-Hochberg adjustment for multiple comparisons. The mean value for the EIS/AD score was used if the child had multiple measurements within the same category of AD status.

Statistical tests were carried out in R version 4.2.1[33] and the packages including, ggeffects 1.1.4,[34] MuMIn 1.47.1[35], car 3.1.0,[36], ordinal 2019.12.10,[37] pROC 1.18.0,[38] and gmodels2.18.1.1[39]. Figures were plotted using the packages ggplot2 3.3.6[40] and ggpubr 0.4.0.[41]

3 | RESULTS

3.1 | Study population

Children without AD ($n=49$) and with AD ($n=66$) did not differ in terms of demographic or early life factors, besides a marginal increase in paternal history of AD in the AD group, as shown in Table 1. Among children who had complete SPT results, there was no difference in the proportions of those who were sensitized to any tested allergen or food allergen between the two groups. The distribution of these factors among the children in the training set ($n=55$) were similar (Table S1).

3.2 | Performance of EIS/AD score

Among the 60 children in the test set (no AD: $n=21$, AD: $n=39$), 31 children had EIS measurements at multiple time points (2 timepoints: $n=17$, 3 time points: $n=13$ and 4 time points: $n=1$) and 29 children had a measurement at only one time point. EIS/AD scores of the individual children in the test set and number of measurements by age are described in Figure S1 and Table S2.

The ROC curve for the EIS/AD score showed that the algorithm was able to differentiate EIS measurements between control and active AD, with an AUC of 0.92 (95% CI 0.85–0.99, Figure 2A). The sensitivity and specificity were each 0.82 and 0.92 for a cutoff of the EIS/AD score at 0.40. The distribution of all EIS/AD scores for control, active AD and non-active AD measurements are shown in Figure 2B. When using the mean value of the EIS/AD score for children with multiple measurements in the same category of AD status, the scores for each category, before onset, after AD and active AD

TABLE 1 Demographic factors, early life factors and clinical characteristics of children with and without atopic dermatitis (AD) in the study.

		No AD	AD	*p*-Value
		n = 49 (%)	*n* = 66 (%)	
Sex	Boy	27/49 (55.1)	35/66 (53.0)	0.98
Maternal history of allergic disease[a]	Yes	26/48 (54.2)	40/65 (61.5)	0.55
Paternal history of allergic disease[a]	Yes	19/48 (39.6)	36/65 (55.4)	0.14
Maternal history of AD	Yes	6/48 (12.5)	10/65 (15.4)	0.87
Paternal history of AD	Yes	2/48 (4.2)	12/65 (18.5)	0.04
Siblings	One or more	19/46 (41.3)	22/65 (33.8)	0.55
Mode of delivery	Vaginal delivery	34/48 (70.8)	43/56 (66.2)	0.75
	Caesarean section	14/48 (29.2)	22/56 (33.8)	
Dog or cat ownership during pregnancy	Yes	9/48 (18.8)	10/65 (15.4)	0.83
Antibiotics use up to 4 months	Yes	7/48 (14.6)	17/66 (25.8)	0.17
Breastfeeding at 4 months	None	10/47 (21.3)	9/61 (14.8)	0.46
	Partial	7/47 (14.9)	14/61 (23.0)	
	Exclusive	30/47 (63.8)	38/61 (62.3)	
Breastfeeding at 1 year old	Yes	16/45 (35.6)	24/60 (40.0)	0.79
Any sensitisation[b]	Yes	19/37 (51.4)	22/38 (57.9)	0.74
Any food sensitisation[b]	Yes	11/37 (29.7)	14/38 (36.8)	0.68
Onset of AD	< 4 months		35/65 (53.8)	
	4 months – 1 year old		17/65 (26.2)	
	1–2 years old		9/65 (13.8)	
	2–3 years old		4/65 (6.2)	

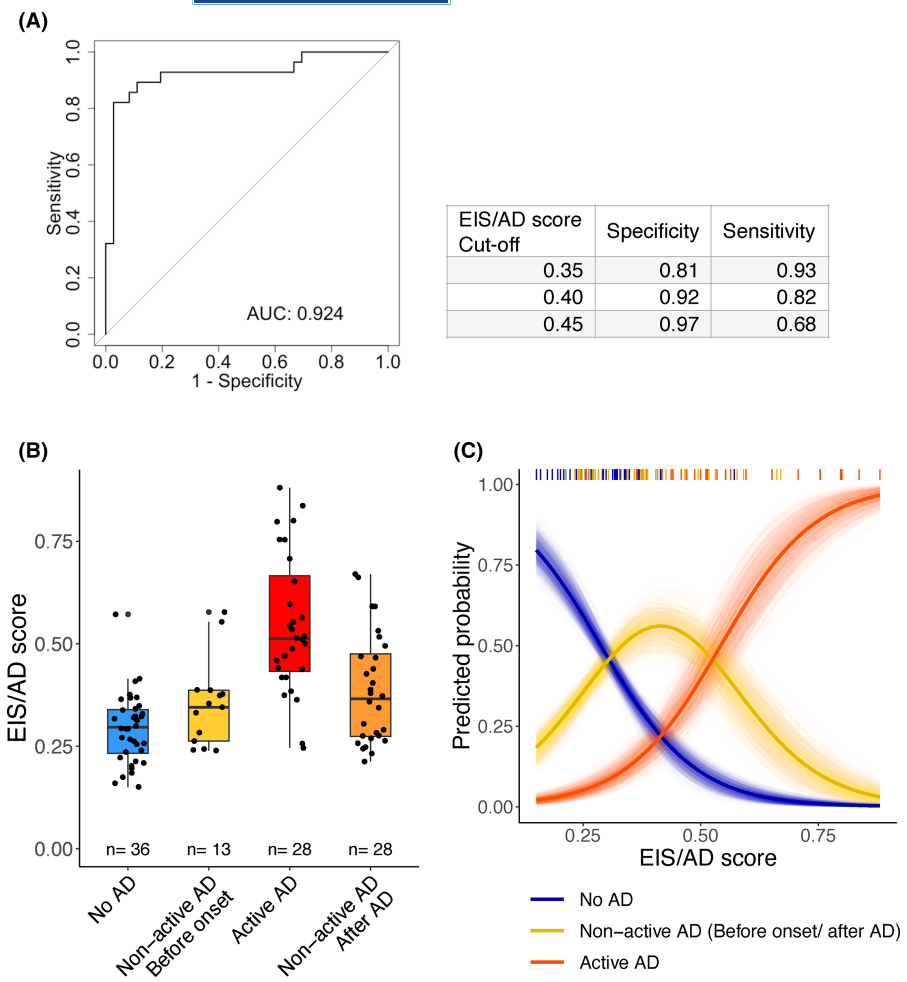
Note: The numbers of children do not add up to total due to missing values.

Note: *p*-value was derived by Fisher's exact test for paternal history of AD, antibiotics use and breastfeeding status at 4 months and by Chi-squared test for the other variables.

Abbreviations: AD, atopic dermatitis.

[a]Maternal or paternal history of atopic dermatitis (AD), asthma, allergic rhinitis or food allergy.

[b]Sensitisation was assessed by skin prick tests at 1, 2 and 3 years old (if followed up to) for the following antigens: wheat, egg, peanut, cow's milk, house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), timothy grass pollen, birch pollen and cat hair. Children who had completed SPT at all relevant timepoints were included here ($n=75$). Children who were sensitised at any time point to the tested allergens are included.



EIS/AD score Cut-off	Specificity	Sensitivity
0.35	0.81	0.93
0.40	0.92	0.82
0.45	0.97	0.68

FIGURE 2 EIS/AD score and its association with AD status. (A) Receiver operating characteristic (ROC) curve describing the performance of the machine learning algorithm to differentiate control (no AD) and active AD measurements, with the specificity and sensitivity for several cutoff values of the EIS/AD score. (B) The distribution of the EIS/AD score of the measurements from children without AD (control) and with AD. Measurements from children with AD were taken when the child had symptoms (active AD), before onset and did not have symptoms (non-active AD, before onset) and after onset but did not have symptoms at the time of measurement (non-active AD, after AD). The number shown on the x-axis is the number of measurements in each category. (C) Prediction plot obtained from 1000 permutated cumulative link models (semi-transparent coloured lines). Solid lines represent median predicted probability of each AD status at a certain value of the EIS/AD score.

measurements were all significantly higher than non-AD measurements (Figure S2, each p-value <.01). ROC curves for the differentiation of active AD vs non-active AD measurements and non-active AD measurements vs control are shown in Figure S3.

Lastly, the permutation cumulative link model indicated that a one standard deviation increase in EIS/AD score (or 0.16 units on the original scale) was typically associated with a 4.94 times increase in the odds of a measurement belonging to a higher rather than lower AD category (active AD vs others and non-active AD vs. no AD, Table S3). Thus, EIS/AD scores can be used to calculate subject-specific probabilities for the three AD categories (Figure 2C). For example, a measurement with an EIS/AD score of 0.25 has median probabilities of 0.60, 0.35, and 0.05 to be non-AD, non-active AD, and active AD, respectively.

3.3 | EIS/AD score, severity of AD, and sensitization status

SCORAD scores of the $n = 28$ active AD measurements (from $n = 21$ children) in the test set ranged from 5 to 38, with a mean and standard deviation of 18 and 7.8. There was no clear correlation between the EIS/AD score and SCORAD ($r_{Pearson} = -0.12$, $p = .56$, Figure 3A).

The distribution of EIS/AD scores did not show a difference between children with and without sensitisation to any of the tested allergens or any food allergens in each category of AD status (Figure 3B,C).

3.4 | EIS/age score and the effect of age on the EIS/AD score

EIS/age score was generated from a separate algorithm to that for the EIS/AD score and represents the probability of a given measurement to be taken at 3 years old. There was a clear difference in the EIS/age score between 4 month and 3-years-old measurements, suggesting that EIS differ by age (Figure 4). Thus, we assessed if there was any effect of age on the EIS/AD score. The difference in the EIS/AD score between no AD and active AD measurements remained when analysed separately for each age group (Figure 5). The difference between no AD and non-active AD measurements and that between non-active AD and active AD measurements were less clear compared to the previous analysis using permutation approach on the whole dataset. The ROC curves for the EIS/AD score for the differentiation of control and active AD measurements had similar AUC using 4 months to 1 year-old and 2- to 3-year-old measurements (Figure S4).

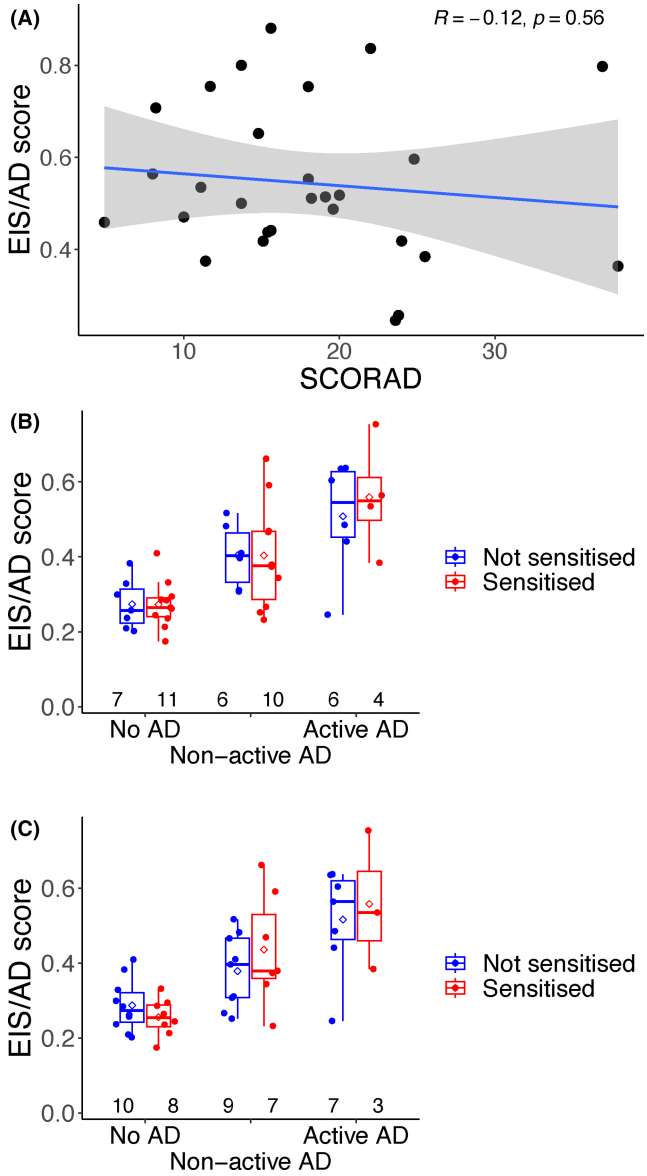


FIGURE 3 EIS/AD score and clinical characteristics. (A) Correlation between EIS/AD score and SCORAD among active AD measurements ($n = 28$ measurements). Solid line represents the linear regression line, and the shaded area describes the 95% CI. p-value was derived by Pearson correlation. EIS/AD score compared by sensitisation status (SPT) to any of the tested allergens (B) and any food allergens (C) among the children who had complete SPT data. The mean EIS/AD score was used if the child had more than one measurement in the same category. The number below the box plot describes the number of children in the category.

3.5 | EIS/AD score and demographic and early life factors

We explored the effects of sex, mode of delivery, family history of allergy, family history of AD, pets, siblings, or antibiotics exposure up to 4 months on the EIS/AD score (Figure S5). There was a marginal difference in the score by maternal allergy, maternal AD and siblings only for non-active AD measurements, which did not remain after adjustment for multiple comparisons. Additionally, we did not find any difference in the EIS/AD score between children with and without the use of any cream or ointment on the measurement site within 12 h before measurement among a subset of children who had this information (Figure S6).

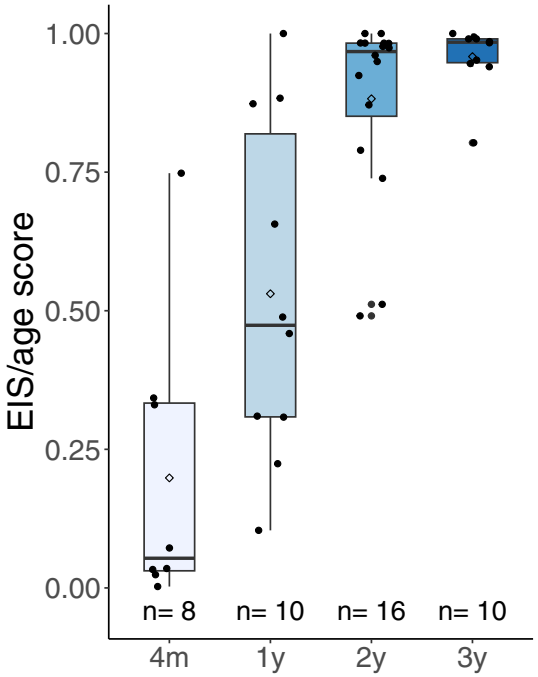


FIGURE 4 The distribution of EIS/age score at each time point. EIS/age score represents the probability that a given EIS measurement was taken at 3y, which was based on a machine learning algorithm that was trained to differentiate 4 months and 3-year-old measurements from children without AD. Measurements from $n = 20$ children without AD were included in the test set for this algorithm and are shown here. The number below the box plot describes the number of children with measurements for each time point.

4 | DISCUSSION

This is the first study showing the ability of EIS to detect skin barrier dysfunction in children, who have developed AD in early life. The score based on the EIS measurements showed a clear difference between healthy skin and clinically unaffected skin from children with active AD, as well as between healthy skin and skin of children with AD when having no active symptoms.

In the previous report using the same technique on adult AD patients, EIS was able to differentiate unaffected AD skin and controls with a higher sensitivity than TEWL.[21] EIS also correlated with serum biomarkers that are associated with inflammatory pathways that influence the epithelial barrier.[21] Our study adds to these findings that EIS is efficient in detecting skin barrier dysfunction or subclinical skin changes in AD in early childhood as well. Our results also suggest the potential further development of this technique to predict future AD by differentiating between skin

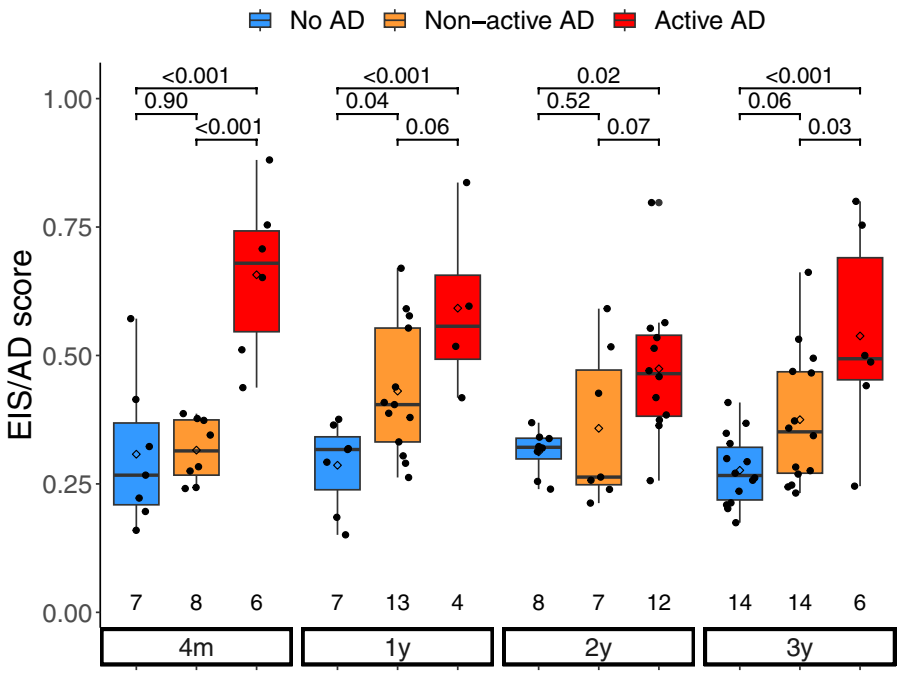


FIGURE 5 EIS/AD score and AD status stratified by age. The number on the x-axis represents the number of measurements (children) included in each category. Statistical analysis was done by ANOVA and posthoc tests, using Benjamini-Hochberg adjustment for all comparisons in the plot.

of healthy children and children with AD even when they did not have active symptoms. Currently, there is no reliable biomarker to predict future AD. Results from studies investigating whether skin barrier function measured by TEWL in early life can predict AD development have shown inconclusive results,[42–45] and although skin barrier dysfunction is a key feature of AD, it is yet unclear if it precedes the development of AD. When using the mean value for EIS/AD scores from the same child in each category, there was a difference in the EIS/AD score between the small number of before onset measurements and healthy skin. Nevertheless, we did not have enough number of measurements that were taken before the onset to test the difference in EIS/AD score separately to those taken after AD for the permutation cumulative link approach across all categories. Likewise, the numbers were small to develop or test an algorithm to differentiate measurements from healthy skin and only before onset. Since EIS detects changes in skin tissue at various depths and captures a large amount of data, it may provide different or additional information regarding the skin barrier to what can be measured by TEWL. Further evaluation using a larger number of EIS measurements taken in early life before the onset of AD will be necessary to confirm if EIS can be used as a predictor of AD development.

We did not observe any correlation between EIS/AD score and the severity of AD, in contrast to the result of the study using EIS among adult AD patients,[21] or studies showing correlation between TEWL and the severity of AD.[14–16] One possible reason for this is that most children in our cohort had a mild form of AD with only a few measurements taken when SCORAD was higher than 25. This may have limited the analysis of the correlation, as well as training of the algorithm to detect differences in severity. More importantly, the information used in the algorithm development to categorize the measurements was only the AD status (whether the measurement is from a child with active AD or not) without severity or other information. This was because the limited sample size and characteristics did not allow a complex model, for example, one that is trained on categories such as mild, moderate, and severe AD. Thus, the current algorithm output (EIS/AD score) reflects the probability of the measurement coming from a child with active AD, but it does not necessarily correlate with the severity, or the degree of skin barrier dysfunction. This may also be the reason for the lack of difference in EIS/AD score by sensitisation status, while increased TEWL has previously been associated with allergen sensitisation.[46,47] Sensitisation status was similar between children with and without AD among our study population, which also contributed to this result. However, it is worth noting that EIS was able to detect a difference even among children with mostly mild disease with possibly small differences in the skin barrier compared to healthy skin.

Newborns have differences in various skin functions compared to adults, and the adaptation occurs in the first few years of life.[48,49] Although we observed difference in EIS when comparing 4 month and 3-year-old measurements in children without AD, the performance of the algorithm for the EIS/AD score did not seem to be greatly affected by age. This suggests that the algorithm for the EIS/AD score correctly detected differences in EIS due to age and was trained to overlook these differences and only look at the differences due to AD, because of the balanced age distribution between the two groups in the dataset. The difference in the EIS/AD score between non-active AD and the other two categories becoming less clear when stratified by age compared to the permutation analysis using the whole dataset, is most likely due to the smaller sample size. However, the direction of association was mostly consistent across age groups.

Finally, we did not find any clear effect on the EIS/AD score by demographic and early life factors, such as family history of allergic disease, siblings, mode of delivery, pet ownership or antibiotics exposure,

which are potential risk factors for developing AD. This is also likely to be influenced by the similar distribution of these factors between the AD and no AD group of our study population and does not rule out the possibility that these factors affect EIS or the skin barrier.

According to the epithelial barrier theory, many toxins and chemicals that humans are exposed to daily damage the epithelium on the surface of our skin, lungs, vagina, and intestine.[50] Defective epithelial barrier has been demonstrated in a wide range of allergic and autoimmune conditions.[51] Microbiota which normally float above the skin and mucosa goes deeper between and beneath damaged epithelial barrier, and colonization of opportunistic pathogens cause microbial dysbiosis. Overall, the local immune response skews to a type 2 immune response and inflammation.[52–54] The continuous and robust epithelial barrier detection is important in many conditions linked to early life exposure.

One of the major strengths of our study is that we have tested the performance of the algorithms among a testing dataset which was sampled from the same dataset but held separate to that used for training the algorithm. This approach has ensured better external validity of the results, as opposed to just using a subset of the training set to assess the performance. Secondly, we have performed EIS measurements from multiple time points during early childhood. This has allowed us to obtain measurements before the child developed AD, as well as investigating the effect of age on EIS. The longitudinal method of data collection has also minimized the possibility of recall bias and selection bias. There are several limitations of our study. We were not able to assess the correlation between EIS measurements and TEWL, FLG gene mutations, markers of inflammation, other skin barrier parameters or histological changes of the skin, due to the lack of such data. Misclassification of AD due to the lack of follow up to assess the chronicity of the disease or response to treatment that usually occurs in clinical practice may have led to an overdiagnosis of AD. However, we have tried to minimize this bias by defining AD based on the clinical diagnosis by trained paediatric allergists. The mild characteristics of the AD among the study population together with this potential overestimation of AD may partly explain the similarity between the children with and without AD, in terms of the proportion of children who were sensitized or with known risk factors of AD. This may have led to the estimation of the true difference in EIS between healthy skin and active AD skin to be lower. As mentioned previously, the sample size may have also limited our investigation into the association between EIS, clinical characteristics and other risk factors. We combined the small number of before onset measurements with after AD measurements as non-active AD measurements to achieve a balanced group size for statistical analysis. Nevertheless, there is currently no evidence that skin barrier function is similarly affected in these two situations, thus further studies are warranted. The use of cream or ointment did not show influence on the EIS/AD score. However, this also needs further investigation since the effect was only assessed among a subset of measurements which included very few children using any cream or ointments. Finally, using machine learning has allowed us to analyse EIS measurements which contain a large amount of information about the skin barrier or skin tissue. However, the interpretation of the output needs to take into account the probabilistic nature of this approach.

In conclusion, we were able to show that EIS can detect skin barrier dysfunction in children with AD, including when they did not have active symptoms. Whether EIS can be used as a predictor of AD development, by itself or in combination with other known genetic or environmental risk factors is an interesting topic of future research.

AUTHOR CONTRIBUTIONS

RL, ReF, CR, CAA and the CK-CARE study group were involved in the conception of the study and RL, ReF and CR designed the study. CAA was involved in the development of the EIS method to assess the skin barrier. RuF, KNH and CR were involved in collecting the data. MSu developed the machine learning algorithms. MSa and EPW performed the statistical analysis. MSa wrote the manuscript. All authors contributed to data interpretation, critically appraised the manuscript, and approved the final version.

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CONFLICT OF INTEREST STATEMENT

M. Sundberg is an employee of SciBase AB. C. A. Akdis has received research grants from the Swiss National Science Foundation, European Union (EU CURE, EU Syn-Air-G), Novartis Research Institutes, (Basel, Switzerland), Stanford University (Redwood City, Calif), Seed Health (Boston, USA) and SciBase (Stockholm, Sweden); is the Co-Chair for EAACI Guidelines on Environmental Science in Allergic diseases and Asthma; Chair of the EAACI Epithelial Cell Biology Working Group is on the Advisory Boards of Sanofi/Regeneron (Bern, Switzerland, New York, USA), Stanford University Sean Parker Asthma Allergy Center (CA, USA), Novartis (Basel, Switzerland), Glaxo Smith Kline (Zurich, Switzerland), Bristol-Myers Squibb (New York, USA), Seed Health (Boston, USA) and SciBase (Stockholm, Sweden); and is the Editor-in-Chief of Allergy. The others have nothing to declare within the scope of this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

CK-CARE Study Group

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