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Original article

Transepidermal water loss measurement during infancy can predict the subsequent development of atopic dermatitis regardless of filaggrin mutations

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Abbreviations:
AD, atopic dermatitis; AU, arbitrary units; CI, confidence interval; FLG, filaggrin; HR, hazard ratio; IRB, institutional review board; NCCHD, National Center for Child Health and Development; SCH, stratum corneum hydration; SD, standard deviation; TEWL, transepidermal water loss

ABSTRACT

Background: Atopic dermatitis (AD) is characterized by skin barrier dysfunction. Few studies have used noninvasive techniques to measure epidermis function in asymptomatic neonates.
Methods: Data of 116 infants from our previous randomized controlled study were analyzed. Skin barrier function was measured through transepidermal water loss (TEWL), stratum corneum hydration (SCH), and pH. The association between skin barrier function and time to AD development was evaluated. Patients were classified with high or low TEWL, and SCH and pH were assessed. The survival function of the time to AD development and hazard ratios were estimated. Allergic sensitization to egg white and ovomucoid at 32 weeks was assessed.
Results: Regardless of a filaggrin mutation, TEWL (optimal cutoff, 6.5 g/m^2/h) of the forehead within the first week of life showed a lower p-value than TEWL of the leg, and the SCH and pH measurements. Baseline TEWL of the forehead was not different between groups, except for the mean gestational age, and it was not affected by humidity. We found a significant difference in the cumulative AD incidence between the high and low TEWL groups for the forehead only ($p < 0.05$). The probability without AD was lower in the high TEWL group than in the low TEWL group. For only the high TEWL group, AD development decreased significantly with daily emollient use. The high TEWL group exhibited a higher rate of sensitization to ovomucoid ($p = 0.07$).
Conclusions: TEWL of the forehead during the first week of life is associated with AD development.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that is characterized by epidermal barrier dysfunction.[1] AD in early infancy is thought to contribute to the development of subsequent allergies, also known as atopic march.[2,3]

A family history of AD and being a carrier for a filaggrin (FLG) gene mutation are risk factors for AD, bronchial asthma, and food allergies.[4–6] *FLG* is a keratin-aggregating protein and plays a major role in the function of the epidermis.[5,6] Previous studies have indicated that allergy development may be associated with a damaged epidermal barrier.[7]

In recent years, noninvasive skin assessment strategies such as measurements of transepidermal water loss (TEWL), stratum

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corneum hydration (SCH), and pH have been used even in infants.[8,9] *FLG* mutations are associated with increased TEWL, which reflects skin barrier dysfunction.[10] Moreover, a recent study by Kelleher *et al.* showed that increased TEWL on the forearm at 2 days and 2 months of age predicted AD development at 1-year-old.[11] Although several studies using noninvasive skin assessment approaches measuring TEWL, SCH, or pH in infants successfully analyzed skin barrier function,[8,9] Kelleher *et al.*'s study only used TEWL measurements on the forearm.[11] Skin barrier function values differ by different body areas[12]; thus, it is possible that the same measurement method reveals different results depending on where the measurement is performed. Moreover, although a correlation between the FLG mutation and allergic sensitization was reported,[13,14] it is unclear if skin barrier function in neonates predicts sensitization to food allergens.

We previously reported the findings of a randomized controlled parallel-group study that indicated that the daily application of emollients in neonates reduced AD risk until 32 weeks of life.[15] In this study, we performed a retrospective post-hoc analysis of the dataset of the same participants. We assessed whether measuring skin function during the neonates' first week of life using several noninvasive skin function tests (TEWL, SCH, and pH) could predict AD development. Furthermore, we determined whether increased TEWL within the first week of life predicted a risk of increased sensitization to egg white and ovomucoid at age 32 weeks.

Methods

Participants and study design

We performed a retrospective post-hoc analysis of prospectively collected data from our previous trial.[15] A total of 116 neonates with a high risk of developing AD, as measured by noninvasive skin measurement techniques within the first 7 days of life, were analyzed. Skin barrier function was measured through TEWL, SCH, and pH. Written informed consent was obtained from the infants' parents before delivery. Our study was approved by the institutional review board of the National Center for Child Health and Development (NCCHD).

Atopic dermatitis diagnosis and skin barrier function measurement

AD was diagnosed by the same blinded dermatologist during the participants' scheduled visits, according to the criteria established in our previous trial.[15] Skin barrier function was tested in neonates within the first 7 days of life (mean ± standard deviation [SD], 3.24 ± 1.37 days). The following noninvasive devices were used: Vapo Meter, SW-4002 (Delfin Technologies, Kuopio, Finland) for TEWL, Moisture Meter, SC-5 (Delfin Technologies) for SCH, and Skin-pH-Meter PH905 (Courage & Khazaka Electronic GmbH, Köln, Germany) for pH. The TEWL and SCH values were measured on the outer side of the lower leg and the forehead, and pH was measured on the cheek. We calculated the median value of three repeated measurements. All measurements were conducted while neonates were resting and not crying, and before any emollients were applied. The room temperature was maintained between 24 °C and 27 °C, and humidity ranged between 11% and 58% (mean ± SD, 38.9 ± 8.31%).

Assessment of food allergen sensitization

Serum egg white- and ovomucoid-specific immunoglobulin E (IgE) levels were measured by using a diamond-like carbon chip with high-density allergen immobilization and high sensitivity.[16,17] After confirming the correlation between the datasets, specific IgE values were converted to CAP-FEIA equivalents.[15,16] The cutoff levels for allergic sensitization were set at 0.35 or 0.70 kUA/L CAP-FEIA equivalents.

Filaggrin mutation analysis

The primer sets for the representative FLG gene mutations found in the Japanese population with AD (p.R501*, p.S2889*, p.S3296*, c.3321delA, p.Q1701*, p.S2554*, and p.K4022*) were used. FLG mutations were genotyped using TaqMan analysis (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA), as described previously.[6,15,18]

Statistical analyses

We used log-rank tests to determine the cut-off points of the skin test (TEWL, SCH, and pH) values measured within the first week of life to evaluate the time to AD development until 32 weeks. Data derived from the skin tests were converted to binary data, allowing us to classify neonates with high or low values, based on an entire series of cutoff values. The cutoff value providing the minimum p-value was considered as the best cutoff value for each test. After determining the cutoff values to classify patients with high or low values, the survival function of the time to AD development was estimated by the Kaplan–Meier method, and the hazard ratio (HR) was estimated by a Cox regression model to assess the association between the skin tests and AD development. Moreover, univariate comparisons were conducted using the chi-square and Mann–Whitney U tests, as appropriate. In addition, groups were further subdivided into two groups based on the use of emollients (daily vs. as needed). SPSS for Windows (version 18.0; SPSS Inc.; Chicago, IL, USA) and the R software (version 3.1.2, http://www.R-project.org) were used for statistical analyses, and $p < 0.05$ was regarded as statistically significant.

Results

In our previous randomized controlled trial,[15] 118 neonates were recruited at the NCCHD (Tokyo, Japan) within 7 days of birth from November 2010 to November 2013. These infants were randomly allocated to two groups: an intervention group receiving daily emollient applications, and a control group receiving emollient applications as needed during the first 32 weeks of life. Significantly fewer infants who received daily emollient applications developed AD than those who received emollient applications as needed. In this post-hoc study, we analyzed data of 116 infants (58 and 58 in the intervention and control group respectively; Supplementary Table 1). One infant was excluded because skin function was not measured by noninvasive devices within the first week of life, and another infant was withdrawn from the study before the skin function examination could be conducted because of a hemangioma.

The log-rank test for TEWL, SCH, and pH values within the first week of life and cumulative AD incidence rates at age 32 weeks showed that TEWL measurements on the forehead were more useful than the other methods (Fig. 1). Thus, only these measurements were used for further analyses. Participants were then divided into two groups: high TEWL (TEWL $\geq$6.50 g/m^2/h; n = 71) and low TEWL (TEWL <6.50 g/m^2/h; n = 45). Besides the mean gestational age, we did not observe any difference in baseline parameters between groups (Table 1).

The log-rank test showed significant differences in cumulative AD incidences between the high and low TEWL groups ($p < 0.05$, log-rank test; HR: 2.00; 95% confidence interval [CI]: 1.05–3.80; Fig. 2). In the control group (in which emollients were only applied

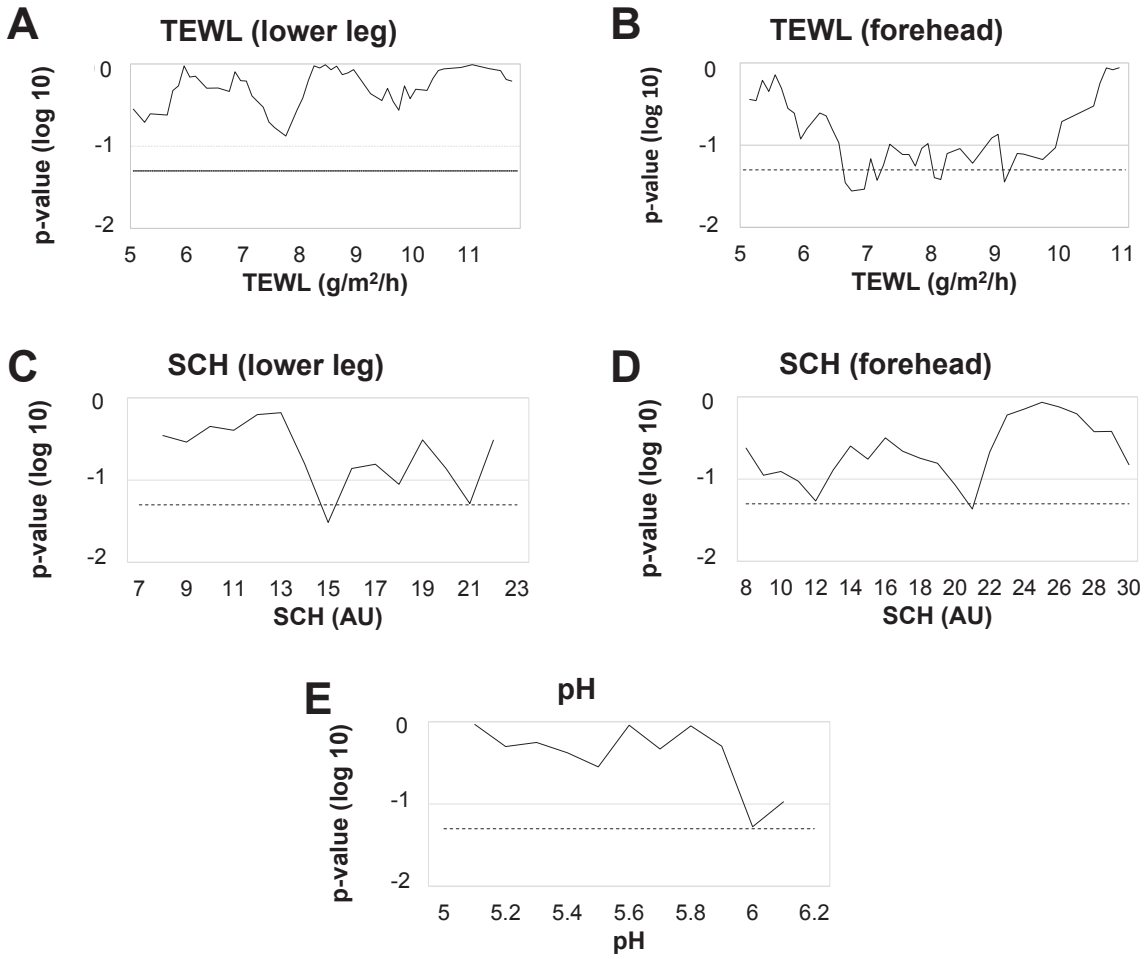

Fig. 1. Correlation between noninvasive skin measurement results and AD development by age 32 weeks. Using the log-rank test, we calculated the p-values for all skin barrier function values measured via transepidermal water loss (TEWL), stratum corneum hydration (SCH), and pH, and the cumulative AD incidence rates at age 32 weeks. **A**, TEWL on the lower leg, 5.0–11.7 g/m^2/h; **B**, TEWL on the forehead, 4.9–10.7 g/m^2/h; **C**, SCH on the lower leg, 8.0–22.0 AU [arbitrary units]; **D**, SCH on the forehead, 8.0–30.0 AU; and **E**, pH on the cheek, 5.1–6.1. The measurements show a significant difference in TEWL measured on the forehead (6.4, 6.5, 6.7, 6.9, 7.8, 7.9, and 8.9 g/m^2/h), SCH on the lower leg (15 AU), and SCH on the forehead (21 AU). TEWL measured on the forehead within the first 7 days of life shows a stronger and more statistically significant correlation with cumulative AD incidence by age 32 weeks than SCH and pH (dashed line, p = 0.05). Based on the results of the log-rank test, the optimal cutoff value for TEWL measured on the forehead is 6.50 g/m^2/h. AD, atopic dermatitis; AU, arbitrary units.

as needed), we observed a statistically significant difference in the risk for developing AD over time between infants in the high and low TEWL groups ($p < 0.05$, log-rank test; HR: 2.65; 95% CI: 1.16–6.06; Fig. 3A). In contrast, no significant difference between the high and low TEWL groups was seen in the intervention group (in which emollients were applied to all infants daily) ($p = 0.368$, log-rank test; HR: 1.60, 95% CI: 0.57–4.49; Fig. 3B). For only the low TEWL group, the log-rank test did not show a significant difference in the cumulative AD incidence between daily emollients and the as needed groups (data not shown).

Lastly, we evaluated the rate of infants that developed allergic sensitization to egg white and ovomucoid in the high and low TEWL groups (using IgE cutoff levels of ≥0.35 and ≥0.70 kUA/L). No significant differences were observed for allergic sensitization to egg white; however, a higher rate of ovomucoid sensitization was seen in the high TEWL group when compared to the low TEWL group ($p = 0.07$ and $p = 0.13$ for cutoff levels of ≥0.35 kUA/L and ≥0.70 kUA/L, respectively; chi-square test; Table 2). The rate of egg white and ovomucoid sensitization between the daily application with emollients and as needed groups was not significantly different (data not shown).

Discussion

To the best of our knowledge, this is the first report to show that TEWL is superior to other noninvasive skin measurements in predicting the development of AD in early infancy. Our study also showed that the daily use of emollients in infants with barrier dysfunction, as estimated by an increased TEWL on the forehead, was associated with a significantly lower risk of developing AD. Asymptomatic infants with increased TEWL in the first week of life were more likely to develop subsequent sensitization to ovomucoid, although this difference was not statistically significant.

Individuals with elevated TEWL are often carriers of *FLG* mutations.[10] TEWL is defined as the insensible water depletion from inside the body via the epidermal layer, which reflects skin barrier function.[19] Meanwhile, although an FLG null mutation, which is associated with epidermal barrier dysfunction, is a risk factor for AD,[10] approximately 54% of FLG null mutation carriers in the United States have never developed AD symptoms.[20] Profilaggrin, the precursor of *FLG* monomers, is cleaved by proteolysis enzymes (e.g., bleomycin hydrolase [BLMH], calpain-1, and caspase-14) and processed into matured *FLG*.[21] BLMH downregulation has been shown

Table 1
Patients' characteristics.

	High TEWL group (n = 71)	Low TEWL group (n = 45)	*p*-value
Intervention, no. (%)	36/71 (50.7)	22/45 (48.9)	NS
Sex (female), no. (%)	31/71 (43.7)	19/45 (42.2)	NS
Mean age of mothers at delivery (y)	35.3 ± 5.09	35.5 ± 4.48	NS
Cesarean section, no. (%)	14/71 (19.7)	14/45 (31.1)	NS
Mean gestational age (wk)	39.3 ± 0.96	38.8 ± 1.0	<0.01
Mean birth weight (g)	3004 ± 356	3129 ± 374	NS
Breastfeeding at age 1 month, no. (%)	33/70‡ (47.1)	23/44‡ (52.3)	NS
Mean no. of siblings	0.29 ± 0.52	0.47 ± 0.69	NS
Born in autumn, no. (%)†	21/71 (29.6)	16/45 (35.6)	NS
Family history			
Food allergy, no. (%)	27/71 (38.0)	17/45 (37.8)	NS
Bronchial asthma, no. (%)	23/71 (32.4)	21/45 (46.7)	NS
Allergic rhinitis, no. (%)	57/71 (80.3)	35/45 (77.8)	NS
Environmental exposures			
Smoking in the family, no. (%)‡	10/69 (14.5)	7/45 (15.6)	NS
Pets, no. (%)‡	15/70 (21.4)	9/45 (39.1)	NS
Dog, no. (%)	8/70 (11.4)	5/45 (11.1)	NS
Cat, no. (%)	4/70 (5.7)	2/45 (4.4)	NS
FLG mutation, no. (%)‡	4/34 (10.8)	3/26 (11.5)	NS
p.R501*	0/34 (0.0)	0/26 (0.0)	NS
p.S2889*	1/34 (2.9)	1/26 (3.8)	NS
p.S3296*	1/34 (2.9)	0/26 (0.0)	NS
c.3321delA	1/34 (2.9)	1/26 (3.8)	NS
p.Q1701*	1/34 (2.9)	0/26 (0.0)	NS
p.S2554	0/34 (0.0)	1/26 (3.8)	NS
p.K4022*	0/34 (0.0)	0/26 (0.0)	NS

No., number; NS, not significant; TEWL, transepidermal water loss; wk, week; y, years.
* Nonsense mutation according to nomenclature for the description of sequence variants in Human Genome Variation Society.
† Autumn was defined as October to December.
‡ We were not able to acquire all data.

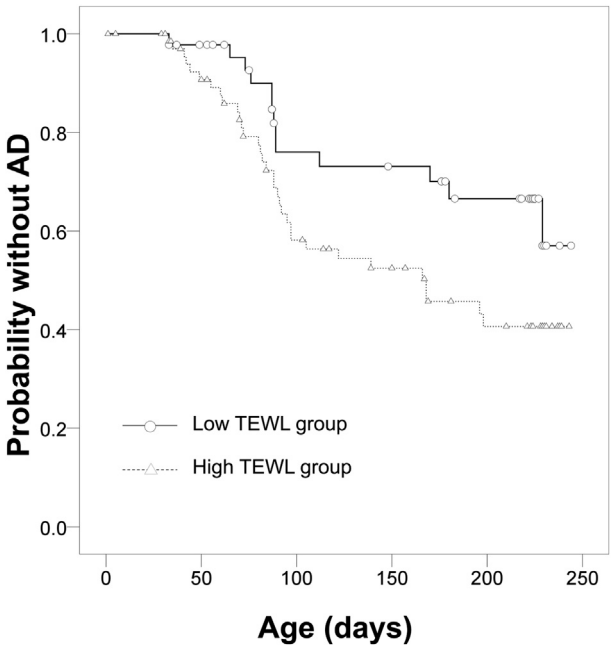

Fig. 2. Kaplan–Meier curves for AD development by age 32 weeks. The probability of AD incidence stratified by TEWL (measured on the forehead) is shown. The high TEWL group (TEWL ≥6.50 g/m^2/h) shows a higher AD incidence than the low TEWL group (TEWL <6.50 g/m^2/h) (log-rank test, p = 0.031). AD, atopic dermatitis; TEWL, transepidermal water loss.

to be associated with a reduction in amino acid production in the epidermis of AD subjects.[22] This indicates that although FLG is a key protein related to epidermis function, various factors are involved in the development of skin barrier dysfunction. Although a previous study showed that the *FLG* mutations status was associated with TEWL by the age of 3 months,[10] a more recent larger-scale study indicated no relationship between FLG mutation and TEWL during the first 2 days of life.[11] Although we could not acquire the FLG mutation data of all study participants, we did not detect a significant difference in TEWL values between FLG mutation carriers and non-carriers during the first 7 days of life (TEWL values of FLG mutation carriers vs. non-carriers, mean ± SD, 6.30 ± 1.77 vs. 8.18 ± 5.22, respectively; Mann–Whitney U test; p = 0.18).

We observed a statistically significant difference in the cumulative AD incidence between infants in the high and low TEWL groups for those who received emollients only as needed. In infants who received emollients daily, those with increased TEWL did not have a significantly different cumulative AD incidence than those with low TEWL. Because it is assumed that infants in the low TEWL group naturally have less barrier dysfunction, preventing AD in early infancy by topically administering emollients may explain why sustained skin barrier function is maintained.

Our data showed that noninvasive skin measurement techniques, in particular measurements of TEWL on the forehead, can contribute to identifying neonates with a high risk of developing AD and thus may be a potential future AD prevention strategy. Because the TEWL value can possibly increase depending on the infant's emotional state, we performed all measurements of TEWL while neonates were resting and not crying. We mentioned that the results should be carefully interpreted; however, AD in infants often develops on the face.[23] Thus, it is possible that TEWL values of the forehead are considered more useful than those of the legs. Concerning the practical guidelines for measuring TEWL, a climate-controlled room is recommended, if available.[19] In our study, we did not have access to such a room; hence, the relative humidity in the room where testing was conducted was affected by the season (data not shown). However, we observed no significant effect of the relative humidity on TEWL measured on the forehead (data not shown).

A recent report showed that TEWL at 2 days or 2 months of age predicted AD development at 1 year of age[11]; this is in line with our findings. However, this study did not report on subsequent allergic sensitization and the use of emollients. A significant positive correlation between increased TEWL and increased sensitization to aeroallergens was found in another previous cross-sectional study.[3] Eczema during infancy has been shown to be associated with the subsequent development of bronchial asthma,[24] allergic rhinitis,[25] and food allergies[26] called atopic march.[2] This is likely caused by eczematous sensitization. We found that infants with a high TEWL at baseline had a higher (but not significant) prevalence of serum anti-ovomucoid IgE sensitization when compared to infants with a low TEWL. TEWL in preterm neonates was shown to be higher than that in term neonates,[27] suggesting that emollient application may be more important in premature neonates. A recent study reported that the application of emollients in preterm neonates starting at birth reduced the risk of infection and mortality, which supports this notion.[28] However, further studies are necessary to address the benefit of emollient application in preterm neonates.

Our study was limited by the inherent limitations of a post-hoc analysis. Significant differences in mean gestational age were found between the high and low TEWL groups at baseline. The reason for this difference is unclear. Further randomized trials using a prospective intervention study design are needed to confirm the association between TEWL measurements at birth and subsequent

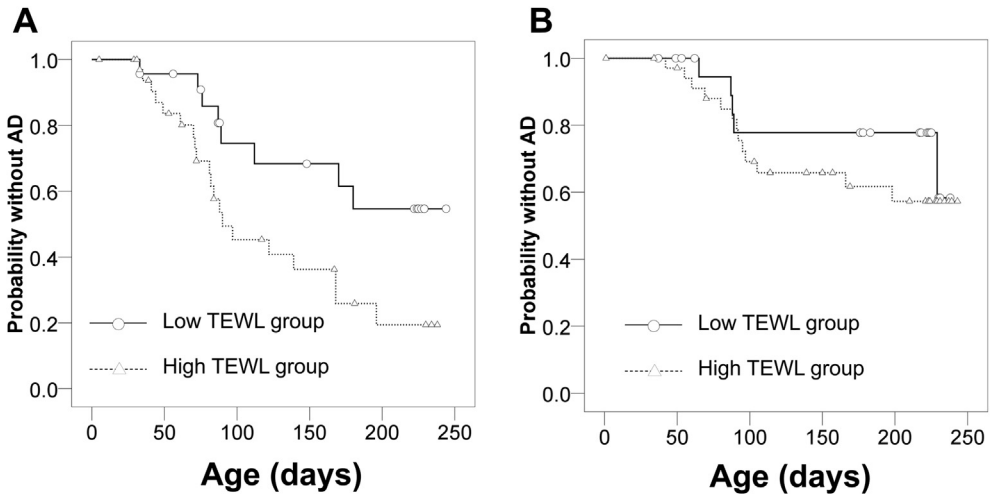

Fig. 3. AD development and emollient use. In the control group, in which emollients were administered only when needed, the high TEWL group is more likely to develop AD by age 32 weeks than the low TEWL group (**A**, log-rank test, p = 0.016). In contrast, no significant differences are observed between the high and low TEWL groups in the intervention group, in which emollients were administered daily to all infants (**B**, log-rank test, p = 0.368). AD, atopic dermatitis; TEWL, transepidermal water loss.

Table 2
Allergic sensitization at age 32 weeks between the high and low TEWL groups.

Specific IgE levels	TEWL on the forehead		*p*-value
	High TEWL group (n = 53)	Low TEWL group (n = 38)	
Egg white (kUA/L)			
≥0.35	43.4%	44.7%	1.00
≥0.70	43.4%	39.5%	0.83
Ovomucoid (kUA/L)			
≥0.35	18.9%	5.3%	0.07
≥0.70	13.2%	2.6%	0.13

IgE, immunoglobulin E; TEWL, transepidermal water loss.

AD development. Another limitation of this study was the lack of complete data for FLG null mutation status. The final limitation of our study was that only individuals with a high-risk family history of AD were included in our analysis, implying that our findings may not be generalizable to the general population. Although our data need to be validated by future larger-scale prospective randomized studies, the results of our post-hoc analysis will be useful for developing suitable interventions for AD prevention.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alit.2015.09.004.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

KH, HN, KMa, YO and HS designed the study. KH, KMo, MN, MK, SK, EI, TS, HN, YO and HS were responsible for data acquisition. KH, EI and HS performed the statistical analysis and interpretation of the results. KH, EI, KMa and HS drafted the manuscript. KH, MN, KMa, YO and HS revised the manuscript critically for important intellectual content. All authors read and approved the manuscript.

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