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Sensitive skin is highly frequent in extrinsic atopic dermatitis and correlates with disease severity markers but not necessarily with skin barrier impairment

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ABSTRACT

Background: Sensitive skin is a condition of cutaneous hypersensitivity to environmental factors. Lactic acid stinging test (LAST) is commonly used to assess sensitive skin and composed of four distinct sensations (pain, burning sensation, itch, and crawly feeling). A link between sensitive skin and barrier dysfunction has been proposed in atopic dermatitis (AD) patients. However, clinical and laboratory factors that are associated with sensitive skin remain unelucidated.

Objective: To investigate relationship between sensitive skin and AD-associated markers.

Methods: Forty-two Japanese AD patients and 10 healthy subjects (HS) were enrolled. AD patients were divided into extrinsic (EAD; high IgE levels) and intrinsic (IAD; normal IgE levels) types. We conducted 1% LAST by assessing the four distinct sensations and calculated the frequencies of sensitive skin in EAD, IAD, and HS. We also performed clinical AD-related tests, including transepidermal water loss (TEWL), visual analogue scale (VAS) of pruritus, and quality of life, and measured laboratory markers, including blood levels of IgE, CCL17/TARC, lactate dehydrogenase (LDH) and eosinophil counts, and concentration levels of serum Th1/Th2 cytokines. *Filaggrin* (*FLG*) mutations were examined in 21 patients. These values were subjected to correlation analyses with each of the four sensation elements.

Results: According to the standard criteria for LAST positivity, the frequencies of LAST-positive subjects were 54.8% and 10.0% in AD and HS, respectively ($P=0.014$). EAD patients showed a significantly ($P=0.026$) higher frequency of positive LAST (65.6%) than did IAD patients (20.0%). Among the four LAST sensation elements, the crawly feeling and pain scores positively correlated with VAS of pruritus, total serum IgE, mite-specific IgE, CCL17/TARC, and/or LDH. There was no association of the LAST scores with serum Th1/Th2 cytokine levels. Notably, neither TEWL nor *FLG* mutations correlated with LAST positivity or any sensation scores.

Conclusions: The frequency of sensitive skin is higher in EAD than in IAD. Sensitive skin is associated with AD severity, but not necessarily with barrier condition.

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Abbreviations: AD, atopic dermatitis; EAD, extrinsic AD; IAD, intrinsic AD; LAST, lactic acid stinging test; TEWL, transepidermal water loss; VAS, visual analogue scale; TARC, CCL17/thymus and activation-regulated chemokine; LDH, lactate dehydrogenase; *FLG*, filaggrin; HS, healthy subjects; DF, dermatophagoides farina; DP, dermatophagoides pteronyssinus; QOL, quality of life; SCORAD, SCORing AD; STAI, state-trait anxiety inventory.

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1. Introduction

Sensitive skin is characterized by the sensations, such as tingling, pricking, heat, burning, pain, or itch with or without erythema. Because of its subjective feeling property, self-assessment may be one of the valuable methods for evaluation of sensitive skin. However, more accurate parameters or objective factors are required for its evaluation [1]. Lactic acid stinging test (LAST) has been widely accepted as an assessment of sensitive skin

[2], although the percentage of lactic acid and the evaluation method are different among studies. In addition to healthy subjects (HS), patients with atopic dermatitis (AD) have been investigated in the frequency and extent of sensitive skin. It was reported that sensitive skin is more common in AD patients than HS [3,4].

AD patients have impaired *stratum corneum* barrier as represented by filaggrin (FLG) deficiency mainly caused by FLG mutations [5]. FLG has an important role in keratinocyte differentiation and skin barrier function. Meanwhile, AD can be classified into two distinct types, extrinsic and intrinsic AD [6]. While extrinsic AD (EAD) shows a high total serum IgE level and an impaired barrier function, intrinsic AD (IAD) has a normal total IgE value with relatively preserved barrier function [7]. The ratio of IAD is approximately 20% with female predominance [6]. We have previously shown that the frequencies of circulating interleukin (IL)-4 or IL-5-positive T helper 2 (Th2) cells in both EAD and IAD were higher than those in HS, and there was no significant difference in them between EAD and IAD [8]. On the other hand, the frequency of interferon- γ (IFN- γ)-positive Th1 cells was significantly higher in IAD than in EAD, suggesting that Th1 cells play a more important role in IAD than in EAD [8].

Thus, it is likely that the sensitive skin is seen in EAD at a higher rate than in HS or IAD possibly due to Th2 cell-induced barrier disruption. In fact, some studies suggested a link of sensitive skin to disruption of the epidermal barrier [9–11]. However, the relationship between each sensation element of sensitive skin and AD severity/barrier markers remains unclear.

In this study, we conducted 1% LAST in AD patients and HS to investigate whether the four distinct sensations are related to AD-associated parameters, including transepidermal water loss (TEWL), visual analogue scale (VAS) of pruritus, quality of life (QOL), peripheral blood levels of IgE, CCL17/thymus and activation-regulated chemokine (TARC), lactate dehydrogenase (LDH), eosinophil counts and Th1/Th2 cytokines, and presence or absence of FLG mutations.

2. Materials and methods

2.1. Participants

Forty-two Japanese patients with AD (aged 31–66 years; 23 men and 19 women), and 10 HS (aged 30–75 years; 6 men and 4 women) were enrolled in this study. AD was diagnosed according to the criteria of Hanifin and Rajka classification [12], and was divided into intrinsic and extrinsic types as previously reported [13]. Intrinsic AD showed serum IgE levels ≤ 200 KU/L or $200 < \text{IgE} \leq 400$ with class 0 or 1 of IgE specific to *Dermatophagoides* (D) *pteronyssinus* (DP) or *D. farinae* (DF). Extrinsic AD showed $400 < \text{IgE}$ levels or $200 < \text{IgE} \leq 400$ with class 2 or more of the specific IgE [13]. All the patients were seen as outpatients or inpatients in April 2015 to January 2016 at Department of Dermatology, Hamamatsu University School of Medicine. This study was conducted according to Declaration of Helsinki Principles, performed after obtaining an informed consent from both the patients and normal volunteers, and approved by the medical ethical committees of Hamamatsu University School of Medicine (No. E-14-126-1).

2.2. AD severity, skin barrier condition, and QOL

Clinical severity of AD was assessed by SCORing AD (SCORAD) index [14,15], and current itch was rated on a 100-mm VAS. Barrier condition of *stratum corneum* of the cheeks, which were the same sites of LAST examination, was assessed by TEWL [7], and was measured by Mobile Tewameter (TM300MP, Integral, Tokyo, Japan). Serum levels of CCL17/TARC, total IgE, IgE specific for DP

and DF, LDH, and peripheral blood eosinophil counts were also assessed in routine clinical work-up.

Skin disease-specific QOL was assessed by Japanese version of skindex-16 [16], consisting of 16 items with each rated on 7-point Likert scale in three scales (symptoms, emotions, and functioning). Each of 16 items was quantified using a linear scale of 100 ranging from 0 to 100. To assess the anxiety, Japanese version of State-Trait Anxiety Inventory (STAI) was completed. The STAI was developed to reveal state and trait anxiety. State anxiety and trait anxiety consist of 20 items with each rated on 4-point Likert scale. The scores can range from 20 to 80, and higher scores mean more anxiety. The STAI form used in our study is the translational version of STAI FORM X [17].

2.3. 1% lactic acid stinging test (LAST)

After cleansing the face with face wash and water, and subsequent gentle drying with tissues, the subjects rested for 15 min in an environment-controlled room (temperature 23.3–27.8 °C, relative humidity 40–50%). Distilled water (DW) was then applied to the left or right side of the cheek by rubbing with cotton bud (4.0 cm $\times$ 4.0 cm). After this step, the subjects reported the types of discomfort (pain, burning sensation, itch, crawly feeling) and their strength (0 to 3 points, increments of 0.5) at three different time points (30 s, 2.5 min, and 5 min) after application of DW according to Discomfort sensation scale for severity assessment (Supplementary Table S1) [18]. Next, 1% lactic acid was applied to the other side and reported the types and strength of discomfort as well. Finally, the maximum score from the three time points for each type of discomfort was analyzed. The difference in one of the maximum discomfort score between 1% lactic acid and DW was defined as LAST score. When the LAST score was ≥ 1.0 , subjects were classified as stingers group (positive), and non-stingers (negative) showed ≤ 0.5 difference in all the maximum discomfort score between them as previously reported [18]. Representative result of LAST scoring data sheet is shown in Supplementary Table S2.

2.4. Measurement of serum cytokine concentration

Patients' sera were stocked at -80 °C to measure cytokine levels [IL-4, IL-5 and IFN- γ] with the Human Cytokine Beads Array (CBA) flex kit (BD) and Cytometric Beads Array Human Soluble Protein Master Buffer kit according to the manufacturer's protocols.

2.5. FLG mutation

In this study, FLG mutation analysis was performed in 21 patients. The former reported FLG mutations specific to Japanese were eight types (R501X, 3321delA, S1695X, Q1701X, S2554X, S2889X, S3296X and K4022X) [19–21]. Genotyping for the 8 types of FLG was performed using polymerase chain reaction (PCR) and restriction enzyme digest analysis [22].

2.6. Statistical analysis

All figures were depicted using the GraphPad Prism software (version 7). Statistical analyses were performed using Statcel3 (Yanai H, 2011) or GraphPad Prism. Differences between two groups were evaluated using Student *t*-test when data were equal variance and normal distribution, if not, Mann-Whitney *U* test was applied to determine calculating differences. Fisher's exact test was also applied to determine calculating differences. Spearman rank correlation was calculated to assess the correlation between the data. *P*-values < 0.05 were considered significant.

3. Results

3.1. Backgrounds of the patients

Total 42 AD patients and 10 HS were enrolled in this study, and their backgrounds are summarized in Table 1. A previous report demonstrated that aging influences on sensitive skin, as the intensity results from 10% LAST inversely correlated with age in HS [23]. In our study, we used 1% LAST, and there were no significant differences in the mean age and sex ratio between the AD and HS groups (Table 1). AD severity was assessed by SCORAD. Considering that SCORAD >50 was estimated as severe [24], the mean SCORAD value of 51.7 in our patients represented the severe disease (Table 1). It was reported that trait-anxiety and state-anxiety are higher in AD than in HS [25]. However, AD patients in this study showed no statistical significance compared to HS (Table 1). Serum levels of total IgE, specific IgE to DF and DP, LDH, eosinophils, and CCL17/TARC were all elevated in the AD group. AD patients generally have impaired barrier function, as represented by increased TEWL or reduced skin surface hydration [26,27]. TEWL in the AD group was markedly higher than that in HS (Table 1). In relation to the barrier condition, 23.8% of the AD patients characterized in this study had FLG mutations (Table 1).

3.2. High frequency of LAST-positive subjects in AD, particularly in EAD

We performed 1% LAST in AD and HS individuals. According to the standard definition of LAST positivity [18], 54.8% of AD patients showed positive LAST, which was significantly higher than the frequency (10%) of HS (Table 1). This is consistent with the previous observation that sensitive skin is more common in AD patients [3,4]. All the four LAST sensation scores, including the pain, itch, burning sensation, and crawly feeling scores, were significantly or tended to be higher in AD patients than in HS (Fig. 1).

Next, we subdivided AD patients into intrinsic (IAD; Total 10, 3 men and 7 women) and extrinsic types (EAD; Total 32, 20 men and 12 women) as previously reported [13]. The ages of IAD and EAD were statistically comparable, but female preponderance, well known as a characteristic of IAD [6], was observed in the IAD patients. EAD patients showed a significantly higher frequency of positive LAST (65.6%) than did IAD patients (20.0%) ($P=0.026$, Fisher's exact test). All four LAST scores tended to be high in EAD, and in particular, the crawly feeling score was statistically higher in EAD than in IAD (Fig. 2).

3.3. Positive correlation of the crawly feeling score and the pain score with AD severity markers

We investigated the relationship between the sensitive skin and the disease severity in our patients. The four LAST sensation elements were analyzed in their relation to the clinical and laboratory severity measurements of AD (Table 2). The pain score was significantly higher in the younger patients (Table 2). None of the four LAST sensation scores correlated with SCORAD, but the crawly feeling score strongly correlated with VAS of pruritus (Fig. 3A and Table 2). It is known that the levels of IgE, LDH, eosinophil count, and CCL17/TARC positively correlate with disease severity of AD [28–31]. Therefore, we further analyzed the relationship between each LAST score and these severity markers. The crawly feeling score positively correlated with total IgE, DF-specific IgE, LDH, and CCL17/TARC, respectively (Fig. 3A and Table 2). The pain score also showed significant correlation with DF-specific IgE, LDH, and CCL17/TARC (Fig. 3B and Table 2). The burning sensation score or itch score was not associated with any of the clinical and laboratory values (Table 2).

Severe AD shows higher scores in the three scales of QOL (symptoms, emotions, and functioning), with a significant positive correlation between the severity and the 3-scale scores [32]. It was also reported that sensitive skin affects QOL [33]. We have previously shown that Japanese version of skinindex-16, a useful tool for the evaluation of QOL, can correlate positively with the severity of AD patients [32]. We here found a tendency of correlation between skinindex-16 symptoms score and the crawly feeling score (Fig. 3A, and Table 2).

These results suggest that the crawly feeling score and the pain score are associated with the disease activity of AD.

3.4. No correlation of TEWL or FLG mutations with sensation scores or LAST positivity

To address the relationship between sensitive skin and barrier condition, the four sensation scores or LAST positivity were analyzed in relation to TEWL or FLG mutations. It was surprising that TEWL did not correlate with any of the four sensation scores (Table 2). We also examined the eight types (R501X, 3321delA, S1695X, Q1701X, S2554X, S2889X and K4022X) of FLG mutations common to Japanese AD patients [19–21] in 21 patients. Heterozygous FLG mutations were found in 5 patients, and the rest 16 patients had wild-type genomes (Table 1). There was no

Table 1
Clinical and laboratory data in AD patients and HS.

	AD	HS	P-value
Patient number	42	10	
Age (years, mean $\pm$ SD)	41.5 $\pm$ 9.0 (n = 42)	47.9 $\pm$ 14.1 (n = 10)	$P=0.15^*$
Sex (M:F,%)	54.8: 45.2 (n = 42)	60: 40 (n = 10)	$P>0.99^{\dagger}$
SCORAD (mean $\pm$ SD)	51.7 $\pm$ 19.7 (n = 31)	N.D.	–
VAS of pruritus (mean $\pm$ SD)	47.3 $\pm$ 24.5 (n = 42)	1.8 $\pm$ 5.7 (n = 10)	$P<0.001^*$
Trait-anxiety (mean $\pm$ SD)	46.0 $\pm$ 7.8 (n = 42)	43.3 $\pm$ 11.9 (n = 10)	$P=0.41^*$
State-anxiety (mean $\pm$ SD)	43.2 $\pm$ 8.1 (n = 42)	39.9 $\pm$ 11.1 (n = 10)	$P=0.29^*$
Total IgE (KU/L, mean $\pm$ SD)	6093.2 $\pm$ 9190.5 (n = 42)	N.D.	–
DP-specific IgE (class, mean $\pm$ SD)	3.3 $\pm$ 2.4 (n = 42)	N.D.	–
DF-specific IgE (class, mean $\pm$ SD)	3.4 $\pm$ 2.4 (n = 42)	N.D.	–
LDH (IU/L, mean $\pm$ SD)	240.6 $\pm$ 100.8 (n = 13)	N.D.	–
Eosinophils (% , mean $\pm$ SD)	8.6 $\pm$ 9.3 (n = 12)	N.D.	–
CCL17/TARC (pg/mL, mean $\pm$ SD)	3621.9 $\pm$ 6419.6 (n = 14)	N.D.	–
Skinindex-16 scale scores (mean $\pm$ SD)	52.4 $\pm$ 25.1 (n = 42)	N.D.	–
TEWL (g/m ² /h, mean $\pm$ SD)	30.4 $\pm$ 14.5 (n = 40)	14.8 $\pm$ 3.2 (n = 9)	$P<0.001^*$
FLG mutations (%)	23.8 (n = 21)	N.D.	–
LAST positive subjects (%)	54.8 (n = 42)	10 (n = 10)	$P=0.014^{\dagger}$

N.D.: not done, SD: standard deviation, † Fisher's exact test, * Student *t*-test or Mann-Whitney *U* test.

The normal ranges are as follows: total IgE, 5–160 KU/L; eosinophils, 1–11%; CCL17/TARC, <450 pg/ml; LDH, 115–208 IU/L.

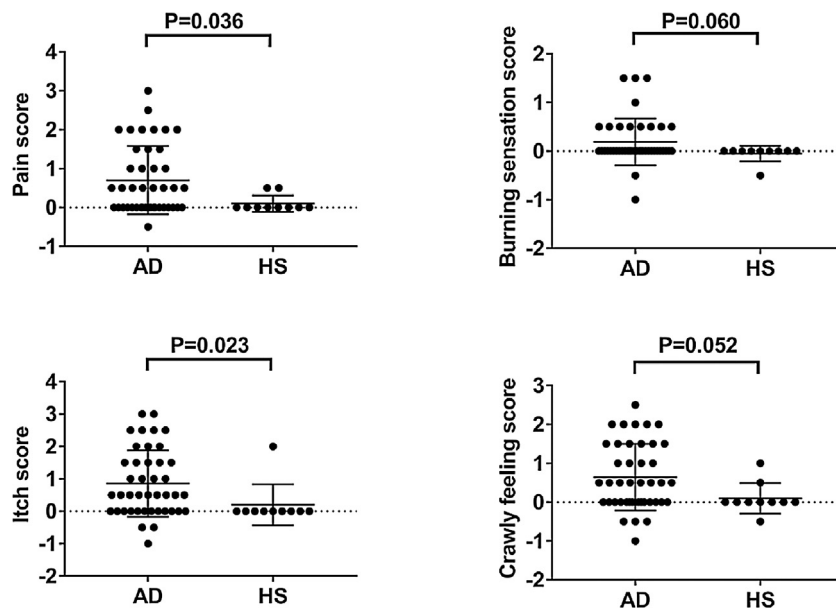


Fig. 1. 1% Lactic acid stinging test (LAST) scores in 42 atopic dermatitis (AD) and 10 healthy subjects (HS). Pain score and itch score are statistically, burning sensation score and crawly feeling score are tended to higher in AD than HS. Mann-Whitney *U* test was applied to determine calculating differences.

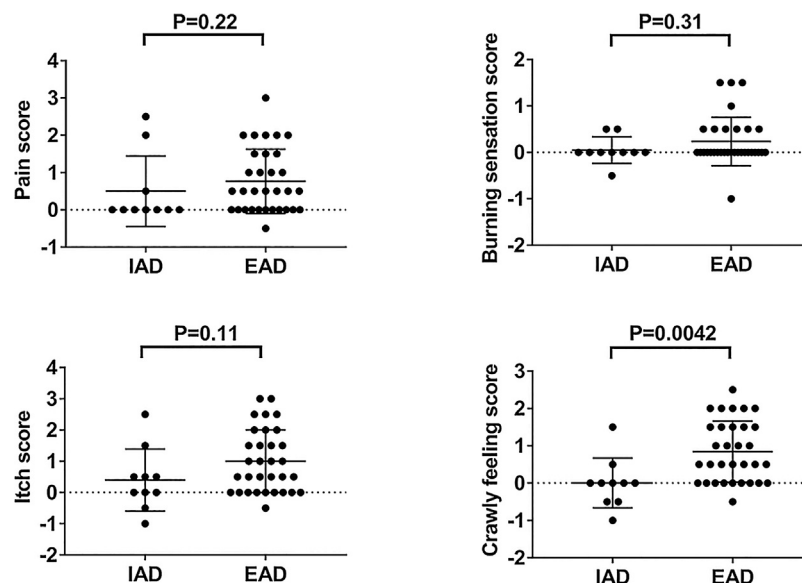


Fig. 2. 1% Lactic acid stinging test (LAST) scores in 10 intrinsic atopic dermatitis (IAD) and 32 extrinsic atopic dermatitis (EAD). Among the four LAST sensation elements, crawly feeling score is statistically higher in EAD than IAD. Mann-Whitney *U* test was applied to determine calculating differences.

significant difference between the *FLG* mutation-positive and negative groups in the frequency of LAST-positive subjects (40.0% vs 68.8%, $P=0.33$, Fisher's exact test). Although the pain score was significantly higher in *FLG* mutation-negative patients than in the positive patients, no significant differences were observed in the other three scores between the two groups (data not shown). Thus, it is unlikely that sensitive skin is associated with the barrier condition and *FLG* state.

3.5. No association of LAST scores with serum Th1/Th2 cytokines

We also investigated the concentration levels of serum IL-4, IL-5 and IFN- γ in 10 AD patients. The mean $\pm$ SD of each cytokine was as follows: IFN- γ , 0.23 ± 0.46 pg/ml; IL-4, 0.26 ± 0.44 pg/ml; and IL-5,

0.41 ± 0.42 pg/ml. We found no significant correlation between these values and each sensation score in the patients (**Supplementary Table S3**).

4. Discussion

By using 1% LAST applied to the cheek as test site, we found that the frequency of LAST-positive AD patients were 54.8%, which was significantly higher than 10% frequency of HS. This confirmed that LAST is useful for evaluation of sensitive skin in AD as well as HS. Since the first description of stinging test by Frosch and Kligman [2], there have been several reports on the frequencies of sensitive skin in HS and/or AD. In HS tested with 5% LAST, the frequencies of sensitive skin were reported as 10.8% (17.6% including intermediate

Table 2

Correlation between each LAST score and clinical/laboratory values in AD patients.

	Pain score	Burning sensation score	Itch score	Crawly feeling score
Age (years, n = 42)	$r = -0.31, P = 0.046$	$r = -0.019, P = 0.90$	$r = -0.046, P = 0.77$	$r = -0.13, P = 0.39$
Sex (male:female, %, n = 42)	$P = 0.92^{\dagger}$	$P = 0.46^{\dagger}$	$P = 0.78^{\dagger}$	$P = 0.84^{\dagger}$
SCORAD (n = 31)	$r = 0.012, P = 0.95$	$r = -0.070, P = 0.70$	$r = -0.17, P = 0.35$	$r = 0.25, P = 0.16$
VAS of pruritus (n = 42)	$r = 0.22, P = 0.16$	$r = 0.24, P = 0.13$	$r = 0.21, P = 0.18$	$r = 0.40, P = 0.0096$
Total IgE (kU/L, n = 42)	$r = 0.21, P = 0.18$	$r = 0.13, P = 0.41$	$r = 0.24, P = 0.28$	$r = 0.33, P = 0.034$
DP-specific IgE (class, n = 42)	$r = 0.28, P = 0.071$	$r = 0.10, P = 0.51$	$r = 0.13, P = 0.41$	$r = 0.27, P = 0.084$
DF-specific IgE (class, n = 42)	$r = 0.34, P = 0.028$	$r = 0.15, P = 0.35$	$r = 0.18, P = 0.24$	$r = 0.32, P = 0.042$
LDH (IU/L, n = 13)	$r = 0.69, P = 0.017$	$r = 0.15, P = 0.61$	$r = 0.084, P = 0.77$	$r = 0.59, P = 0.042$
Eosinophils (% , n = 12)	$r = 0.47, P = 0.12$	$r = 0.042, P = 0.89$	$r = 0.39, P = 0.20$	$r = 0.47, P = 0.12$
CCL17/TARC (pg/mL, n = 14)	$r = 0.68, P = 0.015$	$r = 0.51, P = 0.063$	$r = 0.20, P = 0.47$	$r = 0.48, P = 0.085$
Skindex-16 scale scores (n = 42)				
Symptoms (n = 42)	$r = 0.19, P = 0.22$	$r = 0.036, P = 0.82$	$r = 0.10, P = 0.51$	$r = 0.30, P = 0.051$
Emotions (n = 42)	$r = 0.085, P = 0.59$	$r = 0.097, P = 0.53$	$r = 0.077, P = 0.62$	$r = 0.18, P = 0.26$
Functioning (n = 42)	$r = 0.079, P = 0.61$	$r = 0.15, P = 0.35$	$r = 0.045, P = 0.77$	$r = 0.18, P = 0.26$
Global (n = 42)	$r = 0.13, P = 0.41$	$r = 0.099, P = 0.53$	$r = 0.11, P = 0.49$	$r = 0.24, P = 0.12$
TEWL (g/m ² /h, n = 40)	$r = 0.072, P = 0.65$	$r = 0.019, P = 0.90$	$r = 0.062, P = 0.70$	$r = 0.22, P = 0.18$

Spearman rank correlation was applied to determine calculating differences except sex. [†]Mann-Whitney U test.

The normal ranges are as follows: total IgE, 5–160 KU/L; eosinophils, 1–11%; CCL17/TARC, <450 pg/ml; LDH, 115–208 IU/L.

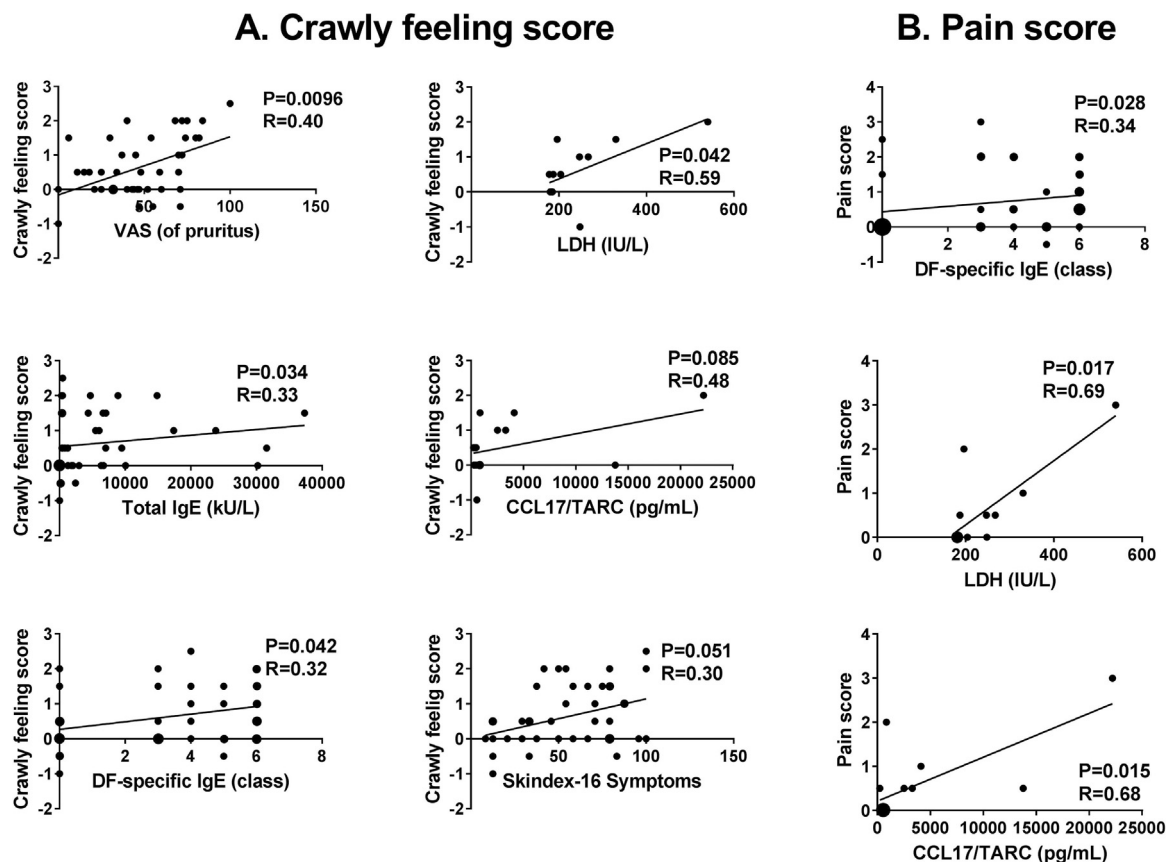


Fig. 3. A. Correlation between 1% Lactic acid stinging test (LAST) crawly feeling score and clinical/laboratory values [visual analogue scale (VAS) of pruritus (n = 42), total IgE (n = 42), *Dermatophagoides farinae* (DF) specific IgE (n = 42), lactate dehydrogenase (LDH) (n = 13), CCL17/thymus and activation-regulated chemokine (TARC) (n = 14), skindex-16 symptoms (n = 42)] in atopic dermatitis (AD). B. Correlation between 1% LAST, pain score and clinical/laboratory values [DF-specific IgE (n = 42), LDH (n = 13), CCL17/TARC (n = 14)] in AD. Spearman rank correlation was calculated to assess the correlation between the data.

group) [34] and 17.4% [35]. Although the percentages of lactic acid used in these and our studies were different, sensitive skin was detected by LAST in 10–18% HS. Meanwhile, epidemiological surveys demonstrated higher frequencies of sensitive skin in HS with difference among ethnic groups, as European countries, 38.4%; USA, 44.6%; and Japan, 54.5% [36–38]. Thus, one can detect sensitive skin with LAST in not all but a certain population of the sensitivity.

In AD patients assessed by 10% LAST, a significantly higher positive frequency (48.4%) was observed than that of HS (6.3%) [4]. It is known that the concentration of lactic acid and evaluation method influence on the frequency of sensitive skin. The sensitivity increases as elevating the concentration of lactic acid, while the specificity decreases [39]. However, the 54.8% frequency in our AD patients using 1% lactic acid is comparable to or rather higher than the 48.4% frequency obtained with 10% lactic acid [4]. The

susceptibility to irritant chemicals is generally lower in older subjects [40], as the intensity of 10% LAST correlates inversely with age in HS [23]. We also showed that the pain score correlated inversely with age in AD patients (Table 2). Therefore, lactic acid percentage, subjects' background, test site, evaluation method may give rise to variations between these studies.

Among the four LAST sensation elements, the crawly feeling score positively correlated with VAS of pruritus, total IgE, DF-specific IgE, CCL17/TARC, and LDH. The pain score also positively correlated with DF-specific IgE, CCL17/TARC, and LDH. The burning sensation or itch score was not associated with any of the clinical and laboratory values. Thus, the crawly feeling seems to be rather more important than the itch sensation to assess the sensitive skin of AD. Nerve growth factor (NGF) is one of the major mediators of cutaneous innervation density and partly involved in itch sensitization [41]. Barrier disruption induces the increased NGF in the skin and nerve growth in the epidermis [42]. In sensitive skin, there is a high content of NGF in *stratum corneum* [43], and NGF and epidermal nerve fiber density are higher in the skin of AD patients than those in HS [44]. From our study, we cannot clarify the mechanism of the crawly feeling, but the factors promoting sensory nerve overgrowth, as represented by NGF, may be involved in this sensation. It is interesting that itch score did not correlate with VAS of pruritus (Table 2), suggesting pruritus may consist of not only itch but also other various discomfort feelings. In our study, we divided the sense of discomfort into four sensation elements, and VAS of pruritus showed a significant correlation with only crawly feeling score. Compared with other sensations, crawly feeling seems to be closely related to pruritus.

Even in their visibly unaffected skin, AD patients have impaired barrier [27]. The impaired barrier function of sensitive skin was detected on the dorsum of hands by using a mathematical model of TEWL desorption curve [10]. In our study, EAD patients, possessing higher barrier impairment than IAD [7], showed a significantly higher frequency of positive LAST than did IAD patients. These observations suggest that sensitive skin is associated with barrier disruption. Meanwhile, however, sensitive skin did not correlate with TEWL or *FLG* mutation in our study. Since we performed LAST and TEWL on the same site of cheek, the correlation of sensitive skin and barrier state was accurately investigated. One of the important predisposing factors to AD is *FLG* mutation [5], leading to *stratum corneum* barrier disruption [45,46]. The presence or absence of *FLG* mutations did not correlate with LAST positivity or any sensation scores, further suggesting that sensitive skin is not necessarily associated with barrier condition. Since the most crucial feeling of sensitive skin, the crawly feeling, was significantly related to total serum IgE, DF-specific IgE, CCL17/TARC, and LDH, the sensitive skin characterized herein seems to be induced not simply by barrier disruption, but rather by multifactorial events where dermatitis severity and Th2 response are involved.

The mechanism underlying sensitive skin remains elusive and we might have a study limitation that seasonal difference affected the result. However, the present study suggests that sensitive skin is highly frequent in EAD, but not simply derived from barrier disruption. In this respect, the mechanism of the crawly feeling is an issue to be elucidated to better understand sensitive skin.

Conflict of interest

The authors have no conflict of interest directly relevant to the content of this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jdermsci.2017.10.011>.

References

- [1] N. Muizzuddin, K.D. Marenus, D.H. Maes, Factors defining sensitive skin and its treatment, *Am. J. Contact Dermatol.* 9 (1998) 170–175.
- [2] P.J. Forsch, A.D. Kligman, A method for appraising the stinging capacity to of topically applied substances, *J. Soc. Cosmet. Chem.* 28 (1977) 197–209.
- [3] C.M. Willis, S. Shaw, O. De Lacharri re, M. Baverel, L. Reiche, R. Jourdain, et al., Sensitive skin: an epidemiological study, *Br. J. Dermatol.* 145 (2001) 258–263.
- [4] R. Darlenski, J. Kazandjieva, J.W. Fluhr, M. Maurer, N. Tsankov, Lactic acid sting test does not differentiate between facial and generalized skin functional impairment in sensitive skin in atopic dermatitis and rosacea, *J. Dermatol. Sci.* 76 (2014) 151–153.
- [5] C.N. Palmer, A.D. Irvine, A. Terron-Kwiatkowski, Y. Zhao, H. Liao, S.P. Lee, et al., Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis, *Nat. Genet.* 38 (2006) 441–446.
- [6] Y. Tokura, Extrinsic and intrinsic types of atopic dermatitis, *J. Dermatol. Sci.* 58 (2010) 1–7.
- [7] T. Mori, K. Ishida, S. Mukumoto, Y. Yamada, G. Imokawa, K. Kabashima, et al., Comparison of skin barrier function and sensory nerve electric current perception threshold between IgE-high extrinsic and IgE-normal intrinsic type of atopic dermatitis, *Br. J. Dermatol.* 162 (2010) 83–90.
- [8] R. Kabashima-Kudo, M. Nakamura, J. Sakabe, S. Kazunari, R. Hino, et al., A group of atopic dermatitis without IgE elevation or barrier impairment shows a high Th1 frequency: possible immunological state of the intrinsic type, *J. Dermatol. Sci.* 67 (2012) 37–43.
- [9] S. Seidenari, M. Francomano, L. Mantovani, Baseline biophysical parameters in subjects with sensitive skin, *Contact Dermatitis* 38 (1998) 311–315.
- [10] P. Pinto, C. Rosado, C. Parreira, L.M. Rodrigues, Is there any barrier impairment in sensitive skin?: a quantitative analysis of sensitive skin by mathematical modeling of transepidermal water loss desorption curves, *Skin Res. Technol.* 17 (2011) 181–185.
- [11] F. Distant, L. Rigano, R. D'Agostino, A. Bonfigli, E. Berardesca, Intra- and inter-individual differences in sensitive skin, *Cosmet Toilettries* 117 (2002) 39–46.
- [12] J.M. Hanifin, G. Rajka, Diagnostic features of atopic dermatitis, *Acta Dermatovera (Stockh)* 92 (1980) 44–47.
- [13] H. Yamaguchi, R. Kabashima-Kudo, T. Bito, J. Sakabe, T. Shimauchi, T. Ito, et al., High frequencies of positive nickel/cobalt patch tests and high sweat nickel concentration in patients with intrinsic atopic dermatitis, *J. Dermatol. Sci.* 72 (2013) 240–245.
- [14] J. Schmitt, S. Langan, H.C. Williams, What are the best outcome measurements for atopic eczema? A systemic review, *J. Allergy Clin. Immunol.* 120 (2007) 1389–1398.
- [15] European Task Force on Atopic Dermatitis, Severity scoring of atopic dermatitis: the SCORAD index. consensus report of the european task force on atopic dermatitis, *Dermatology* 186 (1993) 23–31.
- [16] Y. Higaki, K. Kawamoto, N. Horikawa, M. Kawashima, M.M. Chren, The Japanese version of skindex-16: a brief quality-of-life measure for patients with skin diseases, *J. Dermatol.* 29 (2002) 693–698.
- [17] C.D. Spielberger, R.L. Gorsuch, R.E. Lushene, The State-trait Anxiety Inventory Manual, Consulting psychologists press, 1970.
- [18] N. Mugita, Y. Takahashi, H. Sakaguchi, N. Nishiyama, Development of a useful method for assessing sensory irritancy of cosmetics, *Nihon koshokai gakkaiishi* 36 (2012) 7–12.
- [19] T. Nomura, M. Akiyama, A. Sandilands, I. Nemoto-Hasebe, K. Sakai, A. Nagasaki, et al., Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan, *J. Invest. Dermatol.* 128 (2008) 1436–1441.
- [20] I. Nemoto-Hasebe, M. Akiyama, T. Nomura, A. Sandilands, W.H. McLean, H. Shimizu, *FLG* mutation p. Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema, *Br. J. Dermatol.* 161 (2009) 1387–1390.
- [21] M. Akiyama, *FLG* mutations in ichthyosis vulgaris and atopic eczema: spectrum of mutations and population genetics, *Br. J. Dermatol.* 162 (2010) 472–477.
- [22] H. Yamaguchi, K. Tatsuno, J. Sakabe, Y. Tokura, Second report of *FLG* R501X mutation in Japanese patients with atopic dermatitis, *J. Dermatol.* 40 (2013) 498–499.
- [23] A. Sparavigna, A. Di Pietro, M. Setaro, 'Healthy skin': significance and results of an Italian study on healthy population with particular regard to 'sensitive' skin, *Int. J. Cosmet. Sci.* 27 (2005) 327–331.
- [24] A.P. Oranje, E.J. Glazenburg, A. Wolkerstorfer, F.B. de Waard-van der Spek, Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score, *Br. J. Dermatol.* 157 (2007) 645–648.
- [25] H. Hashizume, T. Horibe, A. Ohshima, T. Ito, H. Yagi, M. Takigawa, Anxiety accelerates T-helper 2-tilted immune responses in patients with atopic dermatitis, *Br. J. Dermatol.* 152 (2005) 1161–1164.

- [26] J.M. Jensen, R. Fölster-Holst, A. Baranowsky, M. Schunck, S. Winoto-Morbach, C. Neumann, et al., Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis, *J. Invest. Dermatol.* 122 (2004) 1423–1431.
- [27] I. Jakasa, M.M. Verberk, M. Esposito, J.D. Bos, S. Kezic, Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients, *J. Invest. Dermatol.* 127 (2007) 129–134.
- [28] N. Laske, B. Niggemann, Does the severity of atopic dermatitis correlate with serum IgE levels, *Pediatr. Allergy Immunol.* 15 (2004) 86–88.
- [29] H. Mukai, T. Noguchi, K. Kamimura, K. Nishioka, S. Nishiyama, Significance of elevated serum LDH (lactate dehydrogenase) activity in atopic dermatitis, *J. Dermatol.* 17 (1990) 477–481.
- [30] J. Thijs, T. Krastev, S. Weidinger, C.F. Buckens, M. de Bruin-Weller, C. Bruijnzeel-Koomen, et al., Biomarkers for atopic dermatitis: a systematic review and meta-analysis, *Curr. Opin. Allergy Clin. Immunol.* 15 (2015) 453–460.
- [31] C.Y. Gu, L. Gu, X. Dou, Serum levels of thymus and activation-regulated chemokine can be used in the clinical evaluation of atopic dermatitis, *Int. J. Dermatol.* 54 (2015) 261–265.
- [32] Y. Higaki, K. Kawamoto, T. Kamo, S. Ueda, J. Arikawa, M. Kawashima, Measurement of the impact of atopic dermatitis on patients' quality of life: a cross-sectional and longitudinal questionnaire study using the Japanese version of skindex-16, *J. Dermatol.* 31 (2004) 977–982.
- [33] L. Misery, E. Myon, N. Martin, S. Consoli, S. Boussetta, T. Nocera, et al., Sensitive skin: psychological effects and seasonal changes, *J. Eur. Acad. Dermatol. Venereol.* 21 (2007) 620–628.
- [34] K. Lammintausta, H.I. Maibach, D. Wilson, Mechanisms of subjective (sensory) irritation, *Derm. Beruf Umwelt.* 36 (1988) 45–49.
- [35] S. An, E. Lee, S. Kim, G. Nam, H. Lee, S. Moon, et al., Comparison and correlation between stinging responses to lactic acid and bioengineering parameters, *Contact Dermatitis* 57 (2007) 158–162.
- [36] L. Misery, S. Boussetta, T. Nocera, N. Perez-Cullell, C. Taieb, Sensitive skin in europe, *J. Eur. Acad. Dermatol. Venerol.* 23 (2009) 376–381.
- [37] L. Misery, V. Sibaud, C. Merial-Kieny, C. Taieb, Sensitive skin in the American population: prevalence clinical data, and role of the dermatologist, *Int. J. Dermatol.* 50 (2011) 961–967.
- [38] R. Kamide, L. Misery, N. Perez-Cullell, V. Sibaud, C. Taieb, Sensitive skin evaluation in the Japanese population, *J. Dermatol.* 40 (2013) 177–181.
- [39] R. Sinaiko, H.I. Maibach, *Irritant Dermatitis P224*, Springer-Verlag, Berlin Heidelberg, 2006.
- [40] M.K. Robinson, Population differences in acute skin irritation responses. Race sex, age, sensitive skin and repeat subject comparisons, *Contact Dermatitis* 46 (2002) 86–93.
- [41] M. Tominaga, K. Takamori, Itch and nerve fibers with special reference to atopic dermatitis: therapeutic implications, *J. Dermatol.* 41 (2014) 205–212.
- [42] M. Tominaga, S. Ozawa, S. Tengara, H. Ogawa, K. Takamori, Intraepidermal nerve fibers increase in dry skin of acetone-treated mice, *J. Dermatol. Sci.* 48 (2007) 103–111.
- [43] T. Yokota, Classification of sensitive skin with analysis of skin physiological parameter, *Nihon koshokai gakkaiishi* 29 (2005) 44–49.
- [44] M. Tominaga, S. Tengara, A. Kamo, H. Ogawa, K. Takamori, Psoralen-ultraviolet A therapy alters epidermal Sema3A and NGF levels and modulates epidermal innervation in atopic dermatitis, *J. Dermatol. Sci.* 55 (2009) 40–46.
- [45] I. Nemoto-Hasebe, M. Akiyama, T. Nomura, A. Sandilands, W.H. McLean, H. Shimizu, Clinical severity correlates with impaired barrier in filaggrin-related eczema, *J. Invest. Dermatol.* 129 (2009) 682–689.
- [46] S. Weidinger, E. Rodríguez, C. Stahl, S. Wagenpfeil, N. Klopp, T. Illig, et al., Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis, *J. Invest. Dermatol.* 127 (2007) 724–726.

Supplementary Table S1. Discomfort sensation scale for severity asses

Score	Degree of discomfort
0	No sensation.
0.5	Slight discomfort, barely detectable.
1	Mild discomfort.
1.5	Mild to moderate discomfort.
2	Moderate discomfort.
2.5	Moderate to severe discomfort.
3	Severe discomfort, intense unplesant feeling.

Supplementary Table S2. Lactic acid stinging test (LAST) data sheet (example).

1% lactic acid	Pain score	Burning sensation score	Itch score	Crawly feeling score
30 seconds	0.5	0	1	1
2 minutes and 30 seconds	0.5	0	1	1.5
5 minutes	1	0.5	1.5	2.5
Maximun discomfort score	1	0.5	1.5	2.5

Distilled water (DW)	Pain score	Burning sensation score	Itch score	Crawly feeling score
30 seconds	0	0	0	0
2 minutes and 30 seconds	0	0	0	0.5
5 minutes	0.5	0.5	1	1
Maximun discomfort score	0.5	0.5	1	1

Comment

The difference of maximun discomfort score between 1% lactic acid and DW	Pain score	Burning sensation score	Itch score	Crawly feeling score
	0.5 (1 - 0.5)	0 (0.5 - 0.5)	0.5 (1.5 - 1)	1.5 (2.5 - 1)

LAST score (The difference in one of the maximum discomfort score between 1% lactic acid and DW)	Crawly feeling score	Evaluation
	1.5 (2.5 - 1) $\geq$ 1.0	Stingers group (positive)

Supplementary Table S3. Correlation between each LAST score and serum Th1/Th2 cytokines in AD patients.

	Pain score	Burning sensation score	Itch score	Crawly feeling score
IL-4 (pg/ml, n=10)	$r = -0.47, P = 0.16$	$r = 0.10, P = 0.76$	$r = -0.36, P = 0.28$	$r = -0.64, P = 0.056$
IL-5 (pg/ml, n=10)	$r = -0.61, P = 0.067$	$r = 0.022, P = 0.95$	$r = -0.42, P = 0.20$	$r = -0.33, P = 0.32$
IFN- γ (pg/ml, n=10)	$r = -0.22, P = 0.51$	$r = 0.41, P = 0.21$	$r = 0.12, P = 0.73$	$r = -0.23, P = 0.49$

Spearman rank correlation was applied to determine calculating differences.