

Comparison of skin barrier function and sensory nerve electric current perception threshold between IgE-high extrinsic and IgE-normal intrinsic types of atopic dermatitis

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Summary

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Conflicts of interest

None declared.

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Background Two types of atopic dermatitis (AD) have been proposed, with different pathophysiological mechanisms underlying this seemingly heterogeneous disorder. The extrinsic type shows high IgE levels presumably as a consequence of skin barrier damage and feasible allergen permeation, whereas the intrinsic type exhibits normal IgE levels and is not mediated by allergen-specific IgE.

Objectives To investigate the relationship between pruritus perception threshold and skin barrier function of patients with AD in a comparison between the extrinsic and intrinsic types.

Methods Enrolled in this study were 32 patients with extrinsic AD, 17 with intrinsic AD and 24 healthy individuals. The barrier function of the stratum corneum was assessed by skin surface hydration and transepidermal water loss (TEWL), and pruritus perception was evaluated by the electric current perception threshold (CPT) of sensory nerves upon neuroselective transcutaneous electric stimulation.

Results Skin surface hydration was significantly lower and TEWL was significantly higher in extrinsic AD than intrinsic AD or normal controls. Although there was no statistically significant difference in CPT among extrinsic AD, intrinsic AD and normal controls, CPT was significantly correlated with skin surface hydration and inversely with TEWL in intrinsic AD and normal controls, but not extrinsic AD. Finally, CPT was correlated with the visual analogue scale of itch in the non-lesional skin of patients with extrinsic but not intrinsic AD.

Conclusions Patients with extrinsic AD have an impaired barrier, which increases the pre-existing pruritus but rather decreases sensitivity to external stimuli. In contrast, patients with intrinsic AD retain a normal barrier function and sensory reactivity to external pruritic stimuli.

Atopic dermatitis (AD) is a chronic inflammatory skin disease with complicated pathophysiological mechanisms and causative agents. Two subtypes of AD have been proposed: extrinsic AD and intrinsic AD. The extrinsic type is the IgE-mediated common form of AD and is associated with respiratory allergies, such as rhinitis and asthma, and high serum levels of IgE.^{1–3} In contrast, intrinsic AD is characterized by the absence of allergen-specific IgE and thus shows normal total IgE levels, although this newly introduced concept is still controversial among academic dermatologists.^{1–3} Approximately 20%⁴ or

fewer⁵ patients are estimated as having intrinsic AD. Its characteristics include female predominance, absence of atopic diseases, later onset of disease, and milder disease severity.^{3–6} A history of atopy, recurrent conjunctivitis, palmar hyperlinearity, keratosis pilaris, pityriasis alba, and hand and/or foot eczema are significantly less present in the intrinsic type, but Dennie-Morgan fold is positively associated with intrinsic AD.³

Several studies have suggested differences in various aspects of pathophysiology between extrinsic and intrinsic AD.

Increased transepidermal water loss (TEWL) and reduced skin surface hydration are hallmarks of atopic skin, and there are some differences in these values between the two types of AD.⁴ Immunologically, surface expression of the high- and low-affinity receptor for IgE and of the interleukin (IL)-4R α chain is elevated in monocytes from patients with extrinsic AD, but serum levels of IL-13 are significantly increased in patients with intrinsic AD.⁷ Skin lesions of extrinsic AD show high levels of chemokines such as CCL18.⁸ Expression of neurotrophins is increased comparably in both types.⁶

The stratum corneum of the epidermis, consisting of more than 10 layers of corneocytes and intercellular lipids, serves as the skin barrier.⁹ In extrinsic AD, impairment of the skin barrier may be the primary condition which facilitates permeation of environmental allergens and leads to immunological responses such as elevation of allergen-specific IgE.¹ A recent finding of filaggrin gene mutations in a high percentage of patients with AD,^{10,11} together with an older finding of ceramide reduction in the stratum corneum,^{12,13} have further suggested the presence of skin barrier damage in extrinsic AD. On the other hand, intrinsic AD shows normal or mildly elevated serum IgE, in striking contrast to extrinsic AD.¹⁴ The mechanisms underlying intrinsic AD remain unclear and more speculative than those underlying extrinsic AD.^{2,6,8,15}

Patients with AD are well known to be sensitive to irritation from the environment due to the impaired skin barrier function. Given that the extrinsic and intrinsic types are different from each other in the skin barrier condition, each type might respond to external stimuli in a different manner. However, little is known regarding the difference in sensitivity to irritants and in elicibility of pruritus between the two types. It appears that most previous studies on sensitivity were performed in patients with extrinsic AD because of its higher incidence.

There are several reported methods to assess the threshold for the itch sensation to various environmental stimuli.^{16–18} Local administration of histamine, either by needle injection or by iontophoresis, is one of the most common procedures for this purpose.¹⁹ Electrically evoked itch is another useful method with the use of a neuroselective transcutaneous electrical stimulator, Neurometer™ CPT/C (Neurotron Inc., Baltimore, MD, U.S.A.), in a noninvasive fashion. Evaluation of electric current perception threshold (CPT) quantifies the sensory threshold to electric stimulation of the sensory nerves.^{20,21} This device has been used mainly by neurologists to demonstrate abnormalities in a variety of neuropathic conditions. It does not measure the sensation only to histamine, but the device directly excites large- and small-diameter sensory nerve fibres in a differentiating fashion, independent of local factors such as skin thickness, temperature and substances involved in the induction of pruritus.^{20,22} The CPT for 250- and 5-Hz frequency current emitted by the Neurometer CPT/C has been reported to enable quantification of the sensory threshold of A δ - and C-fibres, respectively, that are thought to transmit the itch sensation from the skin. Therefore, this instrument allows us to investigate the elicibility of

pruritus in patients with AD. Kobayashi *et al.*²³ have reported that patients with AD showed lower CPT than healthy controls, and CPT was inversely correlated with TEWL after tape stripping in normal subjects.

To address the differences in the mechanisms between extrinsic and intrinsic AD, we measured CPT in patients with AD, together with measurements of stratum corneum function. Our results show that there are prominent differences in the relationship of CPT with the skin barrier function between the two types.

Materials and methods

Participants

Patients over 18 years of age from our department were included in this study. Forty-nine patients with AD (25 men and 24 women), diagnosed in accordance with the Hanifin and Rajka classification,²⁴ and 24 healthy controls were enrolled in this study. The distribution of skin symptoms in all patients was characterized for adult AD. The hands, shoulders, neck, flexures and face were the predilection sites, while the extremities were less involved. Patients who had total serum IgE levels < 220 U mL⁻¹ (normal range for Japanese subjects) were classified as having intrinsic AD, and those with levels > 400 U mL⁻¹ were classified as having extrinsic AD. There was no patient with IgE levels between 220 and 400 U mL⁻¹. IgE RAST for *Dermatophagoides pteronyssinus* was measured in 20 patients. The disease activity was assessed by SCORAD (severity scoring of AD). Patient details are listed in Table 1. All participants provided written informed consent, and the institutional review board approved this study.

Electric current perception threshold and stratum corneum function

C-fibres are sensory nerves conducting itch and pain. Transcutaneous electric current with 5-Hz sine wave stimulates C-fibres, as assessed by the active action potentials of rat dorsal root ganglia.^{21,25} Depending on the body surface site, 5-Hz electric current induces itch and/or pain. In addition, transcutaneous 250-Hz current has also been known to stimulate A δ -fibres, thus inducing itch.²⁵ In this direct stimulation of nerve fibres with transcutaneous electric current, the condition of the stratum corneum possibly modifies the perception by affecting the current or other factors.

We measured CPT, skin surface hydration and TEWL at the nonlesional flexor forearm, the nonlesional lower leg, and at lesional skin on the trunk or extremities. When the patients had skin lesions on the flexor forearms or lower legs we avoided these regions and chose clinically normal areas on the volar skin as the sites to perform measurements on nonlesional skin. The patients did not apply any ointment or cream to the examined sites for at least 2 days before the measurements. Concerning the inflammatory state at the clinically normal sites tested, we have previously demonstrated

Table 1 Patients and healthy individuals enrolled in this study

	Extrinsic AD	Intrinsic AD	Healthy controls
Number of subjects	32 (21 men and 11 women)	17 (four men and 13 women)	24 (nine men and 15 women)
Age (years), mean $\pm$ SD (range)	30.0 $\pm$ 8.1 (19–51)	33.0 $\pm$ 10.4 (18–57)	28.9 $\pm$ 3.8 (23–37)
IgE (U mL ⁻¹), mean $\pm$ SD (range)	5034.8 $\pm$ 7538.0 (436–30 000)	110.5 $\pm$ 66.8 (11–219)	–
SCORAD, mean $\pm$ SD (range)	41.8 $\pm$ 19.0 (4.6–84.5)	27.1 $\pm$ 20.6 (3.5–73)	–
VAS (nonlesional forearm), mean $\pm$ SD	30.9 $\pm$ 20.6	15.8 $\pm$ 22.1	–
VAS (nonlesional lower leg), mean $\pm$ SD	36.0 $\pm$ 24.9	20.4 $\pm$ 28.5	–
VAS (lesional skin), mean $\pm$ SD	55.3 $\pm$ 28.3	47.3 $\pm$ 36.0	–

AD, atopic dermatitis; VAS, visual analogue scale.

that clinically normal-appearing skin of patients with AD has no histological evidence of inflammation.²⁶ As control, we measured CPT, skin surface hydration and TEWL on the mid-flexor forearm and lower leg in healthy individuals. CPT was measured by using the Neurometer CPT/C as described previously.²³ Skin surface hydration was evaluated by capacitance using the Corneometer CM825 (Courage & Khazaka Electronic GmbH, Cologne, Germany) and was expressed as arbitrary units.²⁷ TEWL was measured by detecting the evaporated water using the VapoMeter SWL-2 (Delfin Technologies Ltd, Kuopio, Finland).

Visual analogue scale

All patients rated current itching on a 100-mm visual analogue scale (VAS)²⁸ at the following sites: nonlesional forearm, nonlesional lower leg and lesional skin.

Statistical analyses

Data were expressed as mean $\pm$ SD and assessed for statistical significance. We used Student's *t*-test to compare skin surface hydration, TEWL and CPT. A linear regression analysis was performed for correlations between the skin surface hydration or VAS and CPT, using Pearson's correlation coefficient. For all tests, *P* < 0.05 was considered statistically significant.

Results

Impaired skin barrier function in extrinsic but not intrinsic atopic dermatitis

Patients were classified as having extrinsic or intrinsic AD by means of IgE level (> 400 and < 220 U mL⁻¹, respectively). IgE RAST was scored by index values 0–6 according to the manufacturer's criteria (BML, Tokyo, Japan). An index value > 3 to *D. pteronyssinus* was obtained in 11 of 12 (92%) patients with extrinsic AD, but in only one of eight (12.5%) patients with intrinsic AD. Moreover, 67% of the patients with extrinsic AD showed a RAST score index value of 6, and none of the patients with intrinsic AD showed this highest score. As summarized in Table 1, more patients had extrinsic AD than

intrinsic AD, and women predominated in the intrinsic type, as previously reported.^{3–5} No significant difference was noted in age between the two types. There was a tendency that SCORAD and VAS at the three test sites were higher in extrinsic than intrinsic AD, as reported previously.^{3–5}

As extrinsic AD is caused by external allergens invading through the damaged skin barrier, we initially examined the skin surface hydration (capacitance) and TEWL at the nonlesional forearm and lower leg of patients and normal volunteers in a comparison between extrinsic and intrinsic AD. Skin surface hydration was significantly lower in extrinsic AD than in normal control subjects (Fig. 1). There was no significant difference in the hydration level between intrinsic AD and healthy controls. Extrinsic AD tended to show lower values than intrinsic AD at both sites. TEWL, another assessment of the barrier function, was statistically significantly higher in extrinsic AD than intrinsic AD and normal controls at the nonlesional forearm (Fig. 1). Thus, the skin barrier function was impaired in extrinsic AD and preserved in intrinsic AD, validating this clinical dichotomy.

Correlation between disease severity and pruritus in both extrinsic and intrinsic atopic dermatitis

In advance of analysing CPT, we also examined the correlation between the itch levels and SCORAD in the two types of AD. In both types, VAS scores on the lesional skin were correlated with SCORAD (Fig. 2), suggesting that both types of AD are associated with disease severity-dependent pruritus.

Significant correlation between electric current perception threshold (CPT) and skin surface hydration and between CPT and transepidermal water loss in intrinsic atopic dermatitis as well as in normal individuals

CPT for 5- and 250-Hz current stimuli was measured in patients with AD and normal volunteers. In all experiments, the results from 5- and 250-Hz current stimuli were virtually the same. Figure 3 shows the mean $\pm$ SD CPT in each group, and there was no significant difference between the groups in the nonlesional or lesional skin.

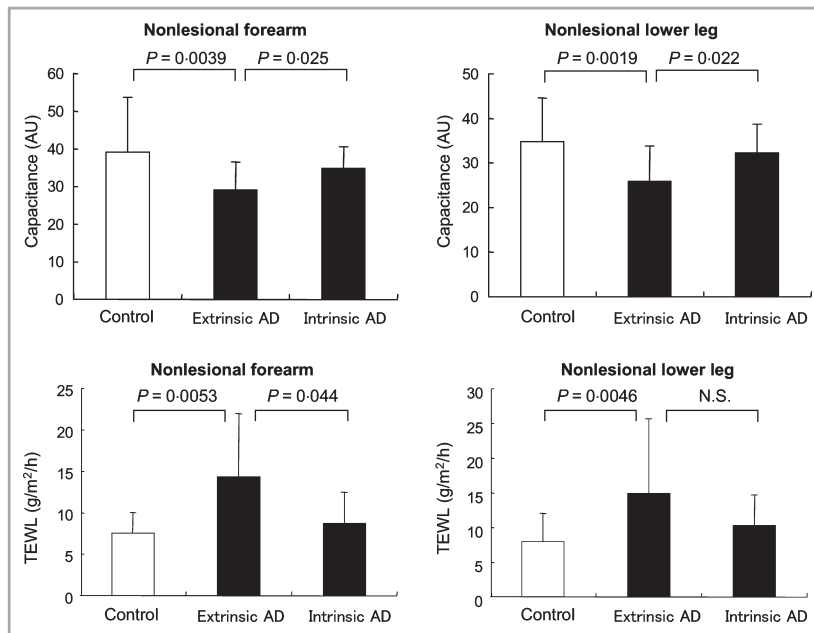


Fig 1. Skin surface hydration on nonlesional forearm and lower leg in extrinsic and intrinsic atopic dermatitis (AD) and healthy controls. Skin surface hydration is represented by capacitance in arbitrary units (AU). Results are shown as mean $\pm$ SD. N.S., not significant.

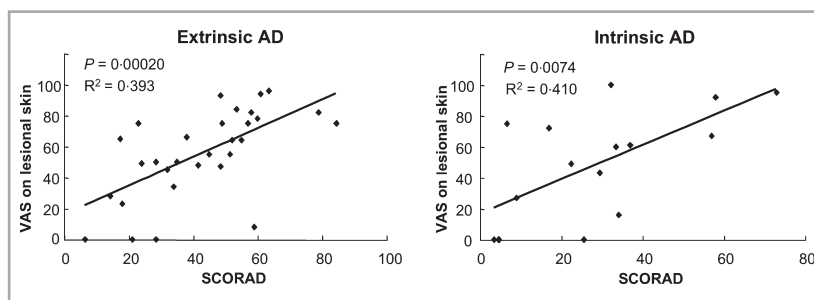


Fig 2. Relationship between SCORAD and visual analogue scale (VAS) score in extrinsic and intrinsic atopic dermatitis (AD).

When CPT was analysed in relation to skin surface hydration, an interesting finding was obtained. In normal subjects (control), CPT was significantly correlated with skin surface hydration (Fig. 4), suggesting that the water-poor cornified layer has a property to evoke pruritus in response to external stimuli. Similarly, the lesional skin of patients with intrinsic AD showed such a significant correlation between CPT and skin surface hydration. However, there was no correlation in extrinsic AD, as large individual variations of CPT were seen in the patients with extrinsic AD and low levels of skin surface hydration.

As to the relation of CPT to TEWL, there was no significant correlation between these two parameters in the lesional skin of patients with AD. However, CPT of nonlesional forearm, as assessed by 250-Hz sensitivity, was inversely correlated with TEWL in intrinsic AD as well as in controls (Fig. 5). The results suggest that intrinsic AD is associated with a normal stratum corneum and no excess elicibility of externally stimulated pruritus, while extrinsic AD does not show such a regular, surface hydration-related irritant perception.

Different electric current perception threshold levels in relation to pre-existing pruritus between extrinsic and intrinsic atopic dermatitis

It is possible that CPT is affected by the itch state in patients with AD. We therefore investigated the relationship between CPT and the pre-existing pruritus assessed by VAS. In the lesional skin of both types of AD there was no correlation between CPT and VAS (data not shown). In the nonlesional lower leg, however, CPT was significantly correlated with VAS in extrinsic but not intrinsic AD (Fig. 6), suggesting that the pre-existing pruritus rather downmodulates the sensitivity to external stimuli in extrinsic AD. The nonlesional forearm exhibited the same tendency but without statistical significance. Thus, the pruritic normal-appearing skin seems to be insensitive to further itchy stimuli in extrinsic AD.

Discussion

The precise concept of intrinsic AD in comparison with extrinsic AD has been a matter of controversy. Extrinsic AD seems

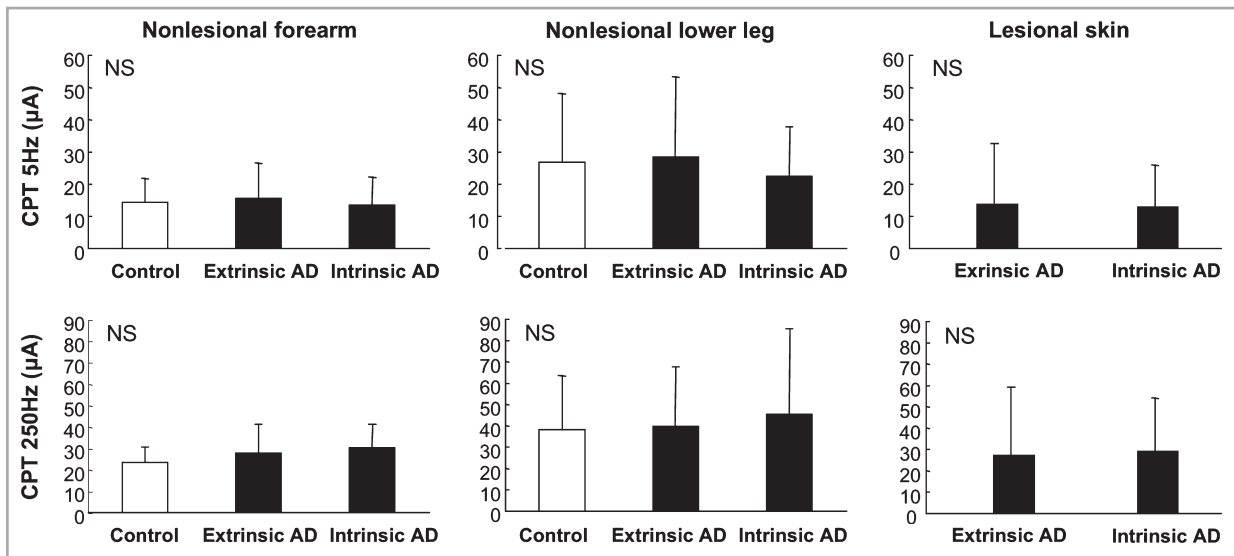


Fig 3. Electric current perception threshold (CPT) on nonlesional forearm or lower leg and lesional skin in extrinsic and intrinsic atopic dermatitis (AD). Results are shown as mean $\pm$ SD. N.S., not significant.

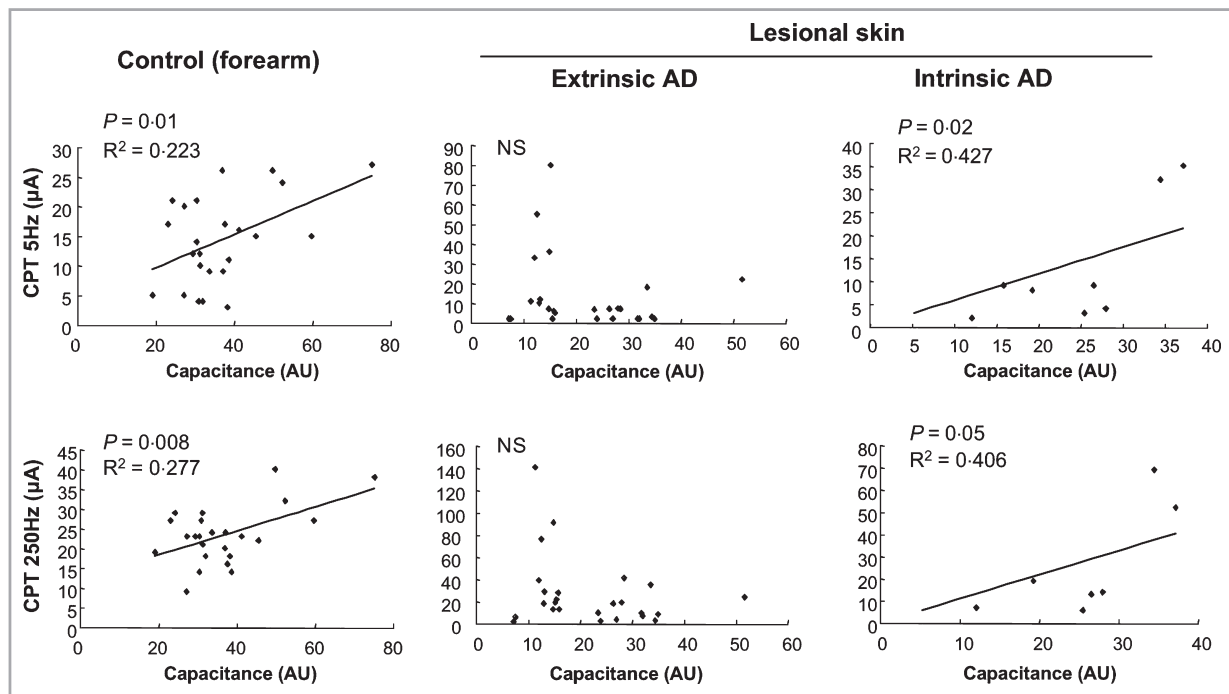


Fig 4. Relationship between skin surface hydration, represented by capacitance in arbitrary units (AU), and electric current perception threshold (CPT) in lesional skin of patients with extrinsic and intrinsic atopic dermatitis (AD) and in nonlesional forearm skin of healthy controls. NS, not significant.

to be induced by sequential events, including impairment of stratum corneum, permeation of external substances, exposure of immunocompetent cells to the allergens, and T cell and IgE responses to the antigenic determinants.¹ Growing evidence has supported this mechanism underlying the extrinsic type.

The recent finding that flaggrin gene mutations are a predisposing factor for AD has clearly demonstrated the presence of barrier impairment in patients with AD.^{10,11} On the other hand, the pathophysiology of intrinsic AD remains obscure, and it may be difficult for clinicians to differentiate the two

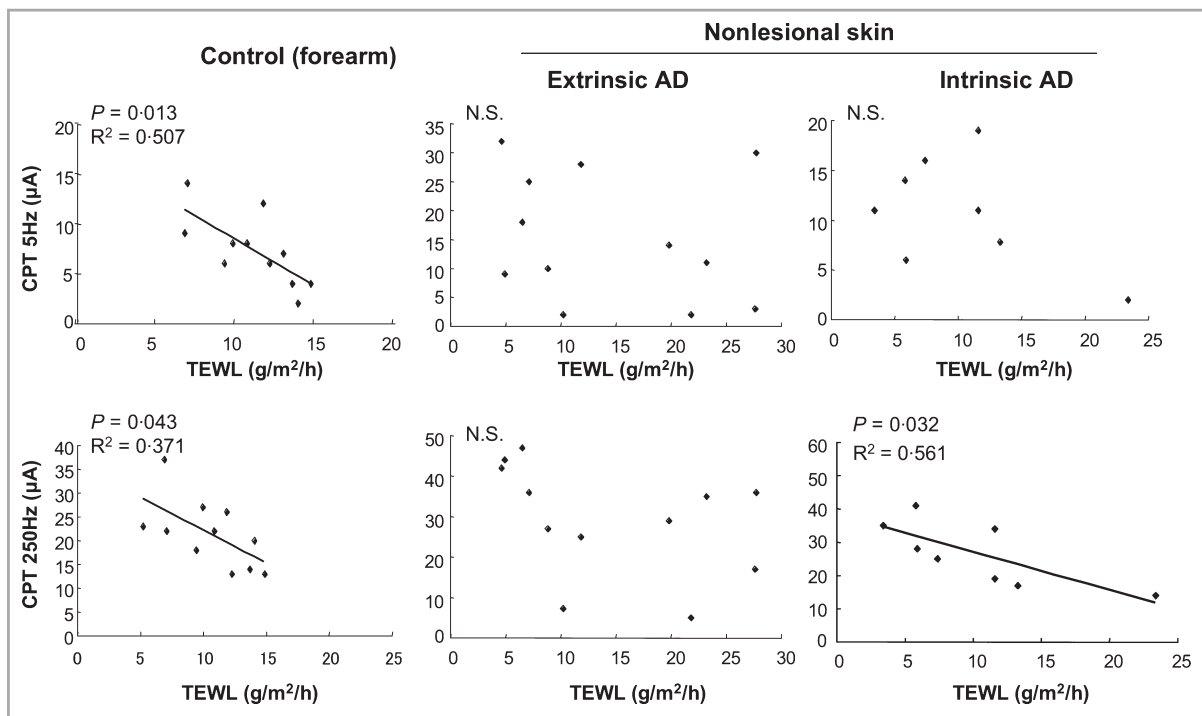


Fig 5. Relationship between transepidermal water loss (TEWL) and electric current perception threshold (CPT) in nonlesional forearm skin of patients with extrinsic and intrinsic atopic dermatitis (AD) and healthy control skin. NS, not significant.

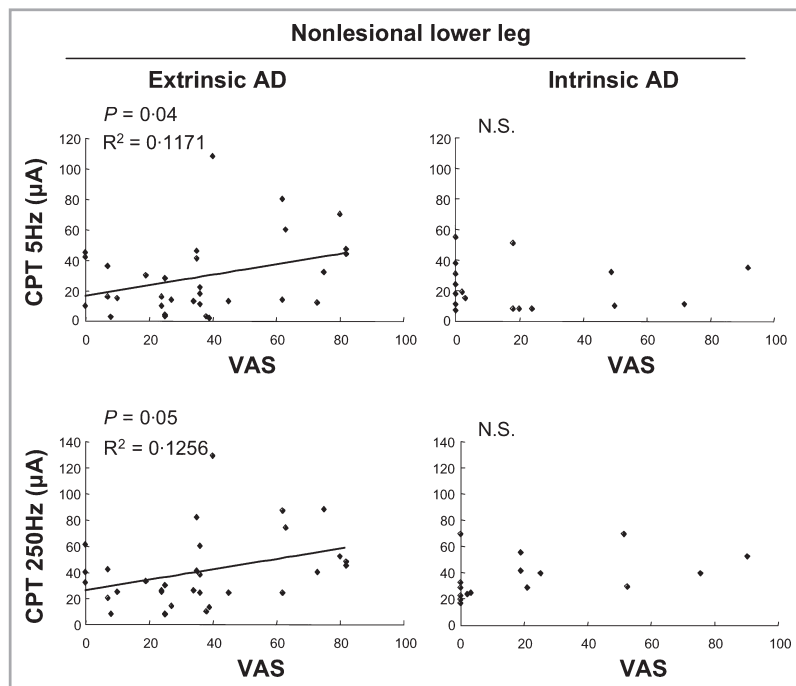


Fig 6. Relationship between visual analogue scale (VAS) score and electric current perception threshold (CPT) on nonlesional skin of patients with extrinsic and intrinsic atopic dermatitis (AD). NS, not significant.

types accurately. Only one clear way to discriminate the two types is the serum levels of IgE,²⁹ but its precise cutoff value has not been determined.

In this study, we tentatively divided the patients with AD into two groups by IgE levels of > 400 and < 220 U mL⁻¹, because the normal range in Japanese individuals is

< 220 U mL⁻¹. This division was confirmed by a high percentage and high scores of positive RAST to *D. pteronyssinus* in extrinsic AD and a low percentage in intrinsic AD. We found that more of our patients had intrinsic than extrinsic AD, and that women were more likely than men to have intrinsic AD, as already reported in previous studies.⁵ In contrast to

extrinsic AD, intrinsic AD is thought to show a normal skin barrier function. We validated this general concept by measuring skin surface hydration and TEWL, and found no significant difference in these values between patients with intrinsic AD and normal individuals, while the patients with extrinsic AD had lower surface hydration levels and higher TEWL levels than the normal subjects.

C-fibres (unmyelinated fibres) are sensory nerves conducting pruritus. A transcutaneous electric current at 5 Hz can stimulate C-fibres.²⁵ Alternating current stimulus at 250 Hz activates A δ -fibres (small myelinated fibres), which may also participate in itch. The condition of the stratum corneum may modify the perception by affecting the current or other factors. In our study, low levels of hydration of the stratum corneum reduced CPT. This suggests that the itch perception to external stimuli is promoted in skin with low hydration.

The difference in the barrier function between the extrinsic and intrinsic types raised the possibility that the elicibility of pruritus differs between them. In normal individuals, CPT and skin surface hydration or TEWL were correlated with each other, suggesting that the barrier-damaged skin is sensitive to external irritants. In normal individuals, Kobayashi *et al.*²³ have reported that CPT is inversely correlated with TEWL levels after tape stripping, providing further evidence that barrier damage leads to elicibility of sensation. In our study, the correlation between CPT and skin surface hydration and the inverse correlation between CPT and TEWL were also found in patients with intrinsic AD, suggesting that intrinsic AD shows a normal skin barrier and elicibility of sensation to external stimuli. In contrast, the patients with extrinsic AD showed different elicibility with individual variations presumably due to the low surface hydration. Kobayashi *et al.*²³ have also shown that the skin of patients with AD is not extremely sensitive as compared with that of normal individuals to the electric stimulation of their A δ - and C-fibres. Their patients seem to include those with both extrinsic and intrinsic AD. Our results suggest that some patients with extrinsic AD have high CPT levels despite the impaired barrier function.

Pre-existing pruritus elevated CPT on the nonlesional skin of patients with extrinsic AD, as CPT and VAS were correlated with each other in the nonlesional sites of the extrinsic type. Accordingly, Ikoma *et al.*³⁰ found that when histamine prick tests are performed in nonlesional skin of patients with AD, itch rating increases more slowly and is significantly lower than in controls.³⁰ Our unexpected finding was not observed in intrinsic AD. It is possible that in the already itchy skin of extrinsic AD, A δ - and C-fibres are in a stimulated state, resulting in insensitivity to external irritants, while the steady-state interaction between the barrier and sensory fibres might be kept in intrinsic AD. The end of sensory fibres in the skin of extrinsic AD seems to be continuously stimulated by the damaged stratum corneum, leading to the elevated CPT.

Our study suggests that the two types of AD are different from each other in the mode of elicibility of pruritus, because of the different skin barrier states between them. Furthermore, it was recently found that IgE autoantibodies

can target keratinocytes in AD; this might promote barrier damage and modify resultant itch elicibility in extrinsic AD.³¹ As the response of sensory nerves to irritants appears to be intact in intrinsic AD, the mechanisms of pruritus underlying this type of AD are an important issue to be elucidated.

Acknowledgments

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