

New Horny Layer Marker Proteins for Evaluating Skin Condition in Atopic Dermatitis

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Key Words

Atopic dermatitis • Albumin • Biomarkers •
Fatty acid-binding protein 5 • Horny layer

Abstract

Background: Atopic dermatitis (AD) has a complicated pathogenesis and its clinical features vary greatly among patients. Although many clinical parameters have been reported, it remains difficult to evaluate AD skin conditions adequately. **Objective:** To support better evaluation of AD patients, we attempted to develop a new, objective and noninvasive method that assesses skin condition in AD using biochemical markers in the skin's horny layer (HL). **Methods:** Thirty-six patients with AD, 8 with psoriasis and 16 healthy volunteers were recruited. HL samples were obtained by tape stripping from involved and uninvolved skin of the forearms. Expression levels of 6 proteins in the HL [fatty acid-binding protein-5 (FABP-5), squamous cell carcinoma antigens 2 (SCCA2), α -enolase, annexin II, apolipoprotein A-I and albumin] were analyzed by immunoblotting and compared with clinical data. **Results:** The 6 proteins were detected at a high level in AD skin lesions, but scarcely in the normal controls. FABP-5 showed correlation with the local severity of the involved skin. Annexin II, apoprotein A-I and albumin

showed correlation with the severity of specific eruptions. SCCA2 correlated significantly with total serum IgE level. Albumin levels in the uninvolved skin of AD patients showed significant correlation with the local severity in the involved skin of the same patient and with the trans-epidermal water loss. Albumin levels in psoriatic skin were very low, even with scratch marks, compared to those in AD skin. **Conclusion:** FABP-5, albumin and some other proteins in HL seem to be useful as biomarkers to evaluate inflammation and skin barrier conditions in AD patients.

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Introduction

Atopic Dermatitis (AD) is a common chronic and relapsing inflammatory skin disorder. It is generally considered that AD is caused by the complex interaction of genetic, immunologic and environmental factors, but its exact etiology and pathogenesis have not yet been entirely elucidated [1]. Many lines of evidence strongly support the hypothesis that the Th2-type immune response is mainly

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involved in the pathogenesis of AD [2–4]. Elevation of blood eosinophil count and serum total IgE level, both of which are fundamental features of AD patients, supports this hypothesis. However, the Th1-type immune response may accompany the chronic phase of AD [5, 6]. Dysfunction of the skin barrier is also known to be a key factor in AD [1, 7]. Because of its complicated pathogenesis, the clinical features of AD patients vary individually.

For the best clinical treatment of AD patients, it is obviously important to correctly evaluate their clinical features and conditions. However, there is a lack of standardization in the assessment of complicated AD activity. Although there are many AD severity scales, most of them rely on subjective assessment of clinical symptoms [8]. Therefore, these assessments often cause interobserver and intraobserver variability. Immunologic states of AD patients are generally determined by measuring some blood parameters, such as the eosinophil number and the serum levels of total IgE and lactate dehydrogenase (LDH), the latter of which is released from impaired skin. These measurements also differently reflect the severity of AD [9]. Taken together, it still remains difficult to evaluate the severity of skin inflammation correctly and objectively. Therefore, it seems important to survey reliable new biomarkers that better reflect the clinical conditions and features of AD skin lesions.

In preliminary studies we have analyzed by mass spectrometry skin proteins which were more highly expressed in the horny layer (HL) of skin in AD patients than in healthy controls. The HL samples were noninvasively obtained by tape stripping from the skin lesions of AD patients. From the several proteins identified, we chose 6 to be candidate biomarkers for AD skin: fatty acid binding protein-5 (FABP-5, or epidermal FABP), squamous cell carcinoma antigen 2 (SCCA2), α -enolase, annexin II, apolipoprotein A-I and albumin (unpubl. data). These candidate biomarkers were detected at higher levels in the HL of AD patients than in healthy controls.

The present study was aimed at assessing whether these proteins can be used as biochemical markers for quantitatively evaluating the skin condition of each lesion in AD patients, because there is no objective marker for such an aim. We analyzed the expression of the 6 proteins in the HL of AD patients and examined the relationship between the expression level of each protein and clinical features or laboratory data. We also compared the HL levels of these proteins between AD and psoriasis. Here we report a new, objective and noninvasive method that assesses the skin condition of AD patients using biochemical markers.

Table 1. Patient baseline characteristics

Characteristic	Control (n = 16)	AD patients (n = 36)	Psoriasis patients (n = 10)
Age (mean $\pm$ SD), years	31.4 $\pm$ 10.8	31.7 $\pm$ 14.1	52.8 $\pm$ 10.7
Male sex, n (%)	10 (63)	22 (61)	7 (70)
Disease duration (mean $\pm$ SD), years	–	21.9 $\pm$ 13.7	23.6 $\pm$ 10.8
Treatment, n (%)			
Antihistamines	–	33 (91)	0 (0)
Topical steroids	–	32 (89)	6 (60)

Materials and Methods

Antibodies and Other Reagents

Antibodies used to detect human skin proteins and their sources are as follows: anti-annexin II rabbit polyclonal antibody (H-50), anti-SCCA2 mouse monoclonal antibody (10C12), and rabbit anti-enolase polyclonal antibody (H-300) from Santa Cruz Biotechnology (Santa Cruz, Calif., USA); anti-apolipoprotein A1 mouse monoclonal antibody (clone 3F10) from MP Biomedicals (Solon, Ohio, USA); anti-human albumin rabbit polyclonal antibody from Inter-Cell Technologies (Jupiter, Fla., USA); anti-FABP-5 rabbit polyclonal antibody from BioVendor Laboratory Medicine (Modřice, Czech Republic), and anti-cytokeratine 8 mouse monoclonal antibody (clone 4.1.18) from Chemicon International (Temecula, Calif., USA).

Patients and Evaluation of Their Clinical Features

The study was performed from November to December 2006, and 36 patients with AD (22 males and 14 females, age range 4–60 years, average 31.7 years) were recruited. AD was diagnosed at Yokohama City University Hospital, based on the criteria of Hanifin and Rajka [10]. The study protocol conformed to the ethical guidelines of the Yokohama City University's ethics committee, and informed consent was obtained from each patient prior to enrollment in the study. Atopic dermatitis patients were all treated with topical ointments (moderate corticosteroids, alclometasone dipropionate and/or moisturizing cream) with or without antihistamines, according to the severity of itching, at least 3 weeks before measuring the proteins. AD patients on systemic immunomodulatory therapy, such as systemic corticosteroids or cyclosporine, were excluded from the study. Topical FK506 was used in some of the patients only on their faces. A severity score for each eruption (from 0 to 4) was evaluated for erythema, papule, dry skin/scale, scratch mark, erosion/crust and lichenification. The local severity score of a measured lesion was presented by the sum of the respective severity scores (0–24) based on the modified scoring scale of each eruption shown in the SCORAD index [11], and clinical features shown by Leung [12]. Clinical characteristics of AD patients are summarized in table 1.

Among the 36 patients, 16 (11 males and 5 females, age range 4–60 years, average 31.9 years) agreed to a blood test at the same time as HL samples were collected. Blood samples were obtained

from these patients to measure the number of eosinophils and the serum levels of total IgE and LDH. No selection of patients was made for the blood analysis.

As normal controls, HL samples were collected from 16 healthy volunteers (10 males and 6 females, age range 22–58 years, average 31.4 years). Healthy volunteers had no current or past history of atopic diseases (including bronchial asthma, allergic rhinitis and conjunctivitis). HL samples were also collected from patients with psoriasis, which is considered to exhibit the Th1-type immune response and clearly differs from AD in the pathogenesis. Ten patients with psoriasis (7 males and 3 females, age range 38–75 years, average 52.8 years) were recruited after giving their informed consent. Since psoriasis occurs mainly in adults and is rarer in infants than is AD, the mean age was higher among psoriasis patients than AD ones (table 1). All psoriatic patients were diagnosed by clinical symptoms and skin biopsy. Patients with pustular psoriasis and psoriatic erythrodermia were excluded from this study. Topical treatments were moderate corticosteroids and/or moisturizing cream at least 3 weeks before measuring the proteins. No patients had received systemic immunomodulatory therapy.

Preparation of HL Samples

HL samples of skin were obtained by tape stripping with skin tapes (25 × 25 mm; HL Checker; Asahi Med, Tokyo, Japan). The tape was attached to a skin lesion on the patient's forearm, weakly pressed with a finger, and detached. An additional HL sample was obtained with a new tape from the same site of the involved skin. To avoid contamination with topical ointments, patients who were recruited to this study had stopped topical treatments 12 h before the HL sample collection. Similarly, a control HL sample was obtained from a corresponding or close region of uninvolved skin of another arm. HL samples were also obtained from the forearms of healthy volunteers and patients with psoriasis. These HL samples on the tapes were immediately stored at –20°C until their use for protein analysis. For the analysis, HL proteins on the gluey surface of each skin tape were extracted with 50 µl of 1% SDS in 10 mM sodium phosphate buffer (pH 6.8) using a plastic scraper. The extracts from 2 tapes were combined and sonicated 3 times for 1 s each. Protein concentrations of the extracts were determined using a Bio-Rad DC protein assay kit (Bio-Rad, Hercules, Calif., USA). In most cases, 60–150 µg of total proteins were recovered in the 100-µl HL extract from each skin site. These samples were directly subjected to immunoblotting analysis of skin proteins, except for α-enolase. In the case of α-enolase, it was often difficult to distinguish the specific band from close nonspecific bands. Therefore, the sonicated extracts were centrifuged at 15,000 rpm for 20 minutes, and the resulting supernatants were used for the analysis.

SDS-PAGE and Immunoblotting Analyses

SDS-PAGE was performed on 12.5 or 14% polyacrylamide gels by Laemmli's method using a Mini-Protein electrophoresis apparatus (Bio-Rad). HL protein samples were diluted with the SDS sample buffer, and 1 protein sample containing 6 µg of protein was applied per lane, except for α-enolase where 2 µg of protein was applied to each lane. For immunoblotting, proteins separated on gels were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, Mass., USA). The membranes were blocked with 5% (w/v) skim milk in PBS at 4°C overnight,

washed 3 times with 0.1% Tween 20 in PBS and incubated with a specific antibody (×1,000 dilution) at room temperature for 1 h. The incubated membranes were washed 3 times, incubated with horse radish peroxidase (HRP)-labeled anti-mouse IgG antibody (×1,000 dilution) or HRP-labeled anti-rabbit IgG antibody (×1,000 dilution; Vector Laboratories, Burlingame, Calif., USA), and washed 3 times. The washed membranes were finally visualized by the enhanced chemiluminescence method (GE Healthcare, Amersham, UK). Relative intensity of specific bands was quantified by the NIH image.

Estimation of HL Hydration and Transepidermal Water Loss

HL hydration and transepidermal water loss (TEWL) were measured using Corneometer® CM825 and Tewameter® TM300 with a Multi Probe Adapter MPA (CK Electronic GmbH, Germany) in an ordinary air-conditioned room at the clinic (temperature 24–29°C, humidity approx. 40–50%).

Statistical Analysis

Statistical analysis of the data was carried out to compare the levels of each skin protein between AD patients and normal controls or to evaluate the correlation between the protein levels and the clinical severity scores, laboratory data, skin moisture or TEWL. The Mann-Whitney U test was used to compare 2 groups. $p < 0.05$ was considered statistically significant for this test. Correlation analysis was performed by the Pearson product-moment correlation test, in which $p < 0.01$ was considered to be statistically significant.

Results

Expression of 6 Proteins in HL of Skin Lesions

Among many keratin and nonkeratin proteins detected in the HL of atopic skin lesions, we chose 6 (FABP-5, SCCA2, annexin II, apolipoprotein A-I, albumin and α-enolase) and compared their expression levels between 36 AD patients and 16 healthy volunteers. For the analysis, the HL samples were obtained by the tape stripping method from the forearms of the volunteers, and the proteins were extracted with a SDS-containing buffer and applied to immunoblotting. The relative quantities of individual proteins in the HL samples were determined from the intensity of the immunostained bands (fig. 1). The 6 proteins were detected at high levels in the AD skin lesions, but scarcely in the normal controls. The average value of α-enolase in the normal controls was relatively high because of the presence of a few samples having high values. The levels of the 6 proteins including α-enolase in the involved skin were significantly higher than those of normal controls. When the uninvolved and involved skin samples of AD patients were compared, there were significant differences in the levels of FABP-5 and SCCA2, suggesting that their expression reflected the severity of

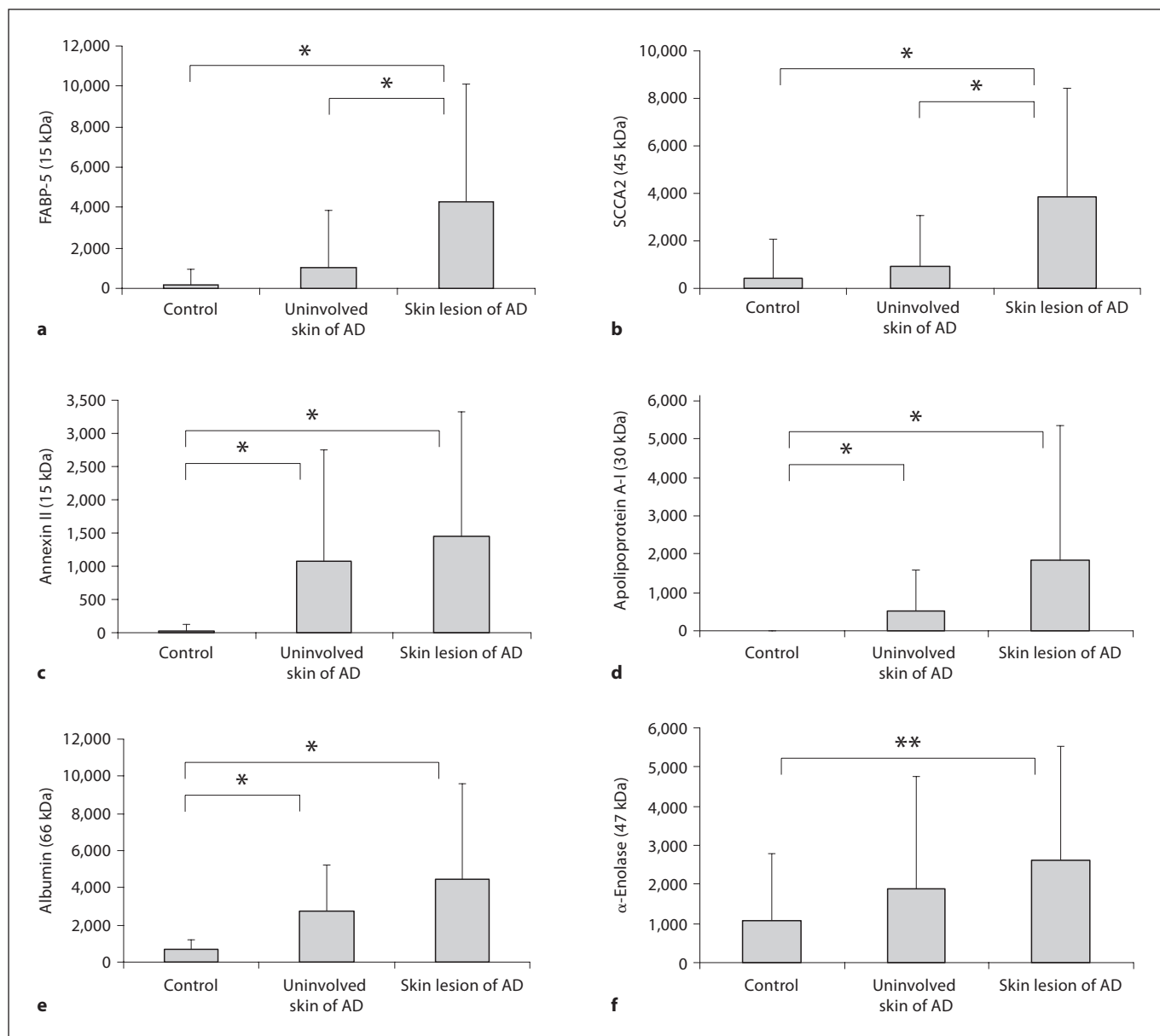


Fig. 1. Comparison of expression levels of 6 proteins in HL samples among normal control skin and uninvolved and involved skin of AD patients. **a** FBP-5. **b** SCCA2. **c** Annexin II. **d** Apolipoprotein A-I. **e** Albumin. **f** α-Enolase. HL samples were obtained by tape stripping from uninvolved or involved skin in the forearms of AD patients and healthy controls, and the HL proteins

were extracted from the tapes and analyzed by immunoblotting. The relative expression levels of individual proteins were determined from the intensity of the immunostained bands. Other experimental conditions are described in 'Materials and Methods'. * $p < 0.001$; ** $p < 0.05$.

the skin lesions. In contrast, annexin II, apolipoprotein A-I, albumin and α-enolase were expressed at relatively high levels even in the uninvolved skin of AD patients, and the first 3 of these proteins were present at significantly higher levels than those of normal controls.

Comparison with Severity of Atopic Skin Lesions

To show the relationship between the expression of the 6 proteins and AD symptoms, the HL level of each protein was compared with the clinical data of atopic skin lesions. FBP-5 ($R^2 = 0.39$, $p < 0.01$) showed statistically

significant correlation with the local severity score of the AD skin, which was determined from the sum of severity scores for the erythema, papule, dry skin/scale, scratch mark, erosion/crust and lichenification (fig. 2a). Albumin in the involved skin showed little correlation with the local severity score ($R^2 = 0.18$; fig. 2b), whereas that in the uninvolved skin correlated with the local severity score in the involved skin of the same patients ($R^2 = 0.25$, $p < 0.01$; fig. 2c).

When compared with the severity score of each eruption, the FABP-5 level showed weak correlation with the scores of erythema ($R^2 = 0.23$, $p < 0.01$), erosion/crust ($R^2 = 0.20$, $p < 0.01$) and lichenification ($R^2 = 0.31$, $p < 0.01$; fig. 3a–c). Similar correlation was obtained for annexin II with erythema ($R^2 = 0.24$, $p < 0.01$; fig. 3d). In addition, apolipoprotein A-I ($R^2 = 0.22$, $p < 0.01$) and albumin ($R^2 = 0.22$, $p < 0.01$) levels in the involved skin tissues showed correlation with erosion/crust (fig. 3e, f).

Comparison with HL Hydration and TEWL

Next, the HL levels of the 6 proteins were compared with HL hydration and TEWL, which are well known as parameters for the barrier function of skin. The albumin level in the involved skin showed good correlation with TEWL ($R^2 = 0.63$, $p < 0.01$; fig. 4a), while that in the uninvolved skin showed reverse correlation with HL hydration ($R^2 = 0.28$, $p < 0.01$; fig. 4c). Similarly, the levels of apolipoprotein A-I in the involved and uninvolved skin samples showed positive or reverse correlation with TEWL ($R^2 = 0.57$, $p < 0.01$) and HL hydration ($R^2 = 0.22$, $p < 0.01$), respectively (fig. 4b, d). The other 4 proteins showed no correlation with HL hydration and TEWL. These results indicate that the levels of the 2 serum proteins in the involved or uninvolved skin reflect the impairment of the skin barrier function.

Comparison with Clinical Data of Blood Samples

Blood samples were obtained from the 16 of 36 patients with AD who agreed to the blood tests, and their data were compared with the HL protein levels in the AD lesions (fig. 5). SCCA2 showed significant correlation with the serum levels of total IgE ($R^2 = 0.52$, $p < 0.01$; fig. 5b) and weak, not significant correlation with the number of eosinophils ($R^2 = 0.20$, $p > 0.05$; fig. 5a) and LDH ($R^2 = 0.23$, $p > 0.05$; fig. 5c). In addition, both FABP-5 ($R^2 = 0.67$, $p < 0.01$) and annexin II ($R^2 = 0.37$, $p < 0.05$) showed good correlation only with the serum total IgE level (fig. 5d, e). Albumin showed weak, insignificant correlation with the number of eosinophils ($R^2 = 0.21$, $p > 0.05$; fig. 5f). None of the other proteins

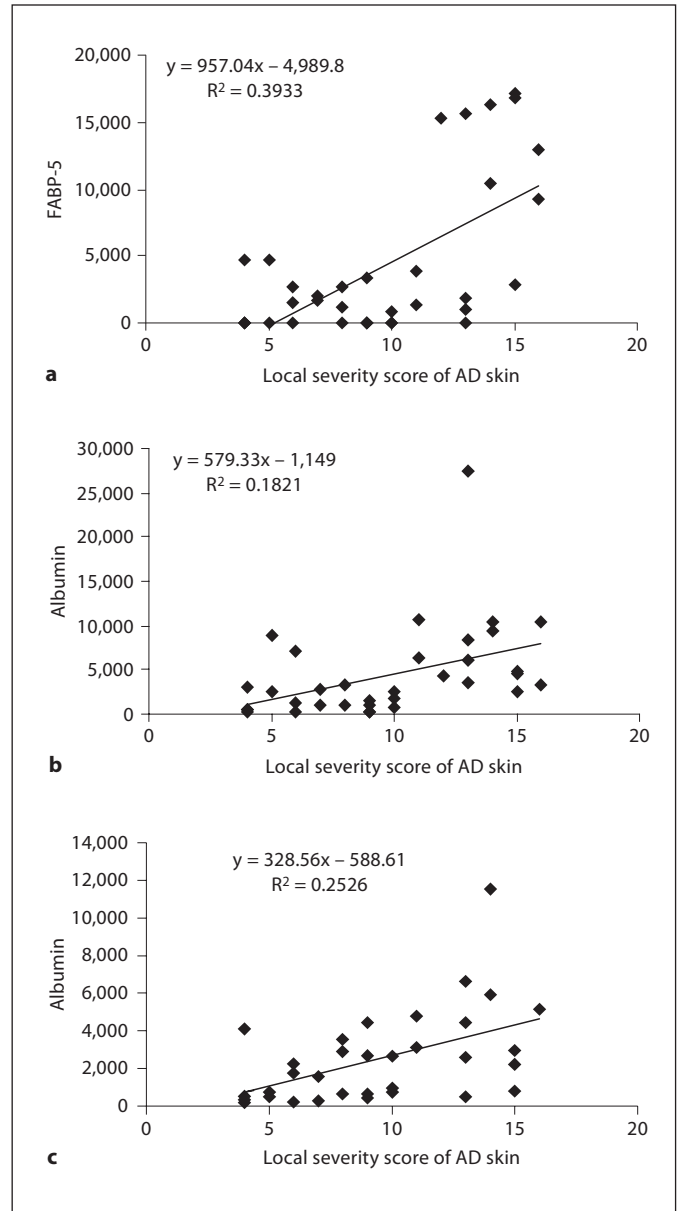


Fig. 2. Correlation of HL levels of FABP-5 and albumin in uninvolved or involved skin of AD patients with local severity scores of the involved AD skin. **a** FABP-5 and local severity score in involved skin. **b** Albumin and local severity score in involved skin. **c** Albumin in uninvolved skin and local severity score of the involved skin in the same patients. Other experimental conditions are described in 'Materials and Methods'.

showed correlation with the blood data. These results suggest that among the 6 proteins tested, SCCA2 might be the most useful biomarker capable of reflecting the laboratory blood data.

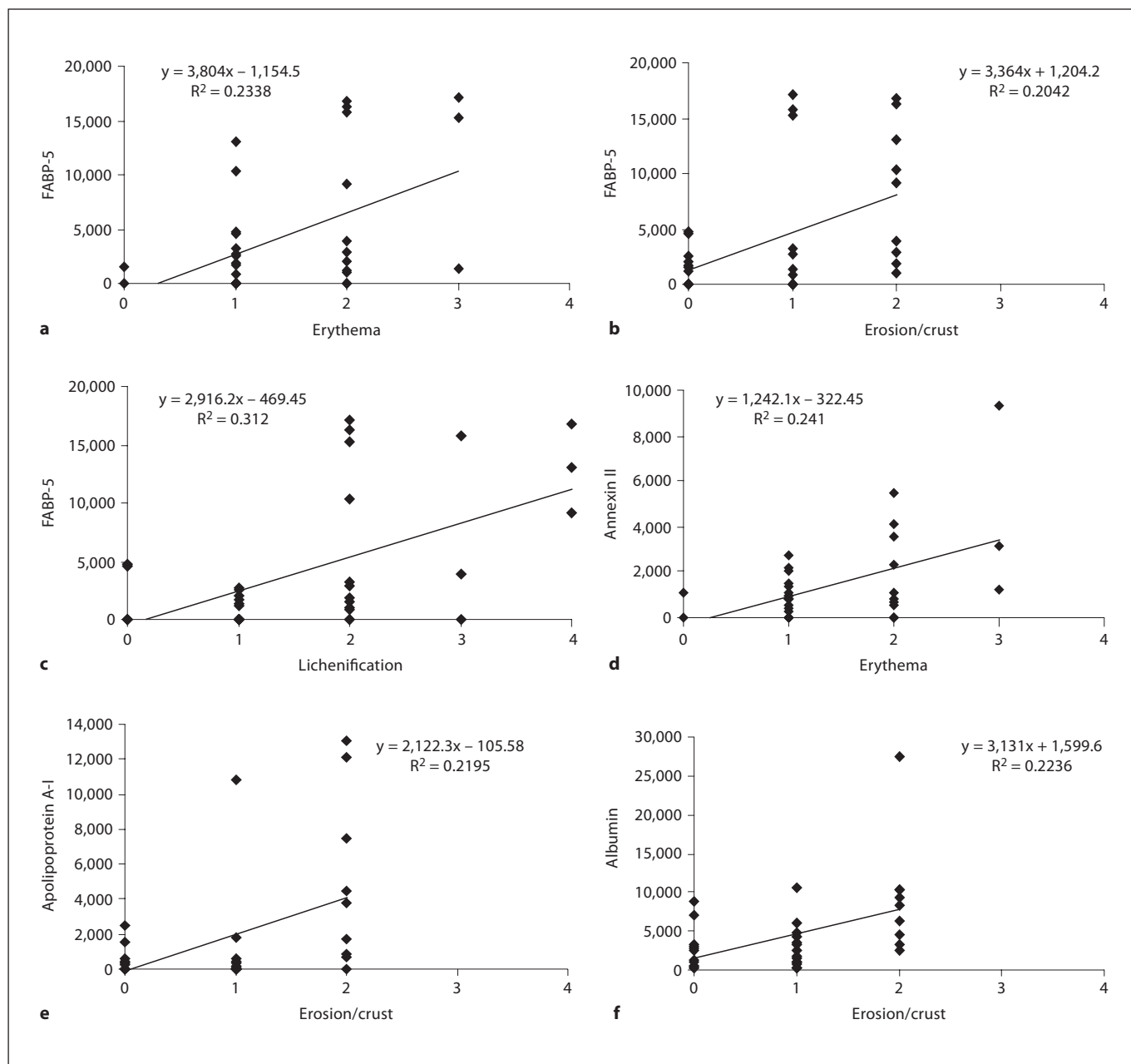


Fig. 3. Relationship between protein levels in involved AD skin and severity score of each eruption. **a** FABP-5 and erythema. **b** FABP-5 and erosion/crust. **c** FABP-5 and lichenification. **d** Annexin II and erythema. **e** Apolipoprotein A-I and erosion/

crust. **f** Albumin and erosion/crust. Proteins other than albumin did not show significant correlation between their levels in the uninvolved skin and the eruptions.

Expression of 6 Proteins in Skin Lesions of Psoriasis

Psoriasis, a common, chronic, inflammatory and proliferative condition of the skin which occurs with or without itching, is a pathologically and clinically different disease from AD. To test whether the HL expression of

the 6 proteins is specific for AD, we analyzed their expression in the HL samples of skin from the patients with psoriasis (fig. 6). The total severity of the psoriasis lesions was scored from 0 to 4. FABP-5 ($R^2 = 0.59$, $p < 0.01$; fig. 6a), α -enolase ($R^2 = 0.49$, $p < 0.05$; fig. 6b) and an-

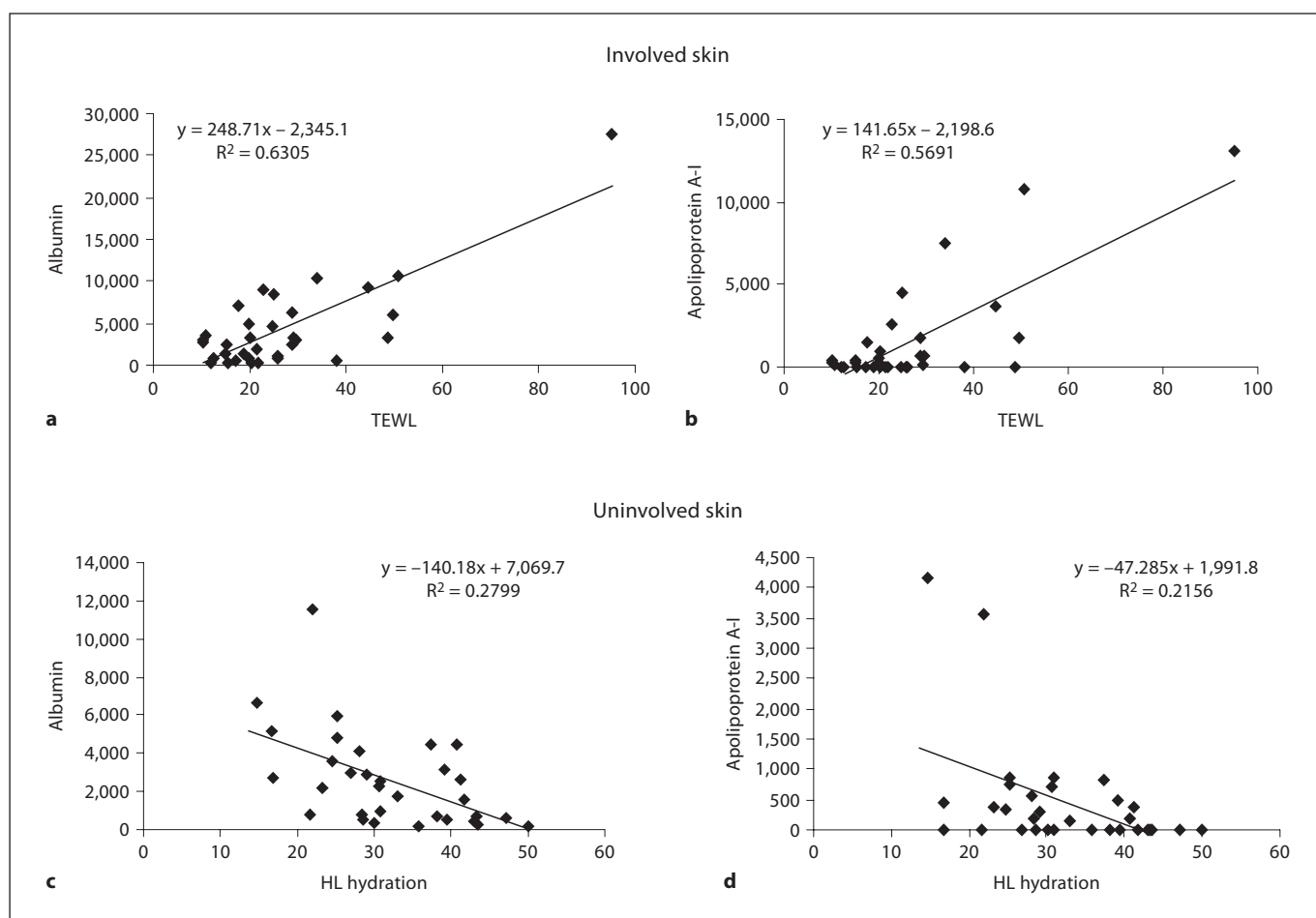


Fig. 4. Correlation of HL levels of albumin and apolipoprotein A-I in uninvolved and involved skin of AD patients with HL hydration and TEWL in the measured areas. **a** Albumin and TEWL of involved skin. **b** Apolipoprotein A-I and TEWL of involved skin. **c** Albumin and HL hydration of uninvolved skin. **d** Apolipoprotein

tein A-I and HL hydration of uninvolved skin. Albumin and apolipoprotein A-I levels in involved skin showed correlation with TEWL levels, while the 2 protein levels in uninvolved skin showed weak reverse correlation with HL hydration.

nexin II ($R^2 = 0.55$, $p < 0.05$; fig. 6c) showed relatively strong correlation with the local severity. SCCA2 showed weak correlation ($R^2 = 0.25$, $p > 0.05$; fig. 6d).

Unexpectedly, albumin levels were very low in the psoriasis skin lesions with severe inflammation even with scratch marks, as compared with those in the involved and uninvolved skin of AD patients. Typical examples from psoriasis and AD patients are shown in figure 7. This implies that the skin barrier is impaired markedly in AD lesions, but not in psoriatic lesions. The high albumin levels in the uninvolved skin of AD patients also suggest that expression of albumin in the HL does not merely result from scratches or other skin damage.

As shown in table 1, the mean age, gender balance and ongoing treatment in the psoriasis group considerably differed from those of the AD group. To correct for the differences, we performed an alternative analysis, in which 2 older psoriasis patients, who had been treated without corticosteroids, were removed from the psoriatic group. This lowered the mean of age from 52.8 to 48.6 and improved the differences in topical steroid treatment (from 60 to 75%) with a little change in the gender balance (from 70% male to 75%) in the psoriatic group. This alteration produced small changes in the correlation between the expression of the marker proteins and the local severity: annexin II ($R^2 = 0.65$, $p < 0.05$), FABP-5 ($R^2 = 0.28$, $p > 0.05$), α -enolase ($R^2 = 0.26$,

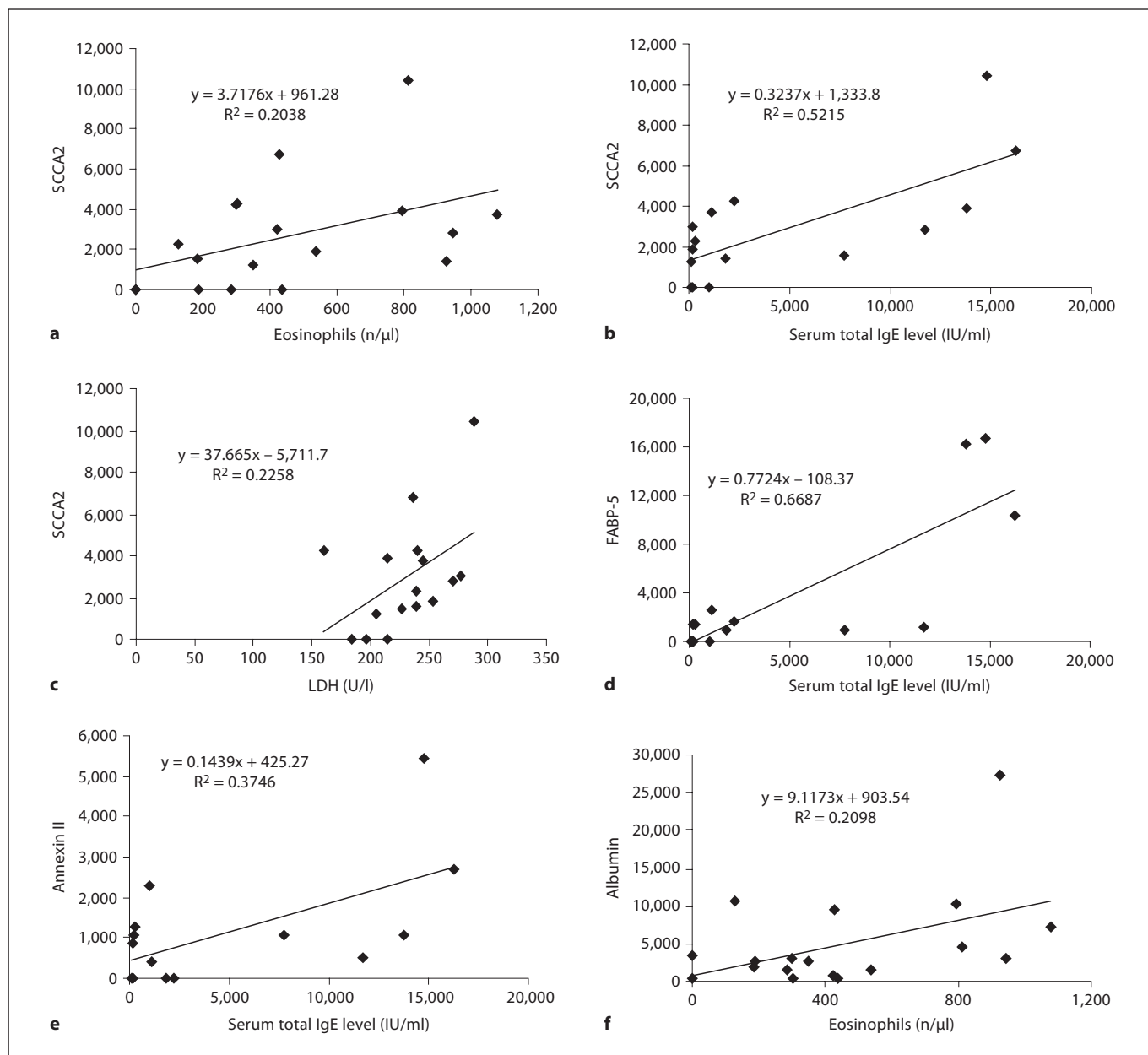


Fig. 5. Correlation of expression levels of 4 HL proteins in AD skin lesions with data of blood samples. **a** SCCA2 and eosinophils. **b** SCCA2 and serum total IgE. **c** SCCA2 and serum LDH. **d** FABP-5 and serum total IgE. **e** Annexin II and serum total IgE. **f** Albumin and eosinophils. SCCA2 showed correlation with all blood

data, eosinophil number and serum total IgE and LDH levels. Both FABP-5 and annexin II showed correlation only with serum total IgE level, while albumin showed very weak correlation with eosinophil number.

$p > 0.05$) and SCCA2 ($R^2 = 0.25$, $p > 0.05$; data not shown). Even in this modified analysis, the averaged albumin level in the psoriasis group (831 ± 733.5) was far lower than that in the AD group ($4,462 \pm 5,172.1$, $p = 0.005$).

Discussion

In this study, we analyzed the expression of 6 proteins in the HL of uninvolved and involved skin tissues of AD and psoriasis patients, as well as healthy controls. All the

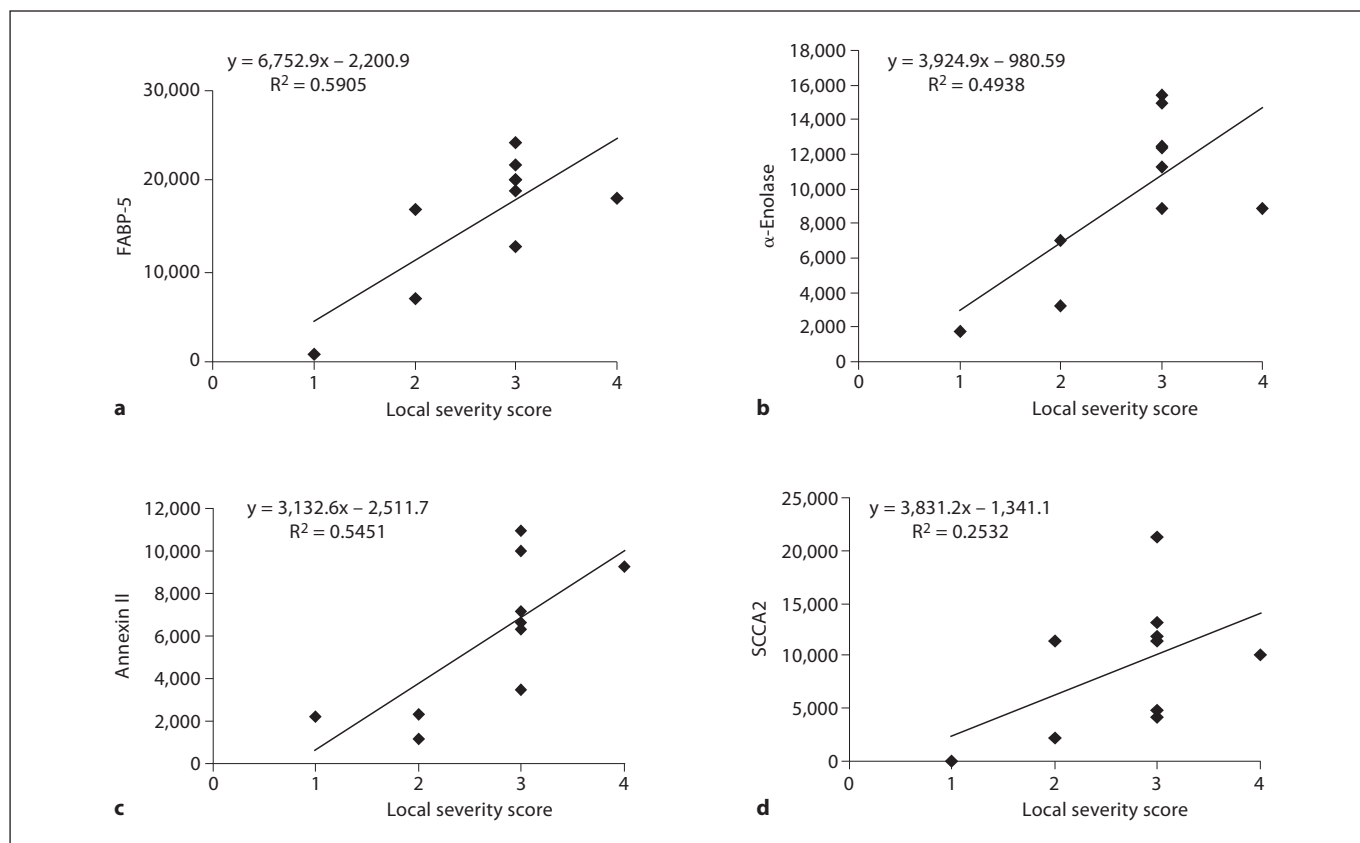


Fig. 6. Correlation of expression levels of 4 HL proteins in skin lesions of psoriasis with local severity score. **a** FABP-5. **b** α -Enolase. **c** Annexin II. **d** SCCA2. FABP-5, α -enolase and annexin II showed strong correlation with the total lesion severity score. SCCA2 showed weak correlation.

proteins showed greatly increased expression in the AD skin lesions as compared with the control samples.

FABP-5, a member of the fatty acid-binding protein family, is mainly expressed in the skin, but also in other tissues such as heart, intestine and adipose tissue [13]. In the skin, FABP-5 is thought to play an important role in the differentiation of keratinocytes. FABP-5 is also known as psoriasis-associated fatty acid-binding protein homolog (PA-FABP) because its expression level is greatly elevated in involved skin lesions of psoriasis [13]. FABP-5 is localized in the epidermis just below the HL, but it is not found in the basal layer of psoriatic skin [13]. FABP-5 is expressed in the epidermal transit amplifying cells [14], and these cells are increased in number in lesions of psoriasis [15, 16]. In the present study, FABP-5 showed close correlation with the local severity and the score of erosion/crust and lichenification in AD lesions, as well as the IgE level in serum. Because of its high expression in psoriasis, FABP-5 is not a specific marker for AD, but seems

to reflect inflammatory activity of skin lesions in both AD and psoriasis. Its aberrant expression may also be related with the enhanced proliferation of keratinocytes. Possible roles of FABP-5 in the Th2-type response and IgE production remain to be clarified.

We also found weak correlation between annexin II and erythema. Annexin II is an intracellular protein, but also exists on the cell surface [17]. It is involved in cytoskeletal regulation. Annexin II on the cell surface is able to bind tissue plasminogen activator, and this binding enhances pericellular proteolytic activity [17]. Such activity may be involved in AD activity.

α -Enolase also showed a relatively high expression in AD skin as compared with normal controls, but its correlation with AD symptoms was not significant. α -Enolase showed significant correlation with the local severity of psoriasis. It was detected at a high level in HL, which may depend on disruption of epidermal cells in AD skin as α -enolase is an intracellular enzyme for glycolysis. Re-

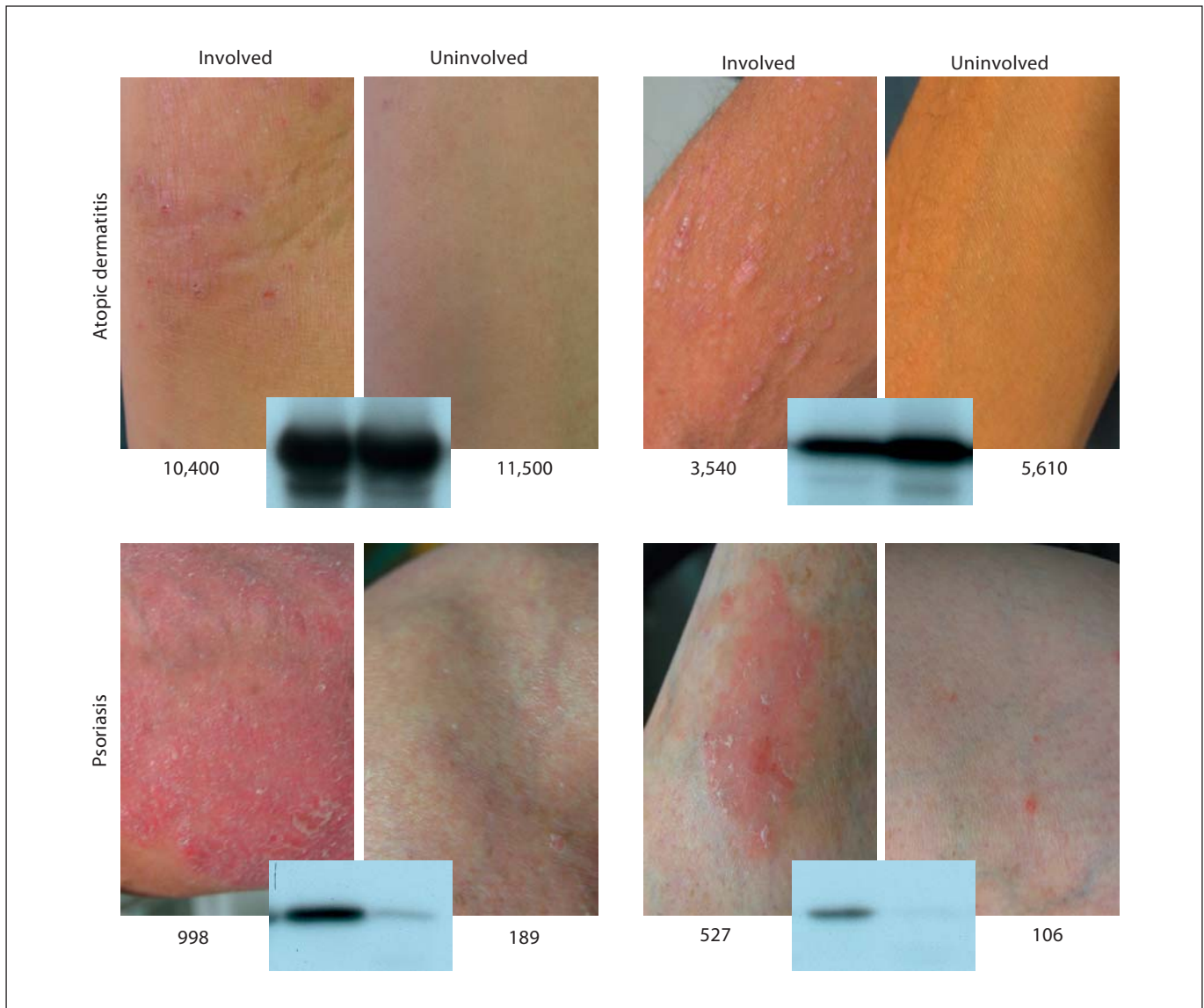


Fig. 7. Typical examples of involved and uninvolved skin conditions of AD and psoriasis patients and immunoblots of albumin in the HL samples. Insets indicate immunoblots of albumin in each HL sample, and the values represent the relative intensity of

albumin bands. Note that the albumin levels were very high even in the uninvolved skin of AD patients, but low in the psoriasis skin lesions with severe inflammation even with scratch marks.

cent studies have shown that α -enolase also acts as a cell surface receptor for plasminogen activator in both mammalian cells and pathogenic bacteria, possibly allowing these cells to invade human tissues [18]. It is also known that autoantibody against α -enolase is involved in autoimmune disease [19]. Many previous studies have shown that sera of patients with severe AD contain autoantibody specific for self-proteins, supporting the hypothesis of autoreactivity as a pathogenic factor in AD [20, 21]. Thus,

it also seems possible that α -enolase is involved in the pathogenesis of AD as an autoreactive factor.

SCCA2 showed relatively poor correlation with AD symptoms, but its level in the HL correlated significantly with that of IgE and weakly with that of LDH and the eosinophil number. Thus, the expression of SCCA2 seems to reflect the immunological state of AD patients. SCCA belongs to the serine proteinase inhibitor family serpin. SCCA1 inhibits papain-like cysteine proteinases such as

cathepsins K, L and S [22, 23], while SCCA2 inhibits chymotrypsin-like serine proteinases such as cathepsin G and mast cell chymase [24]. Past studies have suggested that SCCAs may be involved in psoriasis and AD, probably as proteinase inhibitors [25, 26]. The mRNAs of SCCA1 and SCCA2 are highly expressed in the involved skin of AD patients, and the Th2-type cytokines IL-4 and IL-13 – but not the Th1-type cytokines IFN- γ or TNF- α – induce expression of SCCAs in cultured keratinocytes [26]. Based on the present and past findings, it seems likely that the elevated level of SCCA2 in the HL of AD patients reflects the Th2-type allergic response. SCCA2 may be used as a biomarker for the allergic response.

Both albumin and apolipoprotein A-I are major serum proteins. The HL levels of these proteins showed positive or reverse correlation with TEWL and HL hydration. It has been shown that TEWL and HL hydration correlate well with the severity of AD patients, suggesting that dysfunction of the epidermal barrier is involved in the pathogenesis of AD [27, 28]. The permeability barrier dysfunction is in part attributed to a decreased ceramide content in the HL of AD skin [29]. In addition, recent studies revealed that dysfunction of filaggrin is also associated with AD [30–32]. Filaggrin plays an important role in the barrier function of the skin [33], and the loss-of-function mutations of this gene cause ichthyosis vulgaris [32] and appear to be involved in the pathogenesis of AD [30, 31]. It seems obvious that the increased levels of albumin and apolipoprotein A-I, as well as the high TEWL level, reflect the impairment of the epidermal barrier function. However, the high albumin levels cannot be caused only by scratching or other skin damage because in this study albumin was detected even in the clinically uninvolved skin of AD patients, but scarcely in the severe skin lesions of psoriasis with many scratch marks. It should be noted that the HL level of albumin in uninvolved skin of AD patients correlated well with the total AD activity. This implies that the high level of albumin in the HL of AD patients is an intrinsic skin factor in these patients. Seidenari and Giusti [28] have also reported a correlation between AD activity and TEWL level in uninvolved skin in AD patients. Unlike water, albumin is a high molecular weight protein of 68 kDa. Therefore, in addition to the dysfunction of the epidermal barrier, elevated permeability of blood vessels and the epidermal basement membrane may be required for the high albumin content in HL. These additional factors may limit the albumin level in the HL of psoriatic skin, which often exhibits the dysfunction of the epidermal barrier. The low HL hydration

in AD skin suggests that the osmotic ability of albumin does not affect the HL hydration level.

Because of the low number of psoriasis patients in our study, it is difficult to compare the expression patterns of the marker proteins between AD and psoriasis. Nevertheless, it can be said that FABP-5 and annexin II were highly expressed in both AD and psoriasis skin lesions. Expression of these proteins appears to reflect inflammatory activity or tissue damages of these skin lesions. In contrast, the HL level of albumin in uninvolved skin of AD correlated with the local severity, whereas it was scarcely detected in psoriasis skin. The HL expression of α -enolase appeared to correlate with the local severity of psoriasis skin, but poorly with that of AD skin. It seems possible to discriminate skin conditions between AD and psoriasis by using these different marker proteins.

The clinical conditions of AD patients are generally diagnosed by direct observation of their skin conditions. This subjective method often causes inter-observer and intra-observer variability. In some cases, immunological states of AD patients are assessed by blood analyses such as serum IgE level and eosinophils. In addition, skin conditions can be examined by the measurement of HL hydration and TEWL, which are affected by room temperature and humidity. So far, no methods that use biochemical skin markers have been used for evaluating skin conditions of AD patients. The present study is the first to show that the biochemical markers in the HL can be used to assess the local severity of each lesion in AD patients quantitatively and objectively. Among the 6 proteins tested, FABP-5 seemed to be the best marker to assess the level of skin inflammation. A high serum albumin level in HL appears to be an etiologic factor for AD. Measurements of these and other markers seem to be useful for evaluating the skin conditions of each lesion in AD patients. Since these data indicate directly the level of skin inflammation and barrier function of the measured lesion, more appropriate treatment for each lesion can be chosen using this method. In this point, the present study is distinct from existing criteria, such as blood data and skin scores, which express the total condition of AD.

In our tape-stripping method, HL samples are non-invasively and quickly obtained from the skin lesions of patients at a clinic. Since the immunoblotting analysis of marker proteins required a procedure that is time-consuming (about 2 days) and complicated, we are currently developing a rapid and simple method using ELISA or protein chips by which the whole assay can be completed in a few hours. However, our method obviously requires further validation with an increased

number of HL samples. Moreover, it is unknown whether the elevated expression of FABP-5 and other markers is involved in the pathogenesis of AD or a secondary effect. Aberrant expression of these skin proteins must reflect some specific tissue conditions in AD patients. It seems possible that these proteins, in part, contribute to the development of AD symptoms. When the pathological meanings of the aberrant expression of these marker proteins in AD skin are elucidated, this method would become a more important tool to characterize or evaluate complicated skin conditions of AD patients. Such

biochemical information would be useful for choosing the best clinical treatments for AD patients. Functional studies of the marker proteins are currently under investigation.

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