Increased epidermal filaggrin in chronic idiopathic urticaria is associated with severity of urticaria

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– 국문요약 –

만성두드러기 병인기전에서 피부장벽단백의 역할

만성두드러기는 아토피피부염과 함께 심한 가려움증을 동반한 만성 알레르기 피부질환이다. 그러나 두 질환은 팽진과 습진이라는 각각의 특징적인 피부 병변을 보인다. 최근 아토피피부염은 필라그린의 결핍이 주요 병인기전으로 알려져 있으나 만성두드러기에서 필라그린의 발현 및 기능에 대해서는 아직 밝혀진 바가 없다. 본 연구에서는 만성두드러기 (16 명) 및 성인형 아토피피부염 (11 명) 환자의 피부조직에서 필라그린의 발현을 정상 대조군 (14 명)과 비교하고, 경표피 수분소실률 및 표피 산도 측정 등 피부장벽 기능 표지자 및 두드러기 중증도와의 상관성을 분석하였다.

필라그린에 대한 면역형광염색 결과 만성두드러기 환자의 병변 부위에서 정상 대조군 및 아토피피부염의 병변에 비해 유의하게 증가하였다 (각 P<0.01). 증가한 필라그린 단백발현은 만성두드러기 환자들의 두드러기 중증도 점수와 유의한 상관성을 보였다 (상관계수=0.538, P <0.05). 또한, 필라그린 유전자 발현이 높은 환자들에서 정상 발현 환자에 비해 유의하게 높은 두드러기 중증도 점수를 보였다 (P <0.05). 표피 산도는 만성두드러기 환자에서 정상 대조군 (P <0.01) 및 아토피피부염군 (P <0.001)에 비해 유의하게 낮게 측정되었으며, 경표피 수분소실률은 아토피피부염군에서 정상 대조군 (P <0.01) 및 만성두드러기 환자 (P <0.01)에 비해 유의하게 높았으며, 정상 대조군과 만성두드러기군 간에는 유의한 차이는 없었다. 로리크린 및 인볼루크린 등

표피세포의 말기 분화 단백의 발현 또한 만성두드러기 환자에서 아토피피부염에 비해 유의하게 높았으며 (P <0.05), 미분화 지표인 케라틴 5 는 상대적으로 낮게 발현됨을 확인하였다 (P <0.05). 또한, 필라그린의 분해산물인 히스티딘을 히스타민으로 전환시키는 효소인 히스티딘 탈탄산효소의 단백 및 유전자 발현이 만성두드러기 환자에서 정상 대조군 및 아토피피부염군에 비해 유의하게 증가함을 확인하였다 (각 P <0.05). 필라그린의 최종 분해산물인 피롤리돈 카복실산 및 유로케닌산의 총 함량은 아토피피부염 환자에서는 감소된 (P <0.05) 반면, 만성두드러기 환자에서는 정상 대조군과 차이를 보이지 않았다. 그러나, 유로케닌산의 두 가지 이성질체 중 시스형의 비율이 만성두드러기 환자에서 유의하게 증가되어 있음을 확인하였다 (P <0.05)

만성두드러기 환자에서는 아토피피부염 환자와 달리 피부장벽단백인 필라그린의 과발현이 관찰되었으며, 두드러기 중증도 점수와도 유의한 상관성을 보였다. 또한 변형된 필라그린의 발현은 경표피 수분소실률 및 표피 산도 증 장벽기능에도 영향을 미치며, 이로 인하여 아토피피부염과는 뚜렷이 구별되는 피부양상을 보임을 알 수 있다. 만성두드러기 환자의 피부에서 증가된 필라그린은 히스티딘으로 분해되고, 증가된 히스티딘 탈탄산효소에 의해 히스타민의 과잉 생성으로 이어질 수 있다. 또한, 히스티딘의 분해산물 중 시스형의 유로케닌산이 상대적으로 증가된 만성두드러기 환자의 피부에서는 이로 인한 비만세포의 탈과립, 신경전달물질 분비의 증가로 두드러기 활성도 증가에 기여할 수 있을 것이다.

핵심어 : Chronic idiopathic urticaria, Filaggrin, Skin pH, Trasepidermal water loss, Urticaria activity score

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INTRODUCTION

Chronic idiopathic urticaria (CIU) is defined as the repeated occurrence of transient (≤ 24 hours) wheals and/or angioedema lasting for more than 6 weeks without an eliciting cause (Zuberbier et al., 2009a). Similar to atopic dermatitis (AD), CIU is characterized by immune aberrations, severe pruritus and unpredictable aggravations which lead to a substantial impact on quality of life (O'Donnell et al., 1997; Grob et al., 2005; Powell et al., 2007; Fonacier et al., 2010). As the prevalence of CIU has been reported to reach 1.0% in the general population, the socioeconomic burden of this disease is remarkable (Powell et al., 2007). The cause of CIU is heterogeneous, as many triggering and aggravating factors are involved in its pathogenesis, including physical stimuli, drugs, infections, and autoimmune mechanisms. Recent guidelines (Zuberbier et al., 2009b) recommend to identify and to avoid underlying causes of CIU as the main goal for the treatment. However, it is very difficult to find and eliminate the causes of CIU. Further understanding of the pathophysiology in CIU patients with severe and refractory to conventional treatments is still required.

Although the cutaneous manifestation of CIU, i.e. fleeting wheals is distinct

from AD, some features are shared by these two common skin diseases. Severe pruritus, perivascular inflammatory infiltration and epidermal involvement are observed in both CIU and AD (Hermes et al., 1999; Ying et al., 2002). The epidermal barrier defects associated with filaggrin deficiency play a crucial role in the AD pathogenesis (Kim and Leung, 2012). Not only does genetic impairment lead to skin barrier protein abnormalities and immune dysregulation, but also continuous physical stimulation to the skin by itch and scratch vicious cycle can cause chronic inflammation in patients with AD (Leung, 2013). However, epidermal barrier defects in CIU have not been studied.

The goal of this study was to compare the expression of filaggrin in skin from CIU, AD and normal subjects, and to investigate whether altered filaggrin expression is associated with CIU severity.

METHODS

Subjects

Participants with CIU, AD and non-atopic healthy controls, aged 20 to 70 years, were enrolled at Ajou University Hospital in Suwon, Korea and National Jewish Health in Denver, Colorado. Subjects included 14 Korean healthy controls (mean age, 37.5±8.7 years) with no history of allergic and skin diseases, 16 Korean patients with CIU (mean age, 41.7±10.2 years; mean urticaria activity score (UAS) 12.1±2.9), and 11 patients with AD (8 Korean and 3 European American; mean age, 34.9±11.1 years) whose onset were after the age of 20. Table 1 shows clinical characteristics of the three study groups. None of the subjects had received systemic corticosteroids or immunomodulators including cyclosporine, methotrexate and anti-IgE previously, and none had received antihistamines or topical corticosteroid prior to enrollment in our study. The study was approved by the institutional review board at the Ajou University Medical Center and National Jewish Health. All subjects gave written informed consent before participation in the present study.

To investigate whether increased filaggrin expression can be associated with physiologic function of epidermis, transepidermal water loss (TEWL, Tewameter®

TM300, Courage+Khazaka electronic GmbH, Germany) and skin surface pH (PH900®, Courage+Khazaka electronic GmbH, Germany) on the lesional skin of CIU and AD patients and normal subjects were obtained. Two-millimeter punch biopsies were collected from wheals of CIU patients and eczematous AD lesions and uninvolved skin of the same patients with CIU and AD, and normal healthy skin. The skin biopsies were submerged immediately in either Tri-Reagent (Molecular Research Center, Inc, Cincinnati, OH) or 10% buffered formalin for real-time RT-PCR and immunohistochemical studies, respectively.

Quantitative real-time RT-PCR

Total RNA was isolated from 2-mm skin biopsy samples by chloroform: phenol extraction and isopropanol precipitation according to the manufacturer's guidelines (Molecular Research Center, Inc). RNeasy Mini Kits (Qiagen, Inc) were used according to the manufacturer's protocol to isolate RNA from cell cultures and to purify RNA from skin biopsies further. One microgram of RNA was reverse-transcribed in a 20-mL reaction containing Random Primers (500 mg/mL; Invitrogen, Carlsbad, CA), dNTP (10 mmol/L; Invitrogen), 5X First Strand Buffer (Invitrogen), DTT (0.1 mol/L; Invitrogen), Superscript III enzyme (200 U/mL; Invitrogen) and

RNase inhibitor (10 U/mL; Invitrogen). Real-time PCR was performed and analyzed by the dual-labeled fluorogenic probe method by using an ABI Prism 7300 sequence detector (Applied Biosystems). Primers and probes for human 18sRNA and filaggrin were purchased from Applied Biosystems. Amplification reactions were performed in MicroAmp optical tubes (Applied Biosystems) in a 25-mL volume as previously described (Nomura et al., 2003). Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer's technical bulletin. A standard curve was generated using the fluorescent data from the 10-fold serial dilutions of total RNA of the highest expression sample. This was then used to calculate the relative amounts of target mRNA in test samples. Quantities of all targets in test samples were normalized to the corresponding 18sRNA levels in cultured keratinocytes and skin biopsies.

Filaggrin immunohistochemical staining

Paraffin-embedded tissues were cut at 5 mm and placed on frosted microscope slides. Slides were deparaffinized by using a series of xylene and ethanol washes and then stained using the Cell and Tissue Staining Kit (R&D Systems, Minneapolis, MN). And tissue sections were then blocked with serum blocking

reagent G (R&D Systems) for 1 hour. Slides were stained overnight with mouse monoclonal antibodies against human filaggrin (Vector laboratories (Burlingame, CA) and Abcam (Cambridge, MA) at a 1:500 dilution. We also performed immunohistochemical staining for loricrin, which is another epidermal barrier protein, with mouse monoclonal antibodies (Abcam, Cambridge, MA) at a 1:500 dilution. The secondary antibody was added according to the manufacturer's protocol (R&D Systems). Antibody specificity was confirmed by using purified nonimmune mouse IgG (Southern Biotechnology, Birmingham, AL). All slides were coded before analysis and read blindly to ensure patient anonymity. The intensity of the immunostaining was estimated with the use of microscopy with SlideBook 5.0 (Intelligent Imaging Innovations, Santa Monica, CA).

Measurement of pyrrolidone carboxylic acid (PCA), *cis*-urocanic acid (*cis*-UCA) and *trans*-urocanic acid (*trans*-UCA) from tape stripping samples

Stratum corneum (SC) specimens were obtained with a D-squame standard tape (Cuderm, Dallas, TX, diameter, 2.2 cm) on inner arms. Six consecutive tapes were collected from each subject (10 CIU, 10 AD, and 10 NC). Three amino acid metabolites, PCA and *cis*-/*trans*-UCA were quantitated in SC samples collected from

volar forearm regions (6 consecutive tapes) employing LC/MSMS according to the method previously described (Joo et al., 2012).

Statistical analysis

Descriptive statistics are presented to characterize all subjects included in the analysis. Categorical data are presented as enumerations and percentages. Continuous data are presented as arithmetic mean ± standard deviation or as median (25^{th} percentile, 75^{th} percentile) if the distribution of the data is skewed.

Statistical analysis of gene expression and immunohistochemical staining was conducted by using Graph Pad Prism, version 4.03 (San Diego, CA). Statistical differences between groups were determined by using an unpaired t test with significant differences conferred when $P<0.05$. In cases in which multiple groups were compared with a control, data were analyzed by a 1-way ANOVA, and significant differences were determined by a Dunnett's T3 test.

RESULTS

Filaggrin protein expression is increased in the skin tissue of CIU patients

We compared *FLG* gene expression among three groups using real-time RT-PCR. As illustrated in Figure 1a, relative *FLG* expression was significantly increased in lesional CIU as compared to non-lesional CIU skin ($P<0.01$). In comparison with AD lesions, *FLG* gene expression was significantly increased in lesional skin from CIU patients ($P<0.01$). In contrast, *FLG* gene expression was significantly decreased in both lesional and non-lesional skin from AD patients as compared with normal subjects ($P<0.01$, $P<0.01$, respectively). The *FLG* expression in lesional CIU skin could be classified as two groups (Figure 1a); one group with high *FLG* expression (greater than the mean plus 2 standard deviation of *FLG* gene expression in normal subjects) and one group with normal *FLG* expression. The high *FLG* gene expression group had significantly higher UAS as compared to normal *FLG* gene expression group (13.9 ± 0.90 vs 11.4 ± 3.0, $P<0.05$).

Next, filaggrin protein expression was analyzed in skin biopsies from normal subjects, CIU and AD patients using an immunostaining technique with an antibody directed to filaggrin protein. A significant increase of filaggrin protein expression was observed in lesional CIU skin, without increased expression in non-lesional skin

(Figure 1b). The composite data for filaggrin immunostaining in all samples are shown in Figure 1c. The staining intensity of filaggrin was significantly increased in lesional skin from CIU patients as compared to skin from normal subjects ($P<0.01$) and lesional AD ($P<0.001$). In comparison with non-lesional skin, filaggrin expression was increased in lesional skin of CIU patients, however statistical significance was not noted. It is well recognized that filaggrin expression is decreased in both lesional and non-lesional skin of AD patients, which we confirmed in the present study (Figure 1c). The staining intensities of filaggrin in lesional ($P<0.001$) and non-lesional ($P<0.001$) skin from AD patients were significantly decreased as compared with normal subjects.

Correlation between filaggrin expression and UAS

To examine whether filaggrin expression is associated with UAS, we measured UAS in patients with CIU and investigated correlation between filaggrin expression and UAS. There was a significant positive correlation between UAS and filaggrin staining intensity of lesional CIU skin (correlation coefficient= 0.538, $P<0.05$, Figure 2).

Epidermal physiologic function is enhanced in CIU compared to AD

It has been reported that the deficiency of filaggrin and its acid metabolites results in decreased epidermal hydration and increased TEWL as well as skin pH of stratum corneum (McAleer and Irvine, 2013). Therefore, to investigate whether increased filaggrin expression has an influence on the physiologic function of CIU epidermis, we measured TEWL and pH in the skin from CIU and AD patients. As shown in figure 3a, TEWL was significantly increased in lesional skin of AD patients compared to both skin from normal subjects ($P<0.01$) and lesional skin from CIU patients ($P<0.01$). No significant difference was noted in TEWL between normal subjects and CIU patients. To investigate whether an increase of filaggrin leads to decreased skin pH, we measured skin pH in normal subjects, CIU and AD patients. The mean skin pH was significantly decreased in lesional skin from CIU patients compared to skin from normal subjects and AD patients ($P<0.01$, $P<0.001$, Figure 3b).

Expression of epidermal differentiation markers

During skin differentiation, keratinocytes express different proteins as they

progress upwards forming the different epidermal layers (Candi et al., 2005). We performed immunostaining with loricrin (Fig 4a), keratin 10 (Fig 4b) and keratin 5 (Fig 4c) as markers for the cornified, granular and basal epidermal layers, respectively. As shown in Fig 5, the expression of loricrin was significantly increased in lesional CIU compared with NC and AD, while keratin 5 was significantly decreased in both lesional and nonlesional CIU compared to NC and AD. The expression of keratin 10 was reduced in AD skin, but no significant difference was observed between CIU and NC. These results suggest that the keratinocytes of CIU lesions are at the late differentiation state in general. It also indicates that the reason for increased filaggrin is that there is accelerated skin differentation with upregulation of multiple markers of differentiation, including loricrin, rather than the proliferation of the epidermis is not enhanced.

The proportion of *cis*-UCA was increased in CIU skin

Reduced filaggrin breakdown products, including UCA and PCA leads to decreased skin barrier function of AD skins (McAleer and Irvine, 2013). We measured PCA and both isomers of UCA in skins from NC and patients with CIU

and NC. The concentration of total filaggrin breakdown products (PCA plus UCAs) in CIU lesions (44.5±31.2 μg/mg protein) was not different from NC (63.9±21.5 μg/mg protein), whereas that in AD lesions (21.6±20.2 μg/mg protein, *P* value <0.01) was significantly decreased as compared with NC. The comparison of the concentration of UCA (cis-plus trans-UCA) among three groups was similar with the comparison of total amino acids (10.1±7.8 μg/mg protein in CIU, 14.7±6.3 μg/mg protein in NC, and 4.9±4.9 μg/mg protein in AD (*P* value <0.01 between NC and AD). However, the proportion of *cis*-UCA in CIU lesions (0.44±0.24) was significantly higher than in NC (0.10±0.12, *P* <0.01) and AD lesions (0.14±0.20, *P* <0.001, Fig. 5). In addition, the proportion of cis-UCA was significantly increased in the non-lesional CIU skins (0.51±0.19) as compared with NC (0.10±0.12, *P* <0.001) and AD non-lesions (0.20±0.20, *P* <0.01, Fig. 5).

DISCUSSION

The current study demonstrates, for the first time, that filaggrin expression is increased in lesional CIU skin at both the protein and in RNA level. To increase the validity of our data, we performed immunostaining for filaggrin with two different monoclonal antibodies from Vector Inc. and Abcam Inc. (data not shown), and found that the results are the same. In addition, as loricrin and involucrin expression is also increased in the wheals, a terminal differentiation of the epidermis in CIU lesions appears to be generally accelerated. Interestingly, we also found that protein level of filaggrin was positively correlated with urticaria severity, suggesting that filaggrin may play important roles in CIU. Moreover, among final filaggrin degradation products, cis-UCA, which has been known to induce mast cell degranulation, was relatively increased in CIU patients compared with NC and AD patients. Additionally, we found that TEWL is increased in AD patients compared with normal subjects, and there is no difference in TEWL between CIU patients and normal subjects as we expected.

Filaggrin and its precursor profilaggrin are essential structural proteins in the epidermis (McAleer and Irvine, 2013). It is been well known that they play multiple roles in skin homeostasis and barrier formation against water loss as well as

penetration of allergens and microbes (McAleer and Irvine, 2013). Profilaggrin has a unique structure containing 10-12 filaggrin repeats. It is dephosphorylated and proteolytically processed to release monomeric filaggrin during terminal differentiation of the epidermis. Then filaggrin, as a histidine-rich protein, is deamidated and finally degraded to its component amino acids, including histidine and glutamine. Subsequently histidine and glutamine are metabolized to *trans*-UCA and pyrrolidone-5-carboxylic acid (PCA), respectively. These organic acids, UCA and PCA serve as "natural moisturizing factor (NMF)" which plays an important role maintain epidermal hydration and acidic environment (Brown and McLean, 2012). In addition, filaggrin deficiency causes high skin pH in AD patients (Jungersted et al., 2010). Therefore, we measured pH in the lesional skin from patients with AD and CIU and compared it with normal subjects to examine whether increased filaggrin expression is related to skin pH in CIU patients. Importantly, we found that the skin pH was significantly higher in AD patients and lower in CIU patients compared to normal subjects. This decreased skin pH in CIU lesions supports our finding of increased filaggrin expression leading to an increase of filaggrin breakdown products. Additionally, it may account for reduced staphylococcal colonization in CIU patients

as compared to AD patients despite frequent excoriation in both of these allergic skin diseases. In fact, we demonstrated for the first time the concentrations and the ratio of *cis*-and *trans*-UCA in skin from CIU patients were significantly increased than in skin from AD patients. There have been previous reports evidence that *cis*-UCA is involved in cutaneous inflammation. (Holan et al., 1998; Khalil et al., 2001; Kaneko et al., 2011). It has been reported that *cis*-UCA is a potent inducer of prostaglandin (PG) E2 and cytokines, such as tumor necrosis factor (TNF)-α, IL-6, and IL-8 (Kaneko et al., 2008). Furthermore, *cis*-UCA has a synergistic effect with histamine on PGE2 production from human keratinocytes (Jaksic et al., 1995). As PGE2 has been shown to induce an EP3 receptor-dependent mast cell degranulation and to increase microvascular permeability and infiltration of inflammatory cells into tissues, an increase of PGE2 may contribute to wheal reaction. It has also been reported that *cis*-UCA stimulates mast cell degranulation directly as well as indirectly by inducing neuropeptide release from peripheral sensory nerves in skin (Wille et al., 1999). On the other hand, histidine, the major component amino acid of filaggrin, is a substrate of mast cell histidine decarboxylase (HDC) producing histamine. HDC has been reported to be induced by various stimuli, not only in mast cells and

basophils, but also in neutrophils, T lymphocytes, macrophages and keratinocytes (Laszlo et al., 2001; Inami et al., 2013). As HDC has been observed to be increased in CIU skin (Papadopoulou et al., 2005), greater histidine release may result in the more histamine production in wheals from CIU patients. We also confirmed a significant increase of HDC expression at the levels of both protein (P<0.05 for normal and AD) and mRNA (P<0.05 for normal, P<0.01 for AD) in the lesional skin from CIU as compared to normal and AD skins (data not shown).

Taken together, we prove that increased expression of filaggrin can result in more *cis*-UCA and histamine production, which contributes to increase the disease activity of CIU patients. Even though filaggrin is a structural protein, it turns over quickly and takes only 6 hours to be fully degraded in the skin (O'Regan et al., 2008). Therefore, a transient increase of filaggrin in CIU patients seems to be well correlated with temporary and migrating characteristics of wheals, which don’t last for more than 24 hours at the same skin region of CIU patients (Zuberbier et al., 2009a).

However, the present study has not answered the question why filaggrin expression is increased in wheals from CIU patients yet. We just observed that there

was an accelerated skin differentiation with upregulation of epidermal differentiation markers, such as filaggrin and loricrin, in the wheals from CIU patients. Evans *et al.*(Evans et al., 1993) reported that a positive correlation between extracellular calcium and endogenous PGE2 synthesis during the differentiation of keratinocytes. As calcium is a strong inducer of filaggrin production, an increase of extracellular calcium following PGE2 generation stimulated by *cis*-UCA can enhance filaggrin expression in CIU skin.

Histamine, a major inflammatory mediator in the pathogenesis of CIU, has been found to have an influence on keratinocyte differentiation (Aoyagi et al., 1981; Kanda and Watanabe, 2007; Ishikawa et al., 2009; Gschwandtner et al., 2013). Treating keratinocytes with a lower concentration of histamine (0.1μM – 0.1mM) enhances expression of human beta-defensin D2 and D3, which are late differentiation markers and inhibits proliferation through an increased cAMP and subsequent activation of H2 receptor which leads to calcium increase and keratinocyte differentiation (Aoyagi et al., 1981; Kanda and Watanabe, 2007; Ishikawa et al., 2009). However, incubating keratinocytes with a higher concentration of histamine (10μM – 1mM) reduces skin barrier formation via

activation of H1 receptor (Gschwandtner et al., 2013). In addition, a previous study noted that even with high concentrations of histamine, the expression of differentiation markers is not influenced (Gschwandtner et al., 2013).

CONCLUSION

Taken together, our study suggests future directions to study the potential role of filaggrin in CIU as shown in Fig 6. Filaggrin expression may also be a novel biomarker for CIU severity. The altered filaggrin expression has physiologic effects on TEWL and pH in skin of CIU patients and indicates enhanced barrier function compared to AD patients. Considering that a relatively increased *cis*-UCA among filaggrin breakdown products may contribute to increased urticaria severity, modulating filaggrin expression should be investigated as an alternative treatment modality for severe CIU patients.

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Dermatology F, World Allergy O: EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy* 64: 1417-1426, 2009a

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ABSTRACT

Background: Chronic idiopathic urticaria (CIU) and atopic dermatitis (AD) are common allergic skin diseases associated with severe pruritus. AD skin is characterized by filaggrin deficiency, but it has not been studied in CIU.

Methods: We compared the expression of filaggrin in skin from 16 CIU, 11 AD and 14 normal subjects. Filaggrin expression was evaluated using immunostaining and real time RT-PCR. Urticaria activity score (UAS) was measured in CIU patients to investigate whether altered filaggrin expression is associated with severity of CIU. Additionally, transepidermal water loss (TEWL) and skin pH were measured.

Results: The mean staining intensity of filaggrin was significantly increased in lesional skin of CIU as compared to skin of normal subjects and lesional AD skin ($P<0.01$, $P<0.001$, respectively). A significant correlation was observed between filaggrin staining intensity and UAS in CIU patients (r=0.538, $P<0.05$). CIU patients with high *FLG* gene expression have significantly higher UAS as compared to normal *FLG* gene expression group ($P<0.05$). TEWL was significantly increased in lesional skin of AD patients compared to both skin from normal subjects ($P<0.01$) and lesional skin from CIU patients ($P<0.01$). The mean skin pH was significantly decreased in lesional skin from CIU patients compared to skin from normal subjects

(*P*<0.01) and AD patients (*P*<0.001). Terminal differentiation markers including loricrin and involucrin were also increased in skin from CIU patients. The ratio of *cis*-to-*trans*-UCA was significantly higher in CIU skin compared with AD and NC patients (*P*<0.05).

Conclusions: Filaggrin is overexpressed in lesional CIU skin, and increased filaggrin expression is positively correlated with urticaria severity in CIU. Altered filaggrin expression has physiologic effects on TEWL and pH in skin of CIU patients and indicates enhanced barrier function compared to AD patients. Increased *cis*-UCA, a filaggrin degradation product, can induce mast cell degranuation and neuropeptide release from keratinocytes leads to increased urticaria activity.

Table 1.

	NC n=14	AD n=11	CIU n=16
Age (years)*	37.5±8.7	34.9±11.1	41.7±10.2
Women	12 (85.7%)	7 (63.6%)	9 (56.3%)
Skin Prick Test No. of positive allergens*†	0	5.6±4.7	1.2±1.8
Serum total IgE (KU/L)**	14.8 (6.6-99.3)	192.3 (19-3.829)	138.0 (26-1.188)
Urticaria Activity Score (0-15)	na	na	12.1±2.9

NC, normal controls; AD atopic dermatitis; CIU chronic idiopathic urticaria. *Values given are the mean±S.D. **Values given are the median (min-max). † P=0.014 for AD vs. CIU (Mann-Whitney U test).

Fig 1a.

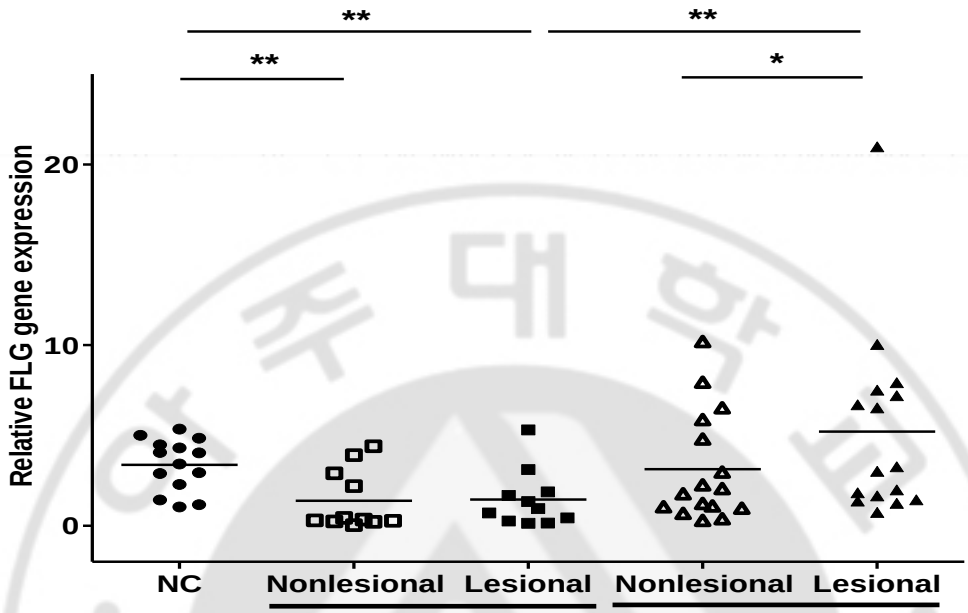

**
**
**
*
Relative FLG gene expression
20
10
0
NC
Nonlesional
Lesional
Nonlesional
Lesional

Fig 1b.

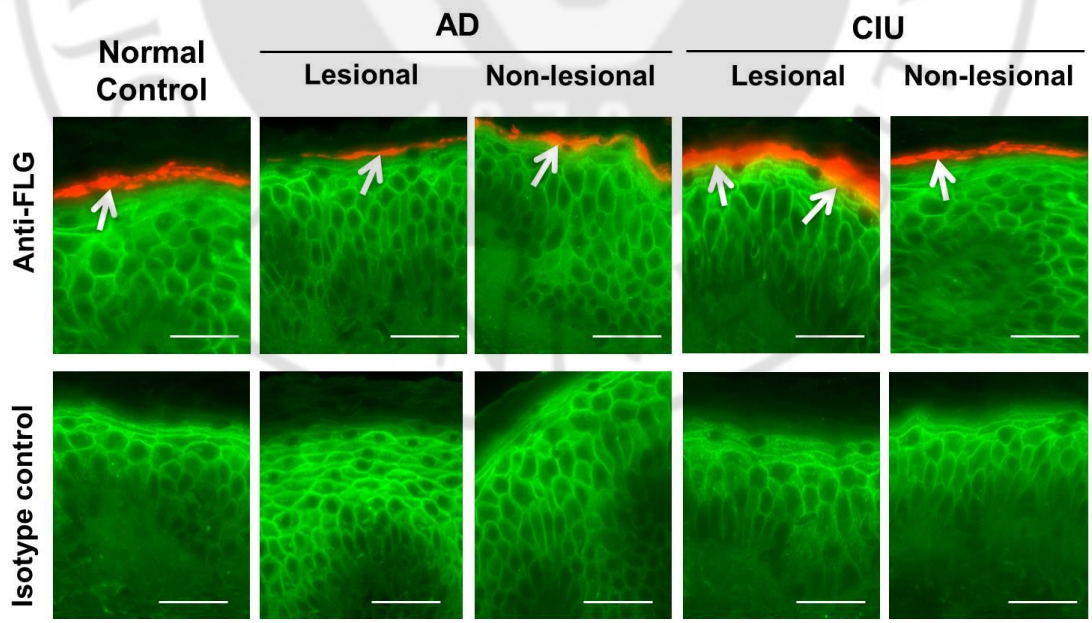

Normal Control
AD
CIU
Lesional
Non-lesional
Lesional
Non-lesional
Anti-FLG
Isotype control

Fig 1c.

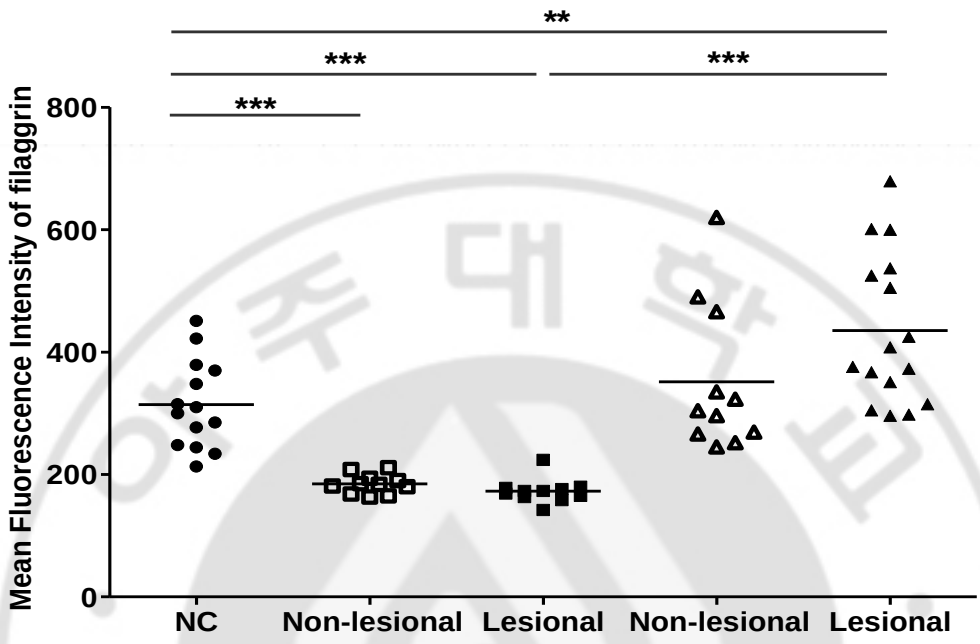

**
***
***
***
800
600
400
200
0
Mean Fluorescence Intensity of filaggrin
NC
Non-lesional
Lesional
Non-lesional
Lesional

Fig 2.

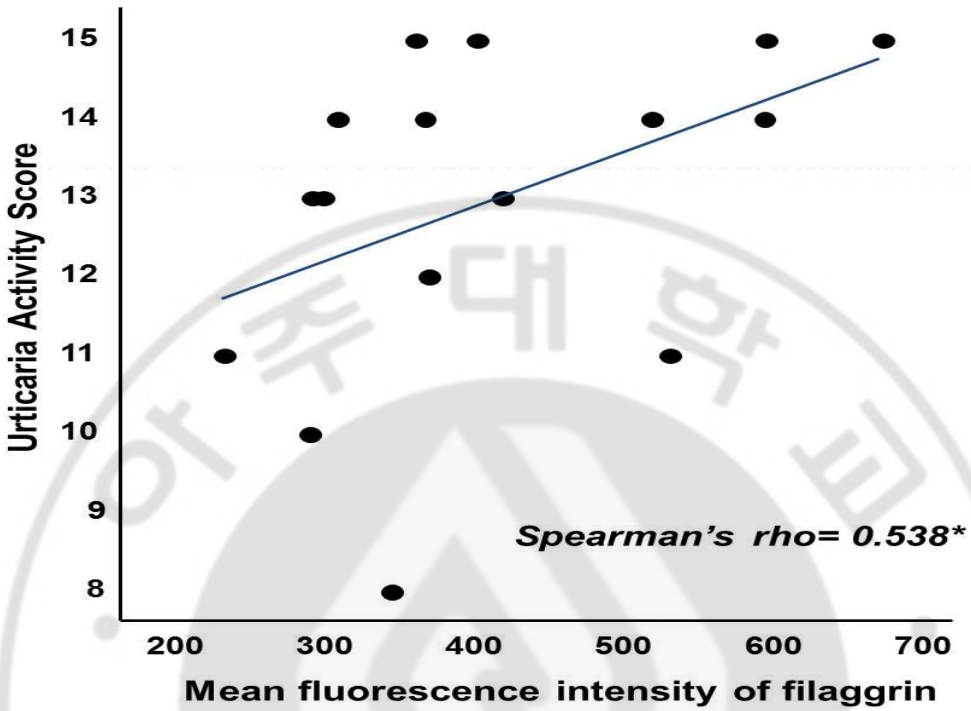

Urticaria Activity Score
15
14
13
12
11
10
9
8
Spearman's rho= 0.538*
200
300
400
500
600
700
Mean fluorescence intensity of filaggrin

Fig 3a.

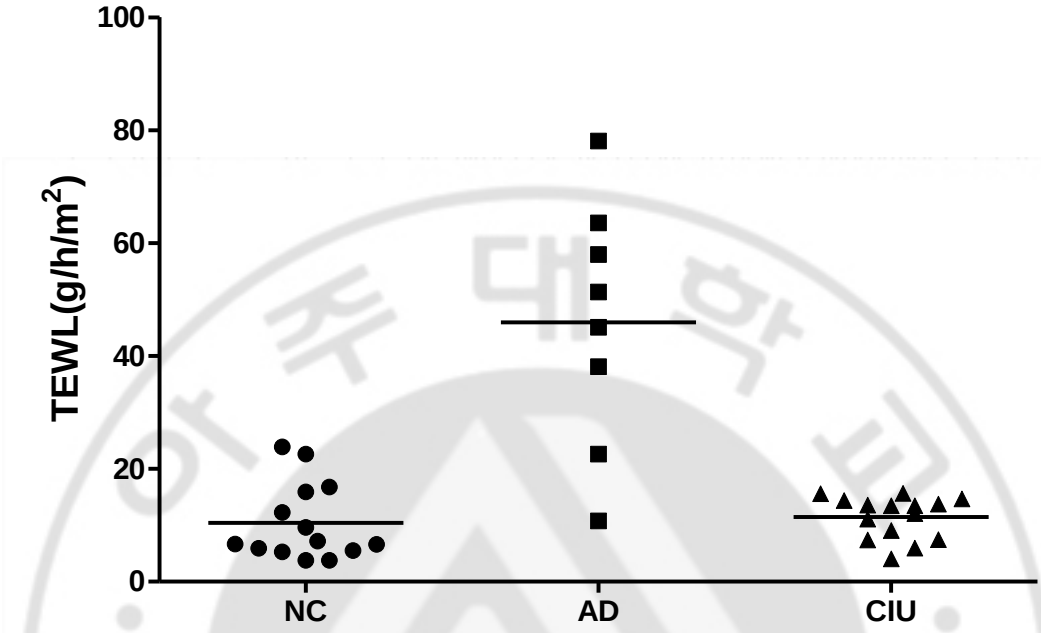

100
80
60
40
20
0
TEWL(g/h/m2)
NC
AD
CIU

Fig 3b.

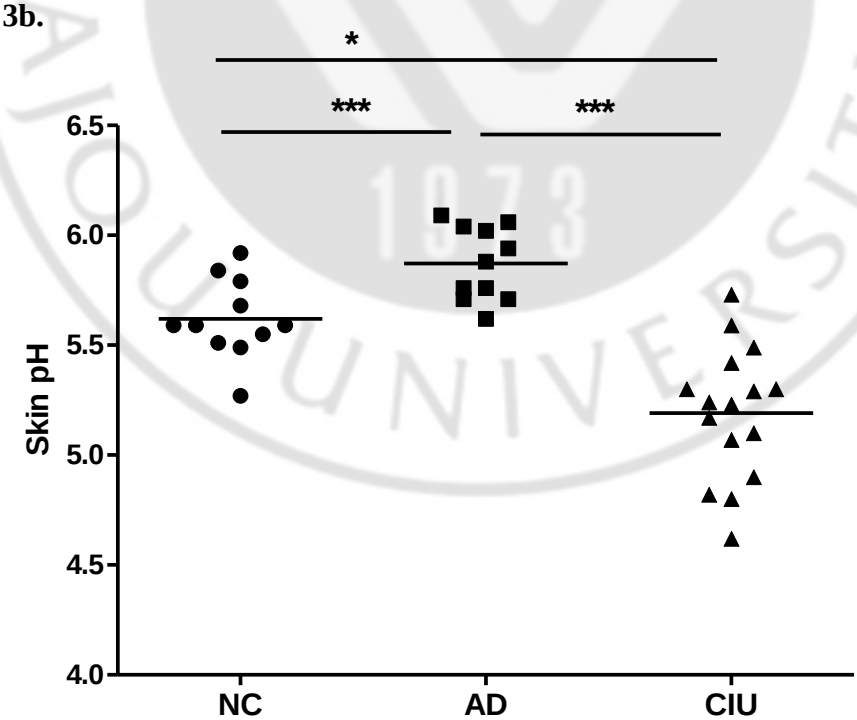

*
***
***
6.5
6.0
5.5
5.0
4.5
4.0
Skin pH
NC
AD
CIU

Fig 4a.

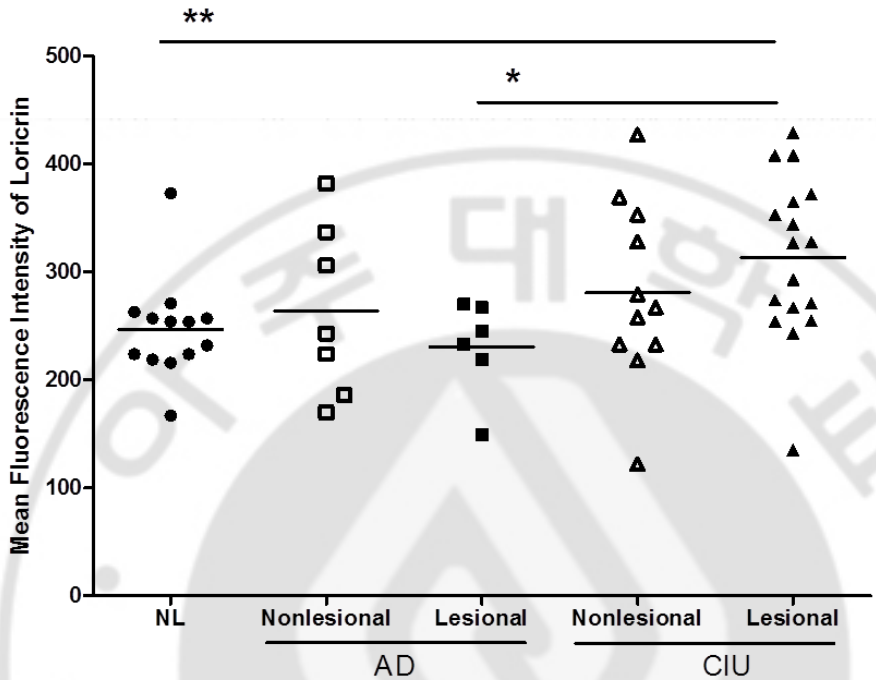

**
*
Mean Fluorescence Intensity of Loricrin
500
400
300
200
100
0
NL
Nonlesional
Lesional
Nonlesional
Lesional
AD
CIU

Fig 4b.

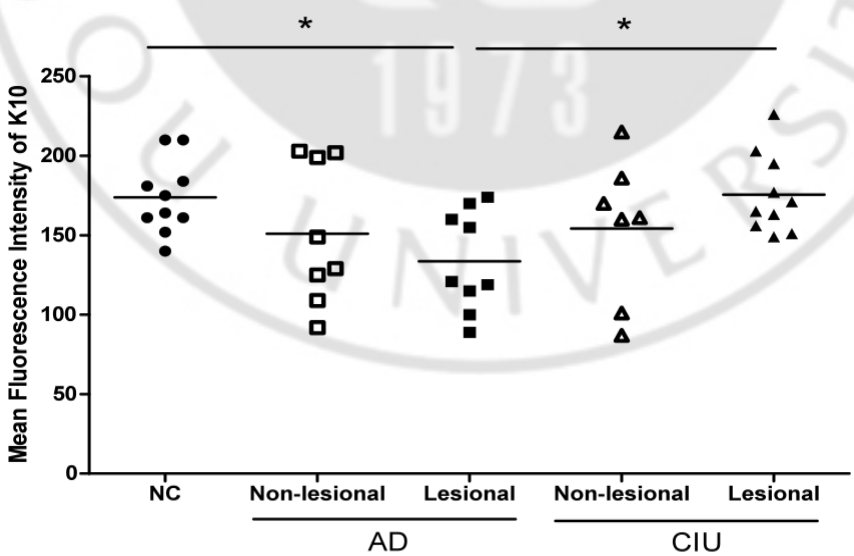

*
*
Mean Fluorescence Intensity of K10
250
200
150
100
50
0
NC
Non-lesional
Lesional
Non-lesional
Lesional
AD
CIU

Fig 4c.

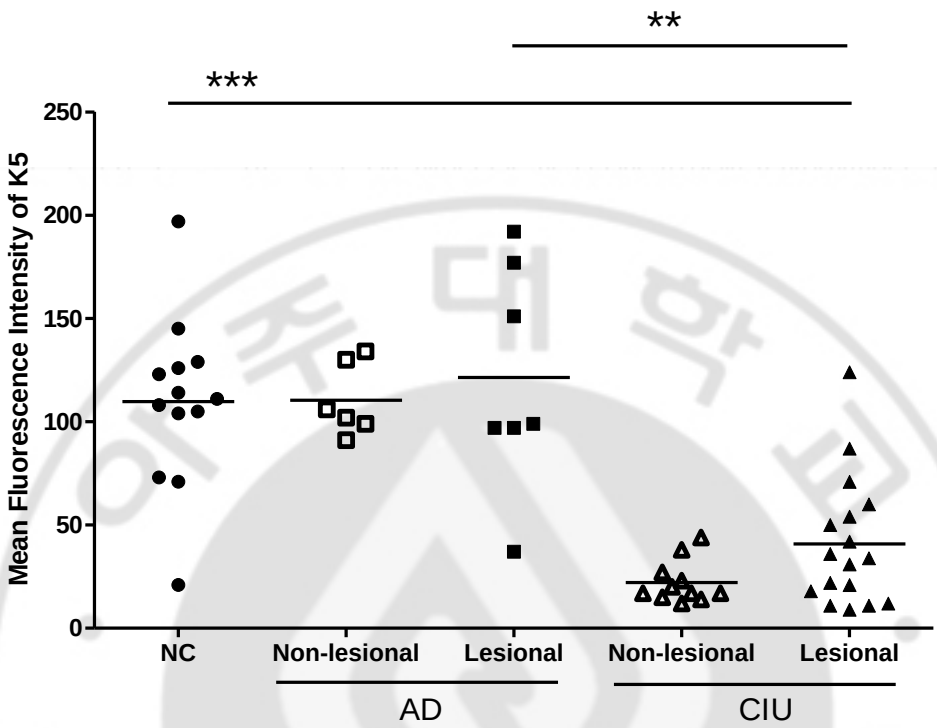

**
***
250
200
150
100
50
0
Mean Fluorescence Intensity of K5
NC
Non-lesional
Lesional
Non-lesional
Lesional
AD
CIU

Fig. 5

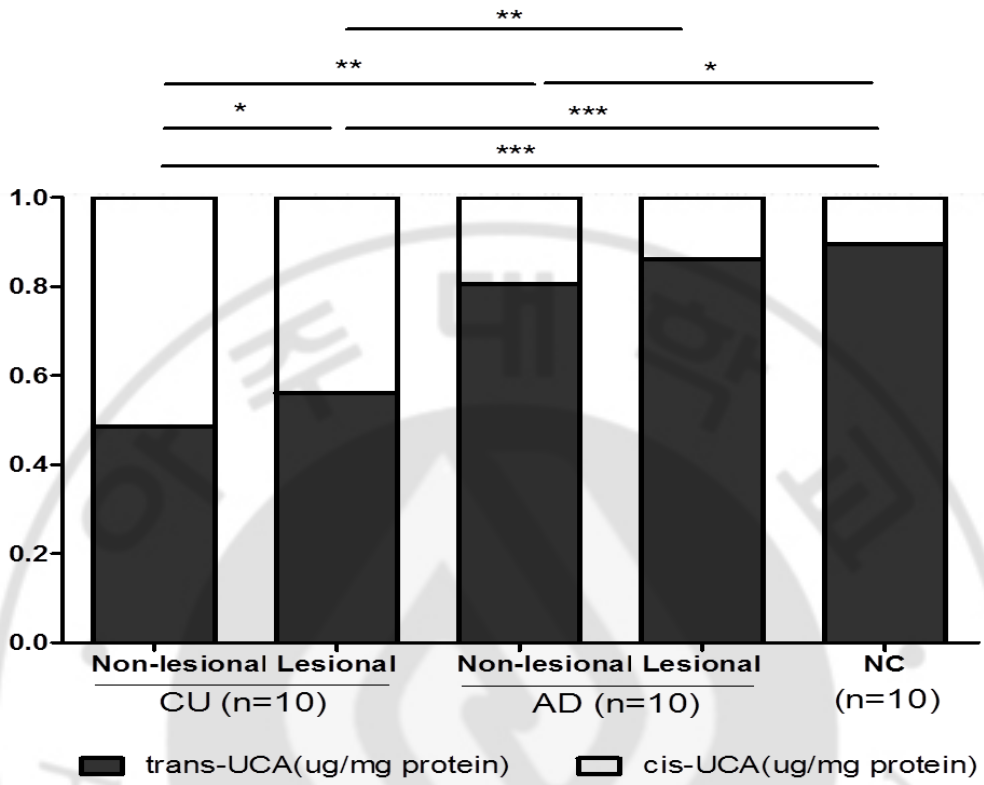
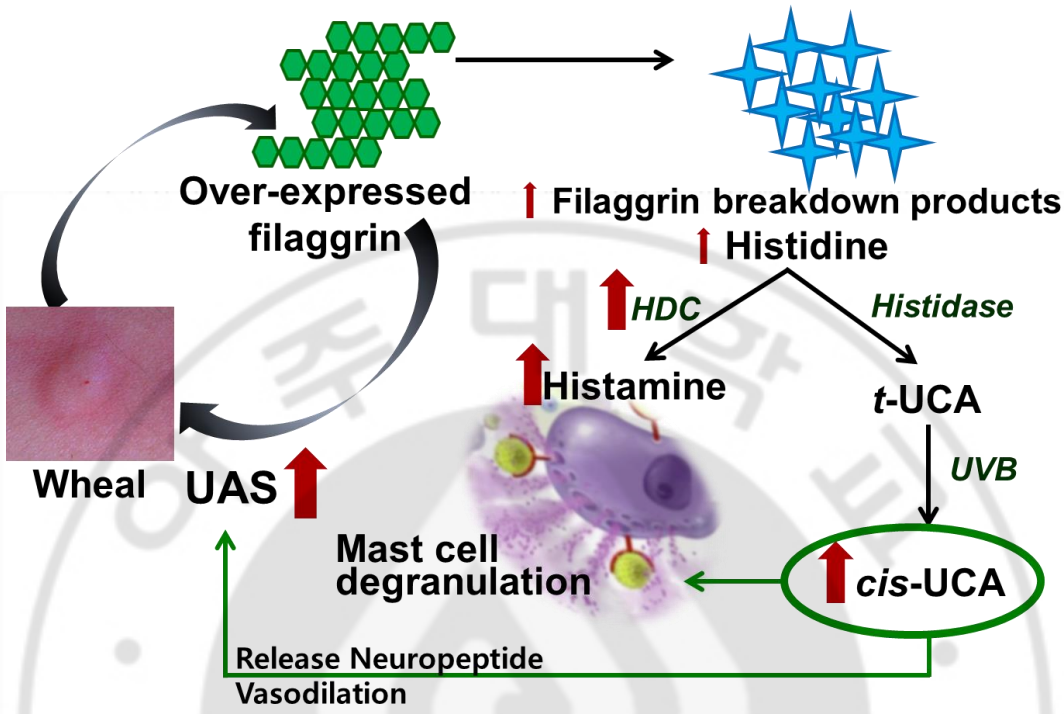

**
**
*
*
***
***
1.0
0.8
0.6
0.4
0.2
0.0
Non-lesional Lesional
CU (n=10)
Non-lesional Lesional
AD (n=10)
NC
(n=10)
trans-UCA(ug/mg protein)
cis-UCA(ug/mg protein)

Fig 6.


Over-expressed
filaggrin
Filaggrin breakdown products
Histidine
HDC
Histidase
Histamine
t-UCA
UVB
Wheal
UAS
Mast cell
degranulation
cis-UCA
Release Neuropeptide
Vasodilation