

# Epidermal Proteinase-Activated Receptor-2 Expression is Increased in End-Stage Renal Disease Patients with Pruritus: A Pilot Study

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Uremic pruritus is a common problem in patients with end-stage renal disease (ESRD), but the underlying mechanisms are not yet fully understood. We aimed to investigate the association between severity of uremic pruritus and cutaneous serine protease activity, as well as proteinase-activated receptor-2 (PAR-2) expression. Twelve ESRD patients with pruritus, 4 ESRD patients without pruritus, and 6 healthy controls were enrolled. Skin biopsies were obtained from the abdomen. Protease activity and PAR-2 expression in the epidermis were examined by in situ zymography and confocal laser microscopy, respectively. All ESRD patients presented more pronounced cutaneous protease activity compared with that in healthy controls. The skin samples from the patients with pruritus showed higher protease activity than either nonpruritic ESRD patients or healthy controls. The epidermis in all samples of ESRD patients presented higher immunoreactivity against PAR-2 versus those of healthy controls. In addition, correlation analysis between PAR-2 expression and VAS pruritus scores showed a significant positive correlation. Our data suggests that levels of serine protease and PAR-2 expression could play important roles in the pathogenesis of uremic pruritus.

**Key Words:** End-stage renal disease, Pruritus, Proteinase activated receptor-2, Serine protease, Uremia

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## Introduction

Uremic pruritus is a common and burdensome problem affecting up to 86% of patients with end-stage renal

disease (ESRD)<sup>1-3</sup>. Although the underlying mechanisms are not yet fully understood, many causative factors have been suggested<sup>1-5</sup>. These include uremic xerosis, secondary hyperparathyroidism, calcium phosphate crystals, uremic neuropathy, and iron-deficiency anemia<sup>4</sup>. In addi-

tion, immune system abnormalities or changes in the opioidergic system have been proposed to explain the pathophysiology of uremic pruritus<sup>5</sup>). Histamine, which is a common mediator of various cutaneous disorders associated with an itching sensation, also plays a pathogenic role in some patients with uremic pruritus. However, because many of these patients are refractory to treatment with antihistamines, there must be additional histamine-independent mediators that need to be identified.

Proteinase-activated receptors (PARs) are G-protein-coupled receptors that are activated by certain proteinases<sup>6,7</sup>. Among the PARs, PAR-2 is cleaved by trypsin-like serine proteases and is associated with acute inflammation<sup>7</sup>. In addition, PAR-2 in spinal apparent neurons has been shown to have an important role in hyperalgesia through its effects on the pain pathway<sup>7</sup>. Furthermore, recent investigations have suggested that PAR-2 is one of the histamine-independent mediators of itching<sup>6,8</sup>. Steinhoff et al.<sup>6</sup> demonstrated that significantly enhanced PAR-2 signaling was found in patients with atopic dermatitis, and intradermal injection of PAR-2 agonists induced enhanced and prolonged scratching response. This suggests a role of PAR-2 in the sensation of pruritus. However, neither the status of epidermal PAR-2 expression nor the relationship between PAR-2 and uremic pruritus in ESRD patients, have been studied. In this study, we investigated cutaneous PAR-2 expression and serine protease activity in ESRD patients with pruritus using confocal laser microscopy and in situ zymography, and these were compared to those of healthy controls and nonpruritic ESRD patients.

## MATERIALS AND METHODS

### 1. Patients

There were 12 ESRD patients with pruritus, 4 nonpruritic ESRD patients, and 6 healthy controls who were enrolled in this study. The study was approved by the Institutional Review Board of Yonsei University College of Medicine, and informed consent was obtained from all participants. The ESRD status was defined as an estimated glomerular filtration rate lower than 15 mL/min/

1.73 m<sup>2</sup>, as calculated by the Modification of Diet in Renal Disease equation. No participants had a prior history of any dermatologic disease other than pruritus. Medications related to pruritus except anti-histamine were discontinued at least two weeks before starting the baseline evaluation. At baseline, visual analog scale (VAS) and pruritus grading score for assessing severity of pruritus were recorded<sup>4</sup>.

### 2. Epidermal serine protease activity

Skin biopsy specimens from all participants were obtained from a similar area of the abdomen without remarkable excoriations or crusts. Serine protease activity in the epidermis of 5- $\mu$ m-thick frozen sections were examined by in situ zymography using the EnzCheck<sup>®</sup> Protease assay kit (Invitrogen Inc.; Grand Island, NY, USA) as previously described<sup>9</sup>.

### 3. Confocal laser microscopic study

Epidermal PAR-2 expression in biopsy material was visualized by immunofluorescence study. Briefly, paraffin-embedded samples were cut into 4- $\mu$ m-thick sections and deparaffinized. The deparaffinized sections were incubated for 2 hours at room temperature with a 1:250 dilution of a rabbit anti-human PAR-2 polyclonal antibody (Santa Cruz Biotechnology, Inc.; Santa Cruz, CA, USA) in blocking buffer (1% w/v BSA and 0.1% w/v cold-water fish gelatin in PBS). The sections were washed with PBS and then incubated for 1 hour at room temperature with a 1:100 dilution of fluorescein isothiocyanate-labeled goat anti-rabbit IgG antibody (Santa Cruz Biotechnology, Inc.) in blocking buffer. Stained tissue sections were washed with PBS and then examined with a C1 Plus confocal laser microscope (Nikon; Tokyo, Japan). Fluorescence intensities were analyzed using Metamorph software version 7.7.1.0 (Molecular Devices Inc.; Sunnyvale, CA, USA).

### 4. Statistical analysis

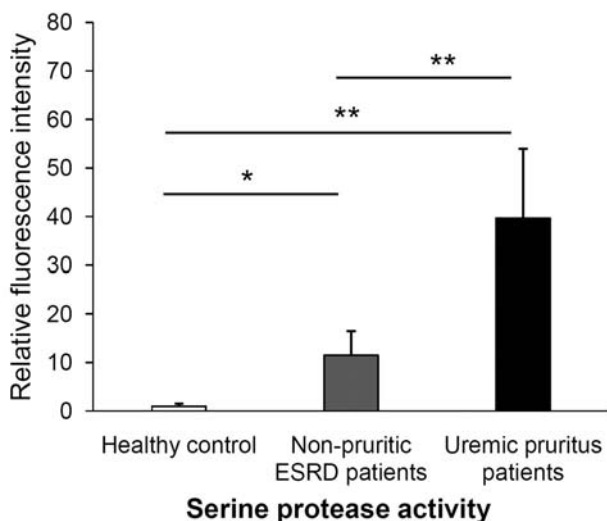
Statistical analyses were performed by the nonparametric Kruskal-Wallis test for multiple comparisons. Signifi-

cant differences obtained by Kruskal-Wallis analysis were confirmed by Mann-Whitney U test. The relationship between PAR-2 expression and VAS pruritus scores was assessed by spearman's correlation analysis. All analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) and p-values of <0.05 were considered to be significant.

## RESULTS

### 1. Patients characteristics

Baseline characteristics of the participants are summarized in Table 1. The median age of ESRD patients with



**Fig. 1.** Computer-based fluorescence intensity analyses. Comparison of serine protease activity was done in healthy controls (n=6), nonpruritic ESRD patients (n=4), and uremic pruritus patients (n=12). \*p<0.01, \*\*p< 0.001.

pruritus was 66 years (ranges from 41 to 79), and male gender was 75%. The median VAS and pruritus score were 5.25 (ranges from 1 to 10). The median age and gender ratio of ESRD patients without pruritus and healthy control were comparable with ESRD patients with pruritus.

### 2. Epidermal serine protease activity

All ESRD patients without pruritus presented more pronounced cutaneous protease activity by computer-based analysis of relative fluorescence intensities, compared with that in healthy controls (Fig. 1, p<0.01). In addition, the skin samples obtained from uremic pruritus patients showed higher protease activity than either non-pruritic ESRD patients or healthy controls (p<0.001).

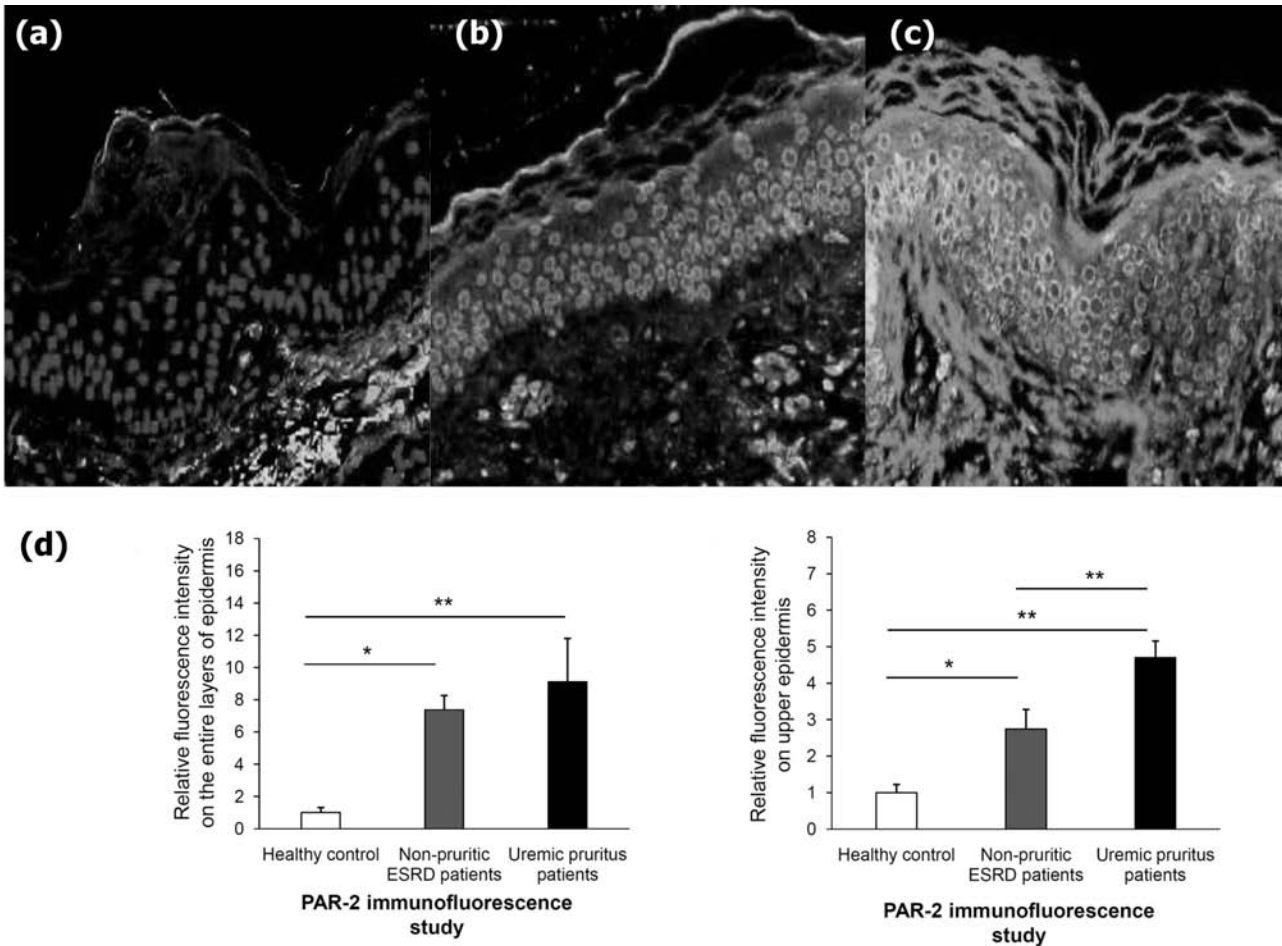
### 3. Confocal laser microscopic study of epidermal PAR-2 expression

Regardless of pruritus status, the epidermis in all samples of ESRD patients presented higher immunoreactivity against PAR-2 versus those of healthy controls (Fig. 2). Computer-based fluorescence intensity analysis revealed that significantly higher PAR-2 expression relative to healthy controls were found in samples from nonpruritic (p<0.01) and pruritic ESRD patients (p<0.001). Somewhat higher PAR-2 expression were found in samples from pruritic versus nonpruritic ESRD patients, but the difference was not statistically significant. However, PAR-2 expression in the upper half of epidermal layers of skin were significantly higher in samples from pruritic versus nonpruritic ESRD patients (p<0.001) (Fig. 2D).

**Table 1.** Baseline characteristics of study participants

	Healthy control individuals	Nonpruritic ESRD patients	Uremic pruritus patients
Number of patients (male/female)	6 (2/4)	4 (3/1)	12 (9/3)
Median age in yr (range)	67 (45-75)	58 (49-66)	66 (41-79)
Time on dialysis (months)	N.A	28±17	31±12
Diabetes mellitus (n)	0	3	8
VAS*	0	0	5.25 (2-10)
Pruritus grading score*	0	0	8.5 (6-18)

\*Scores are expressed as median values (range from minimum-maximum). ESRD, end-stage renal disease; N.A, not applicable; VAS, visual analog scale.



**Fig. 2.** Confocal laser microscopic study of epidermal PAR-2 expression. The skin samples obtained from (A) healthy controls, (B) nonpruritic ESRD patients, and (C) uremic pruritus patients indicate there was higher PAR-2 expression in uremic pruritus patients than in healthy controls or nonpruritic ESRD patients (original magnification  $\times 400$ , Bars=100  $\mu\text{m}$ ). (D) Computer-based relative fluorescence intensity analyses of PAR-2 expression in the entire epidermis and the upper half of epidermis were done in healthy controls ( $n=6$ ), nonpruritic ESRD patients ( $n=4$ ), and uremic pruritus patients ( $n=12$ ).  $*p<0.01$ ,  $**p<0.001$ .

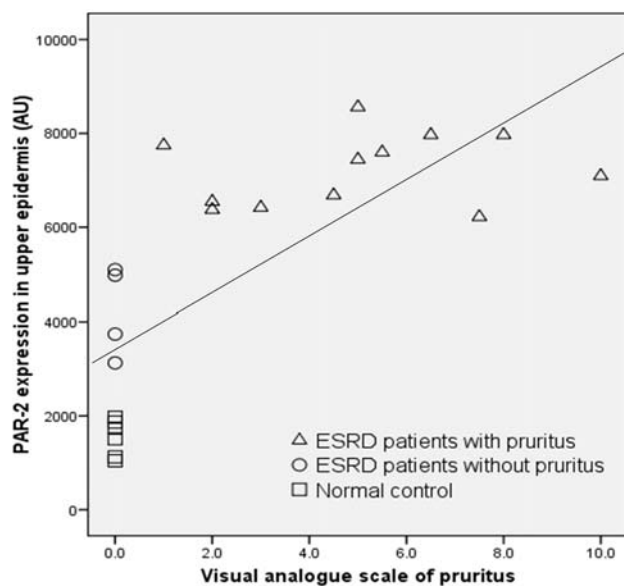
Interestingly, correlation analysis between PAR-2 expression and VAS pruritus scores in ESRD patients showed a significant positive correlation (Spearman's  $\rho=0.670$ ;  $p=0.005$ ) (Fig. 3).

## DISCUSSION

The PAR proteins are G-protein coupled receptors characterized by self-activation following specific proteolytic cleavage of their extracellular domains<sup>10</sup>. Among the four identified PAR members, PAR-2 is activated by trypsin-like serine proteases; whereas, the others are activated by thrombin<sup>11</sup>. In skin, PAR-2 is abundantly expressed and is

known to be a sensor for endogenous and exogenous proteases, and to play numerous pathophysiological roles<sup>11,12</sup>. In atopic dermatitis, PAR-2 in the skin is markedly expressed on primary afferent nerve fibers and significantly related to pruritus<sup>6</sup>. Provocation of an itching sensation by intracutaneous injection of endogenous PAR-2 agonists has suggested that this receptor is one of the histamine-independent pruritic mediators, and that PAR-2 might be a new therapeutic target for treatment of itching in atopic dermatitis<sup>6</sup>.

Uremic pruritus is a common complication in ESRD patients<sup>3</sup>. Various treatment modalities, such as phototherapy, i.e., with broad-band ultraviolet B and narrow-band



**Fig. 3.** The correlation analysis between epidermal proteinase-activated receptor-2 expression and visual analogue scale of pruritus. Correlation analysis showed a significant positive correlation (spearman's  $\rho=0.670$ ;  $p=0.005$ ).

ultraviolet B, and oral medications, including antihistamines, gabapentin, gamma linolenic acid, or kappa-opioid receptor antagonist, have been used for the treatment of uremic pruritus with various treatment outcomes<sup>4,13,14</sup>. In this study, we have investigated the association between the severity of pruritus in ESRD patients and levels of cutaneous serine protease activity, as well as PAR-2 expression. From the results, we hypothesized that uremic pruritus might be related to PAR-2 expression, as in atopic dermatitis. Our data revealed that epidermal PAR-2 expression was significantly increased in ESRD patients compared to healthy controls. In addition, *in situ* zymography confirmed that protease activity was upregulated in uremic skin. Pruritic ESRD patients showed significantly higher levels of PAR-2 expressed in the skin, especially in the upper epidermis, than that in ESRD patients without pruritus. Previous studies have demonstrated that ESRD patients presented increased serum trypsin levels and decreased trypsin inhibitory capacity<sup>15,16</sup>. In addition, the protein-energy wasting, which is commonly found in ESRD patients, is attributable to increased levels of specific proteolytic activities, including activation of caspase-3 and the ubiquitin-proteasome system<sup>17</sup>. We suggest that

the pathological milieu in uremic skin might increase proteolytic activity that results in the increased PAR-2 expression in the skin of ESRD patients, and that this mechanism provokes pruritus in such patients.

There are several limitations in this study. First, various etiologic factors associated with uremic pruritus were not fully investigated. Biochemical parameters such as hemoglobin, serum calcium, phosphorous, ferritin, vitamin D, and intact-parathyroid hormone were not measured. Second, the basic investigation about the pathogenesis of how uremia induces PAR-2 expression was not performed. Inflammatory cytokines that might increase protease activities should have been done. Lastly, the number of patients enrolled in this study were very small. However, this is a pilot study so that further evaluations including basic research for pruritus pathway and large scaled clinical trials will be conducted.

In this report, we have demonstrated that protease activity and PAR-2 expression in the epidermis were significantly increased in uremic pruritus patients compared with nonpruritic ESRD patients and healthy controls. In addition, PAR-2 expression and VAS pruritus scores in ESRD patients showed a significant correlation. Although the exact pathogenesis of uremia-induced cutaneous PAR-2 expression remains to be more fully explained, our data does suggest that serine proteases and PAR-2 in the skin could have an important function in the pathogenesis of uremic pruritus.

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## Conflicts of Interest

The authors have no financial conflicts of interest.

This manuscript or any portion thereof, is not under consideration by another journal or electronic publications and has not been previously published.

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