



# Principal Component Analysis to Differentiate Patients with Palmoplantar Pustulosis from Those with Palmoplantar Pustular Psoriasis

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**Background:** Palmoplantar pustulosis (PPP) is initiated from the acrosyringium. However, it is unclear whether PPP should be considered a distinct entity or should be classified into the spectrum of pustular psoriasis, also known as palmoplantar pustular psoriasis (PPPP).

**Objective:** We evaluated the differences in immunohistochemical staining in patients with PPP to determine whether they can be classified into two groups based on psoriatic properties or acrosyringal properties.

**Methods:** Nineteen punch biopsy specimens diagnosed with PPP were collected. Antibodies were chosen for identifying the acrosyringal properties of  $\alpha$ -3-nicotine acetylcholine receptors ( $\alpha$ -3-nAChR), psoriatic properties of interleukin (IL)-23 and IL-36R, inflammatory cell properties of human cathelicidin antimicrobial peptide 18/LL-37, IL-8, lipocalin-2 (LCN2), and CD3. The degree of staining of the epidermis was evaluated using the ordinal scale (0~3). The principal component analysis was used to derive principal components (PCs) of common variation between the stains, and the two groups were divided using PCs and cluster analysis.

**Results:** Three main PCs explained 64% of the total variance in PPP. PC1 (pustular psoriasis properties) showed a higher correlation with IL-36R. PC2 (acrosyringal/inflammatory properties) showed a higher correlation with  $\alpha$ -3-nAChR, IL-8, LCN2, and CD3. PC3 (psoriasis properties) showed a higher correlation with IL-23. PC1 showed a statistically significant difference ( $p=0.0284$ ) between the two groups. We identified three PCs associated with the pathomechanisms of PPP.

**Conclusion:** Although PC1 showed a statistically significant difference between the two groups, we did not identify differential protein expression related to the pathogenesis between PPP and PPPP.

**Keywords:** Palmoplantar pustulosis, Principal component analysis, Psoriasis, Pustular psoriasis

## INTRODUCTION

Palmoplantar pustulosis (PPP) is a chronic and refractory disease characterized by sterile pustules on palms and soles. Palms and soles have distinct characteristics of increased eccrine glands and thick stratum corneum. The eccrine gland and duct have nicotine acetylcholine receptors (nAChRs) that

are stimulated by smoking, and smoking can alter nAChR expression and result in inflammation<sup>1</sup>. It is known that this inflammatory process begins in the acrosyringia, arising from precipitating factors such as focal infection, smoking, and metal allergy<sup>2</sup>.

It is debatable whether PPP should be considered a distinct entity or should belong to the spectrum of pustular psoriasis<sup>3</sup>,



also known as palmoplantar pustular psoriasis (PPPP). PPP and PPPP have similar characteristics, such as the female dominance, association with smoking, and Koebner phenomenon<sup>4</sup>. In some reports, PPP has been differentiated from PPPP based on the sites of the lesion, where PPPP patients might have a plaque psoriasis lesion on any sites outside the palms and soles<sup>5-7</sup>. Other studies have reported that PPP can have an extra-palmoplantar lesion that is milder than psoriasis<sup>4</sup>. However, an extra-palmoplantar skin lesion site is not an absolute standard for the differentiation of PPP from PPPP.

Recently, Murakami and Terui<sup>8</sup> suggested a possible pathomechanism of PPP arising from the acrosyringium, initiated by an increase in human cathelicidin antimicrobial peptide 18/LL-37, which promotes the production of interleukin (IL)-36, followed by IL-23 and IL-17 production. Based on this pathomechanism, we hypothesized that inflammation from the acrosyringium can proceed to pustular psoriasis-like inflammation associated with IL-23 and IL-36.

This study aims to evaluate the differences in the immunohistochemical staining (IHS) in patients with PPP and determine whether they can be divided into two groups based on acrosyringial (PPP) or psoriatic properties (PPPP).

## MATERIALS AND METHODS

### Case selection

The medical records and punch biopsy specimens of patients with PPP who visited Ajou University Hospital between January 2015 and August 2019 were retrospectively evaluated. Forty patients with PPP who had pustules composed of neutrophils in the intra-epidermis were screened. Patients who did not have intact pustule and those who concomitantly had another differential diagnosis, such as generalized pustular psoriasis or chronic eczematous dermatitis, were excluded. Pompholyx can be excluded by vesicles with spongiosis compared with PPP which showed vesicles lacking spongiosis<sup>9</sup>. Overall, 19 patients (thirteen female and six male) were enrolled.

### Immunohistochemistry of paraffin-embedded specimens

For immunohistochemistry, paraffin embedded 4  $\mu$ m thick tissue was used to the avidin-biotin complex method which was visualized by aminoethyl carbazole (GBI Labs, Bothell,

WA, USA). Antigen retrieval was carried with pressure cooker of Cuisinart CPC-600 (Cuisinart Corp., East Windsor, NJ, USA) for 10 minutes in citrate buffer pH 6.0. All punch biopsy specimens from pustular skin lesions were stained with seven antibodies: anti- $\alpha$ -3-nAChR (Sigma, St. Louis, MO, USA; at 1:1,000) for the acrosyringia, anti-IL-23 for plaque psoriasis (BioLegend, San Diego, CA, USA; at 1:400), anti-IL-36R for pustular psoriasis (Abcam, Cambridge, UK; at 1:200), anti-LL37 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; at 1:200) for antimicrobial peptides, anti-IL-8 (Abcam; at 1:2,000), anti-Lipocalin-2 (LCN2) (Abcam; at 1:8,000) for neutrophils, and anti-CD3 for lymphocytes (Novocastra, Newcastle upon Tyne, UK; at 1:300). All slides were independently examined by two dermatologists for evaluating stain degree.

### Evaluation of histopathological findings

The degree of staining of the epidermis of the surrounding pustule was evaluated using ordinal classification (0, negative; 1, weak; 2, moderate; 3, strong). The epidermis was subdivided into the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale, and each layer was evaluated. Each degree of staining of the epidermal layers was summed and categorized into three categories: the upper epidermis (stratum corneum and stratum granulosum), the lower epidermis (stratum spinosum and stratum basale), and the total epidermis (upper epidermis and lower epidermis).

### Statistical analyses

Principal component analysis (PCA) makes simple the complexity in high-dimensional variables while keeping trends and patterns by transforming the data into fewer dimensions, which act as summaries of features. To identify the underlying common latent structure among the seven stains pattern, we performed PCA, which is a multivariate statistical method that downsized the number of variables into fewer new variables, named as principal components (PCs) and all patients were allotted their PC score by each PC. The eigenvalues measure the amount of variation retained by each loading factor, and eigenvalues  $>1$  were selected. All patients were clustered into two groups using K-means clustering ( $k=2$ ) based on PC scores. The Wilcoxon rank-sum test analysis was then performed to compare PCs between the two groups.  $p$ -values  $<0.05$  were considered statistically significant. All analyses were performed using the R program (version 3.6.3; R Devel-

opment Core Team, 2020; <http://www.r-project.org>).

## Ethics

The institutional review board of Ajou University Hospital approved the study protocol (IRB No: AJIRB-MED-MDB-19-422) before study commencement. The requirement for written informed consent was waived by the ethics committee.

## RESULTS

### Epidemiologic data

The demographic and clinical characteristics of study populations are presented in Table 1. Six male and thirteen female were included, with the mean age of 45.8 years. All patients had variable-sized pustules on the palms and soles; two patients had extra-palmoplantar lesions that might be associated with PPP. Of the 19 patients, 10 patients (52.6%) had smoking history.

### Principal component analysis based on the ordinal scale

The main PCs acquired by the PCA are presented in Table 2. The first three PCs accounted for 64% of the variance, and the first five PCs had eigenvalues  $\geq 1$  and accounted for 85% of the variance. Both 3 PCs and 5 PCs equally clustered our patients into two groups, however, five PCs cannot be defined any dermatological meaning, we selected three PC to explain the IHS pattern. The first component (pustular psoriasis component, PC1) strongly correlated with IL-36R with correlations  $>0.6$

**Table 1.** Demographic and clinical characteristics of the study population

Characteristic	PPP (n=19)
Sex	
Male	6 (31.6)
Female	13 (68.4)
Mean age (yr)	45.8
Location	
Palm and sole	17 (89.5)
Palm and sole, extra palmoplantar	2 (10.5)
Smoking	
Yes	10 (52.6)
No	7 (36.8)
Unknown	2 (10.5)

Values are presented as number (%). PPP: palmoplantar pustulosis.

(Table 3). The second component (acro-syringial/inflammatory component, PC2) correlated with  $\alpha$ -3-nAChR, LCN2, CD3, and IL-8. The third component (plaque psoriasis component, PC3) strongly correlated with IL-23 with correlations  $>0.7$ .

**Table 2.** Principal component analysis and total variance showed by components

Component	Initial eigenvalues		
	Total	% of variance	Cumulative (%)
1	4.32*	27.00	27.00
2	3.27*	20.46	47.46
3	2.58*	16.15	63.61
4	2.31	14.41	78.02
5	1.18	7.38	85.10
6	0.84	5.28	90.68
7	0.77	4.82	95.50

\*Three principal components account for 64% variation.

**Table 3.** Rotated components

Stain	Epidermis*	PC1	PC2	PC3
LCN2	Total	0.4780	0.6733	0.0792
	Upper	0.4308	0.6558	0.1961
	Lower	0.2499	0.0822	-0.6443
CD3	Total	0.5154	0.5476	-0.2709
	Upper	0.0000	0.0000	0.0000
	Lower	0.5154	0.5476	-0.2709
$\alpha$ -3-nAChR	Total	-0.5795	0.6633	0.2222
	Upper	-0.2245	0.4895	0.5087
	Lower	-0.5775	0.5317	-0.0138
IL-8	Total	-0.7126	0.4445	0.1525
	Upper	-0.7126	0.4445	0.1525
	Lower	0.0000	0.0000	0.0000
IL-23	Total	0.3773	-0.2494	0.7313
	Upper	0.2370	0.0390	0.8291
	Lower	0.3918	-0.3926	0.5068
IL-36R	Total	0.6994	0.2942	0.0054
	Upper	0.6324	0.1542	0.3270
	Lower	0.5798	0.3121	-0.1919
IL-37	Total	0.0000	0.0000	0.0000
	Upper	0.0000	0.0000	0.0000
	Lower	0.0000	0.0000	0.0000

Correlation between seven immunohistochemical stains and the three main PCs. PC: principal component, IL: interleukin.

\*Epidermis was classified into three categories: the upper epidermis (stratum corneum and stratum granulosum), the lower epidermis (stratum spinosum and stratum basale), and the total epidermis (upper epidermis, lower epidermis).

## Cluster

Using the PC scores acquired from the three main PCs (PC1, PC2, and PC3) as dependent variables, we divided the patients into two groups of seventeen and two using K-means clustering ( $k=2$ ) (Table 4). There was more rate of patients with extra-palmoplantar skin lesions in group 2 than in group 1. PC1 showed a statistically significant difference between the two groups ( $p=0.0284$ ) based on the Wilcoxon rank-sum test, with no significant differences in other PCs (Table 4). Representative patients in each group showed a stain tendency according to PCs (Fig. 1).

## DISCUSSION

In our study, seven IHS antibodies were used to differentiate PPPP from PPP. Using PCA, three PCs associated with inflammatory pathomechanisms of PPP were identified. However, after patients were clustered into two groups, PPPP could not be differentiated from PPP using three PCs, including acrosyringial and psoriatic properties.

The acrosyringium plays a role in the initiation of inflammation in PPP. The acrosyringium and keratinocytes have nAChR. Hagforsen et al.<sup>1</sup> proved that PPP patients showed a higher intensity of  $\alpha$ -3-nAChR staining in the epidermis than

healthy control. Additionally, cholinergic materials, such as nicotine, can cause keratinocytes to proliferate and release cytokines that sustain the chronic inflammatory process, suggesting the involvement of the eccrine glands in the pathogenesis of PPP<sup>10-13</sup>.

LL-37 is the sweat antimicrobial peptide detected in the fluid of the vesicle/pustule in PPP<sup>13</sup>. It can induce proinflammatory cytokines, including IL-36 $\gamma$ , IL-8, IL-23, and IL-17C, in keratinocytes<sup>8,14,15</sup>. Because LL-37 did not show staining in the epidermis except for the pustule, it did not show any correlation with the PCs in our study.

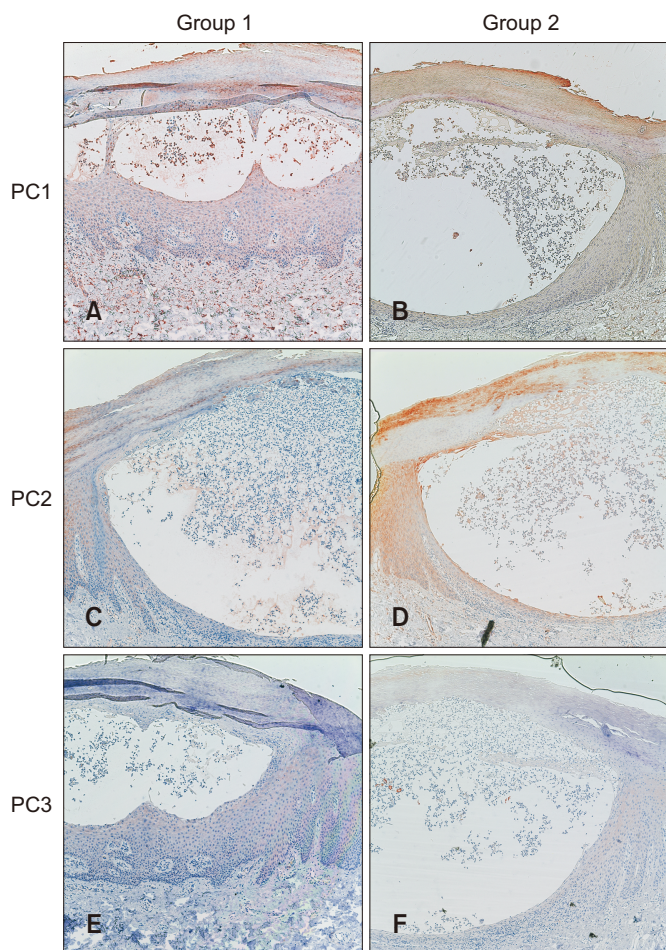
Our results showed that PC2 correlated with acrosyringial inflammation ( $\alpha$ -3-nAChR) followed by other inflammatory factors (LCN2, CD3, and IL-8). The chemokine IL-8 associated with neutrophil activation may be a key player in the formation of pustules and is more strongly stained than healthy control and patients with psoriasis vulgaris<sup>16</sup>. The increase in LCN2 produced by granulocytes and the promotion of neutrophil activation and infiltration in the keratinocytes of PPPP lesions have been previously reported<sup>6,17,18</sup>. However, in our study, PC2 did not show a statistically significant difference between the two groups, which indicates that PPP and PPPP more likely share the series of the inflammatory process rather than being a separate entity. In other words, inflamma-

**Table 4.** Groups divided using principal components (PCs) and clusters

Variable	Group 1 (n=17)	Group 2 (n=2)	p-value <sup>†</sup>
PC			
PC1	0.403 (-0.335, 1.360)	-4.875 (-5.190, -4.560)	0.0284*
PC2	-0.851 (-1.467, 1.148)	1.341 (0.932, 1.750)	0.2588
PC3	0.495 (-1.260, 1.050)	0.617 (0.409, 0.825)	0.9470
Clinical characteristics			
Sex			
Male	5 (29.4)	1 (50.0)	
Female	12 (70.6)	1 (50.0)	
Mean age (yr)	46.6	39.0	
Location			
Palms and soles	16 (94.1)	1 (50.0)	
Extra palmoplantar	1 (5.9)	1 (50.0)	
Smoking			
Yes	9 (52.9)	1 (50.0)	
No	6 (35.5)	1 (50.0)	
Unknown	2 (11.8)	0	

Values are presented as number (%) or median (interquartile range). Group 1 and 2 were divided by K-means clustering ( $k=2$ ) based on PC scores. \*Statistical significance ( $p<0.05$ ). <sup>†</sup>Wilcoxon rank-sum test.





**Fig. 1.** Histopathologic findings of the representative patient in each group. (A) Interleukin (IL)-36R stain in group 1 (magnification  $\times 100$ ). (B) IL-36R stain in group 2 ( $\times 100$ ). (C)  $\alpha$ -3-nAChR stain in group 1 ( $\times 100$ ). (D)  $\alpha$ -3-nAChR stain in group 2 ( $\times 100$ ). (E) IL-23 stain in group 1 ( $\times 100$ ). (F) IL-23 stain in group 2 ( $\times 100$ ). Group 1 and 2 were divided by K-means clustering ( $k=2$ ) based on PC scores. PC: principal component, PC1: pustular psoriasis component, PC2: acrodermatitis/inflammatory component, PC3: plaque psoriasis component.

tion starting from the acrodermatitis might progress to have a psoriatic inflammatory pathway. The association of PC2 with IL-8 from the upper epidermis and CD3 from the lower epidermis showed that lymphocytes have a different route than neutrophils.

Our results showed that PC3 had a correlation with IL-23. Anti-IL-23 monoclonal antibody (Guselkumab) has been approved for the treatment of PPP and has shown improvement in the PPP severity index total score at week 16, with the effects maintained until week 24 when compared with a placebo<sup>19</sup>. The IL-36 $\gamma$  level increased in PPP and pustular and

non-pustular psoriatic lesions but not in healthy skin; it plays a key role in accumulating neutrophils with IL-8. In contrast to generalized pustular psoriasis, which showed a decrease in IL-36 receptor antagonist (IL-36Ra), PPP showed an increase in both IL-36 $\gamma$  and IL-36Ra<sup>20</sup>. IL-36R activation by IL-36 $\gamma$  can induce psoriasis-associated cytokines of IL-23, which is vital role in psoriasis pathogenesis<sup>21</sup>. Our results showed that PC1 had a correlation with IL-36R and presented statistically significant differences between the two groups. Considering the role of IL-36, this pathway is likely to have a therapeutic target for PPP and other pustular inflammatory diseases.

This study has several limitations. First, PCA is a statistical method and may not reflect the reality, and interpretation of the PCs is mainly research dependent. Second, our IHS result analysis was performed by a semi-quantitative. Third, the study involved a very small group. To differentiate PPP from PPPP in future studies, a larger patient population for the investigation of immunological changes in chronological order compared with psoriasis is needed.

In conclusion, although this statistical study could not clearly distinguish PPP from PPPP, we identified three meaningful PCs, namely PC1 (pustular psoriasis component), PC2 (acrodermatitis/inflammatory component), and PC3 (plaque psoriasis component) associated with the pathomechanism of PPP. PC1 showed a statistically significant difference in the two groups, and differential protein expressions related to pathogenesis between PPP and PPPP could not be identified. These results suggest that PPP and PPPP are not classified by a complex series of processes mediated by  $\alpha$ -3-nAChR, LL37 to IL-36, and IL-23.

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## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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## DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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