Elevated Human Tissue Kallikrein Levels in the Stratum Corneum and Serum of Peeling Skin Syndrome-Type B Patients Suggests an Over-Desquamation of Corneocytes

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Background

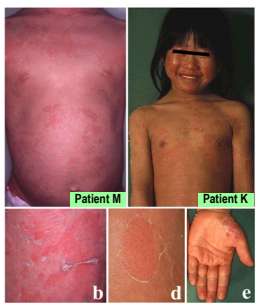
- **Peeling skin syndrome type B (PSS-type B) is a very rare congenital skin disease associated with continual skin peeling & ichthyotic erythroderma.**
- **PSS-type B displays various clinical similarities with Netherton syndrome caused by genetic defects of *serine protease inhibitor Kazal-type5* (*SPINK5*).** (Wile, 1924; Traupe, 1989; Magert HJ et al, 1999; Chavanas et al, 2000).
- **Human tissue kallikreins are a family of 15 trypsin-like or chymotrypsin-like secreted serine proteases (hK1-hK15) found in a variety of tissues** (Yousef and Diamandis, 2001). **At least 8 different hKs have been identified in the stratum corneum & sweat as desquamation-related proteases** (Komatsu et al, 2005b, 2006).
- **hKs may contribute to the overall SC protease activities and the degradation of intercellular adhesion molecules resulting in desquamation of corneocytes** (Simon et al, 2001; Komatsu et al, 2002; Caubet et al, 2004). **SPINK5 inhibitory domains are believed to be negative regulators of desquamation related proteases including hKs** (Mitsudo et al, 2003; Descargues et al, 2005; Egelrud et al, 2005; Schechter et al, 2005).
- **The gene(s) responsible for PSS pathogenesis, as well as the relationship between kallikrein (hK) expression and skin manifestations in PSS, have not as yet been determined. The reasons for the similarities between Netherton syndrome & PSS-type B, despite the intact *SPINK5* in the latter, are still unknown.**

Aim

- **To clarify pathogenesis of PSS-type B**
- **To elucidate the relationship between PSS-type B & kallikrein expression**
- **To explain the reason for the clinical similarities between PSS-type B & Netherton syndrome**

Results

I. Clinical features & pathological findings



Patients

- Two unrelated 8 yr-old female Japanese patients (Patient M & Patient K) were born with erythroderma accompanied by scaling. Their lesions have shown no improvement to date.
- *SPINK5* has been proved to be intact, therefore, Netherton syndrome was ruled out, leaving PSS-type B as the most likely diagnosis.

Informed consent was obtained from all patients, their parents and normal volunteers, and our studies were performed according to the Declaration of Helsinki. The Medical Ethics Committee of the Graduate School of Medical Science, School of Medicine, Kanazawa University; and Juntendo University School of Medicine, approved all described studies.

Features	Peeling Skin Syndrome type B			Netherton syndrome[b]
	Patient M	Patient K	PSS-type B[a]	
Cutaneous features				
Erythema	Yes	Yes	Yes	Yes
Peeling	Yes	Yes	Yes	No
Blistering	No	No	?	No
Scales	Sheet	Sheet	Sheet	Small Flakes
Pruritus	Very Strong	Very Strong	Yes	Yes
Pain in Denuded Skin	Yes	Yes	Yes	Yes[c]
Bleeding in Denuded Skin	No	No	?	No
Skin Infection	Herpes	No	Yes	Yes
Lichenification	Yes (Joints)	Yes (Joints)	Yes?	Yes (Joints)
Corneocyte Turnover	1-3 Days	1-3 Days	1-8 Days	?
Percutaneous Absorption	Over-Absorption	?	?	Over-Absorption[d]
Lesions				
Skin	Generalized	Generalized	Generalized	Focal to Generalized
Palms/Soles	Peeling	Peeling	No	Peeling in Severe Cases[e]
Mucosa	Normal	Normal	?	Normal[c]
Hair	Normal	Normal	NP/possibly bamboo hair[f]	Bamboo Hair
Nail	Normal	Normal	?	Normal[c]
Teeth	Normal	Normal	?	Normal[c]
General Conditions				
General Condition	Good	Good	Good to Lethal	Good to Lethal
Physical Problem	No	No	?	No[c]
Neurological, Mental Problem	No	No	?	Yes or No[g]
Sexual Dysfunction	?	?	No?	?
Growth Retardation	Yes (<-2SD)	No	Yes or No	Yes
Temperature Instability	Yes	Yes	?	Yes
Sweat Secretion	Very Low	Low	?	Very Low
Allergic Diseases	Asthma	No	No?	Yes
Other Complications	Astigma	No	?	Astigma[c]
Onset	Birth	Birth	Birth	Birth
Clinical Course	No Changes	No Changes	Improves with Age	Improves with Age
Laboratory Findings	Eo 2090x10^6/L	Eo 720x10^6/L	Elevated IgE	Eosinophilia
	IgE 10608 IU/ml	IgE 27570 IU/ml		Elevated IgE
Inheritance	AR?	AR?	AR?	Autosomal Recessive (AR)
Gene Locus	?	?	?	5q31-2
Genetic Defect	?	?	?	*SPINK5*

- Clinical **similarities** or **differences** between PSS-type B & Netherton syndrome are indicated in **red** or **blue**, respectively.

II. Quantification of kallikreins (hKs) in the stratum corneum & serum by ELISA

Stratum Corneum

hK (ng/mg dry weight)	Normal Mean ± SD	Patient M	Patient K
Chymotrypsin-like hK			
hK7	10.9 ± 6.0	**65.8** *	**130.7** *
Trypsin-like hKs			
hK8	5.8 ± 1.8	**62.9** *	**63.7** *
hK11	8.7 ± 4.1	**37.6** *	**56.9** *
hK5	3.1 ± 1.4	**8.3** *	**13.1** *
hK10	0.67 ± 0.41	**21.7** *	**29.5** *
hK14	0.34 ± 0.13	**2.3** *	**3.2** *
hK6	0.28 ± 0.12	**73.2** *	**30.0** *
hK13	0.17 ± 0.14	**24.1** *	**15.9** *
Total of Trypsin-like hKs	19.1 ± 5.4	**230.1** *	**212.3** *

Serum

hK (ng/ml)	Normal Mean ± SD	Patient M	Patient K
Chymotrypsin-like hK			
hK7	5.1 ± 2.1	**17.0** *	**35.5** *
Trypsin-like hKs			
hK6	4.4 ± 1.5	**15.7** *	**10.4** *
hK8	1.9 ± 0.77	**15.2** *	**7.1** *
hK10	1.2 ± 0.56	**5.6** *	**5.4** *
hK5	0.68 ± 0.15	**2.0** *	**0.47**
hK11	0.54 ± 0.16	**0.38**	**0.67**
hK14	0.22 ± 0.091	**0.43**	**2.6** *
hK13	<0.01	**0.26** *	**0.14** *
Total of Trypsin-like hKs	4.5 ± 5.4	**23.9** *	**16.4** *

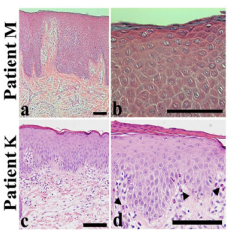
(*), Smirnov test showed significant differences between normal SC samples & individual patients (P<0.05). The amount of hK13 in normal samples usually is so low or undetectable that the value for hK13 in normal serum was described as < 0.01 ng/ml without SD. The statistics for hK13 was performed estimating as hK13 values for normal subjects were 0.01 ng/ml.

III. Stratum corneum serine protease enzymatic activities

Substrate Released AMC or pNA (nmol/mg dry weight)	Time (hr)	Normal mean ± SD	PSS-type B Patient M	PSS-type B Patient K
Trypsin-like Activity				
Phe-Ser-Arg-AMC	2	**15.5** ± 1.5	**21.0**	**15.5**
Pro-Phe-Arg-AMC	2	**5.7** ± 3.1	**24.0** *	**18.6** *
Chymotrypsin-like Activity				
Arg-Pro-Tyr-pNA	4	**13.9** ± 5.5	**11.9**	**13.7**
Furin-like Activity				
Pyr-Arg-Thr-Lys-Arg-AMC	2	**3.0** ± 1.3	**43.0** *	**28.5** *
Plasmin-like Activity				
Val-Leu-Lys-AMC	2	**1.7** ± 1.0	**8.9** *	**19.6** *

- (*) indicates significant differences (P<0.05) between the mean of normal samples & each patient at the specified time (Smirnov test for extreme values).
- Trypsin-like (FSR-) & chymotrypsin-like (RPY-) activities are known to be necessary for desquamation.
- Since SPINK5 protein possesses anti-"plasmin" function (Mitsudo et al, 2003), overall SC "plasmin-like (VLK-) activity" was predicted.

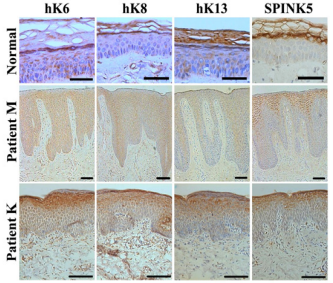
- SPINK5 pro-protein can be proteolytically processed at (R-KR↓) by furin-like activity to 15 individual bioactive domains (Seidah & Chretien, 1999; Komatsu et al, 2002; Mitsudo K et al, 2003).
- According to their kinetic properties, hK5, hK6, hK8, hK13 & hK14 strongly display trypsin-like (FSR-) activity. hK7 may be largely responsible for the chymotrypsin-like (RPY-) activity.
- The identity of enzymes contributing to PFR- & VLK-activities in the skin is unknown.



Histopathology

- HE staining. Skin sections were obtained from an erythematous & scaling lesion 1 month after birth for both patients.
- The findings are: 1) absence of the SC or a few layers of parakeratosis, which tended to be separated from the stratum granulosum; 2) psoriasisforme acanthosis; 3) perivascular infiltration with mainly mononuclear leukocytes (a & c), and some eosinophils (d,▲).

Scale bars: 100μm (a, c & d) & 50 μm (b).



Immunohistochemistry for hKs & SPINK5 proteins

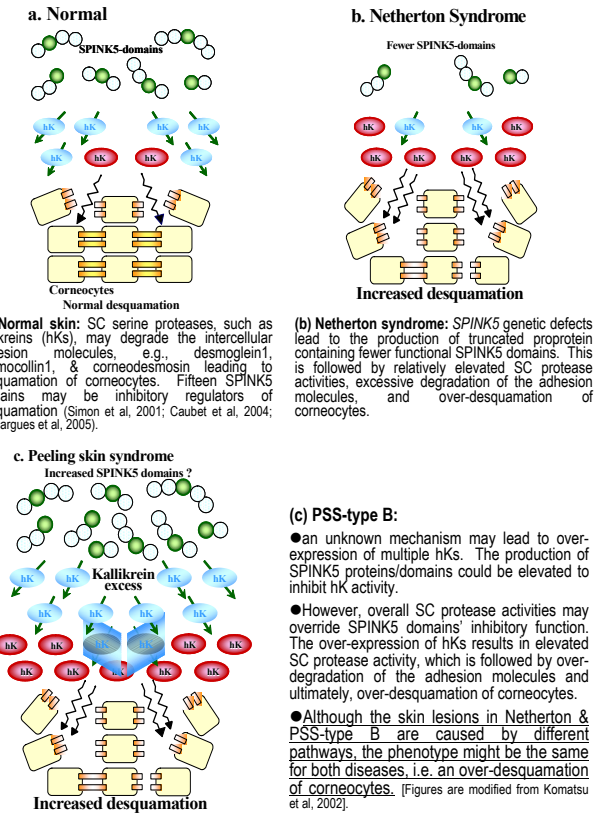
- In PSS-type B patients, hKs & SPINK5 protein expressions were deeply expanded into the lower epidermis compared with those in normal skin.
- It was previously demonstrated that NS patients show absent or only faint staining in the skin epidermis when the same anti-SPINK5 protein Ab is applied (Raghunath et al, 2004).

Scale bars: 50μm (normal) & 100μm (patients).

Clinical features

- NP, no problem; ?, not determined; a, PSS-type B, Peeling skin syndrome type B, referred from (Traupe, 1989; Mevorah et al, 1987); b, c, d & e, referred from (Griffiths et al, 1998; Komatsu, 2003; Allen et al, 2001; Judge et al, 1994), respectively.
- Although it has been suggested that palmoplantar lesions are usually absent in PSS-type B (Traupe, 1989), the two patients does have mild SC peeling in the palms & soles (see pictures).

IV. A hypothetical model for desquamation regulation in normal, Netherton syndrome & PSS-type B



(a) Normal skin: SC serine proteases, such as kallikreins (hKs), may degrade the intercellular adhesion molecules, e.g., desmoglein1, desmocollin1, & corneodesmosin leading to desquamation of corneocytes. Fifteen SPINK5 domains may be inhibitory regulators of desquamation (Simon et al, 2001; Caubet et al, 2004; Descargues et al, 2005).

(b) Netherton syndrome: *SPINK5* genetic defects lead to the production of truncated proprotein containing fewer functional SPINK5 domains. This is followed by relatively elevated SC protease activities, excessive degradation of the adhesion molecules, and over-desquamation of corneocytes.

(c) PSS-type B:

- an unknown mechanism may lead to over-expression of multiple hKs. The production of SPINK5 proteins/domains could be elevated to inhibit hK activity.
- However, overall SC protease activities may override SPINK5 domains' inhibitory function. The over-expression of hKs results in elevated SC protease activity, which is followed by over-degradation of the adhesion molecules and ultimately, over-desquamation of corneocytes.
- Although the skin lesions in Netherton & PSS-type B are caused by different pathways, the phenotype might be the same for both diseases, i.e. an over-desquamation of corneocytes. [Figures are modified from Komatsu et al, 2002].

Summary & Conclusions

- **PSS-type B may not be an ichthyosis characterized by the retention of thick adherent scales, but an over-desquamation disease due to an over-expression of hKs and an elevation of the SC protease activities.**
- **The over-desquamation of corneocytes may explain the clinical similarities between PSS-type B & Netherton syndrome.**
- **The elevated SC enzymatic activities may be good therapeutic targets for PSS-type B patients.**

Materials & Methods

Refer to JID, 125(6):1182-9, 2005
BJD, 153:274-81, 2005

For further information

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