

Article type: Letters to the Editor

Title: A case of Netherton syndrome/*SPINK5*-syndromic epidermal differentiation disorder evaluated by serial tape-stripping: Persistent elevation of serine protease activities despite clinical improvement

Running title: NS/*SPINK5*-sEDD and tape-stripping

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Ethics Statement: The authors obtained written consent from patients for photographs and medical information to be published in print and online and with the understanding that this information may be publicly available. Patient consent forms were not provided to the journal but are retained by the authors.

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Main text

Netherton syndrome/*SPINK5*-syndromic epidermal differentiation disorder (NS/*SPINK5*-sEDD) is a rare autosomal recessive disorder caused by loss-of-function mutations in the *SPINK5* gene encoding the serine protease inhibitor Lympho-epithelial Kazal-type-related inhibitor (LEKTI).¹ LEKTI deficiency results in increased serine protease activities in the stratum corneum, including kallikrein-related peptidase (KLK)5 and KLK7, leading to barrier disruption and atopic dermatitis-like skin symptoms.¹

We report a case of NS/*SPINK5*-sEDD evaluated by serial tape-stripping to assess serine protease activity and inflammation. The patient was previously reported as a 19-year-old woman with NS/*SPINK5*-sEDD complicated with persistent MRSA bacteremia and adrenal insufficiency.² Initially, she presented with erythroderma (Fig A, B, Ichthyosis Area and Severity Index (IASI)=44.4). Management included systemic corticosteroid replacement (oral hydrocortisone 90mg/day administered from day 1, gradually reduced until completion on day 90), intravenous daptomycin and ceftazidime (from day 1 to day 14), and topical hydrocortisone butyrate (from day 1, continued throughout). No systemic immunomodulators or biologics were given. Clinical improvement followed management of infection and adrenal insufficiency. On day 647, her condition was stable with scattered erythema and scaling (Fig C, D, IASI=18.2). Whole-exome sequencing revealed compound heterozygous mutations, c.377_378delAT(p.Tyr126Ter) and c.1220+1G>C, in *SPINK5*, confirmed by

76 Sanger sequencing.

77 Trypsin- and chymotrypsin-like serine protease activities were serially measured in scales
 78 collected by tape-stripping from her forearm. This was approved by the Ethics Committee of Okayama
 79 University (approval no. 1611-002). Trypsin-like serine protease activity remained consistently higher
 80 than those observed in AD patients or healthy volunteers, regardless of clinical improvement (Fig E,F).
 81 Chymotrypsin-like serine protease activity was also elevated but to a lesser extent (Fig G, H).
 82 Additionally, RNA sequencing was performed on two scale samples (approval no. 2210-012)—during
 83 erythroderma (IASI=44.4) and clinical improvement (IASI=18.2)—revealing increased expression of
 84 IL-1 and IL-17 family cytokines including IL-1 α/β , IL-18, IL-36 α/γ /Ra and IL-17C/F, during active
 85 disease. (Fig. I). In contrast, *KLK* transcripts were not significantly upregulated.

86 This is the first report of serial serine protease activity measurement in NS/*SPINK5*-sEDD
 87 using tape-stripping. Despite clinical improvement, serine protease activity remained elevated.

88 Our RNA sequencing of scale samples is also novel in NS/*SPINK5*-sEDD. Although RNA
 89 sequencing has been used in studies using psoriasis and AD scales, our RNA sequencing data further
 90 support the involvement of IL-1/IL-36/IL-17 cytokines in NS/*SPINK5*-sEDD pathogenesis,
 91 consistent with biopsy-based analyses in previous studies.³ These cytokines may serve as non-invasive
 92 biomarkers of disease activity. Their reduction after infection treatment and adrenal replacement likely
 93 reflects systemic control of inflammation, while persistent LEKTI deficiency explains sustained

94 protease activity.

95 We acknowledge the reviewer's concern that SPINK5 and serine protease transcripts were
96 not detected in our samples. This may reflect both biological mechanisms—since LEKTI deficiency
97 primarily causes protein-level dysregulation—and methodological limitations of tape-stripping in
98 capturing low-abundance transcripts. Biopsy-based RNA sequencing studies in NS/SPINK5-sEDD
99 have shown complementary findings.³ Thus, while tape-stripping offers a practical, non-invasive
100 approach, it has inherent limitations.

101 Although limited to one patient, this study highlights tape-stripping as a feasible tool for
102 longitudinal assessment in NS/SPINK5-sEDD. Further studies in larger cohorts are required to
103 validate biomarkers and develop effective targeted therapies.

Figure Legends

A, B. Ichthyosiform erythroderma on the extremities and the trunk on Day 1. The IASI was 44.4. **C, D.** Ichthyosis linearis circumflexa on the extremities and the trunk on Day 647. The IASI was 18.2. **E, F.** Trypsin-like and chymotrypsin-like serine protease activities in tape-stripping samples from forearm of normal healthy volunteer (NHV, n=9), atopic dermatitis patients (AD, n=49), and our NS/*SPINK5*-sEDD patient (NS). Each point for NHV and AD represents one individual, based on a single measurement per person. Six serial measurements from the NS/*SPINK5*-sEDD patient are shown as individual data points. **G, H.** Time-course of trypsin-like and chymotrypsin-like serine protease activities of the NS/*SPINK5*-sEDD patient. **I.** Heatmap illustrating the changes in mRNA expression in tape-stripping samples from the forearm on Day 1 (IASI:44.4) and Day 647 (IASI:18.2).

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (OpenAI) solely for language editing and proofreading of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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