Journal of Dermatological Science xxx (2019) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Letter to the Editor

A heterozygous *SERPINB7* mutation is a possible modifying factor for epidermolytic palmoplantar keratoderma

To the Editor

Recently, causative genes for the various types of palmoplantar keratoderma (PPK) have been found, resulting in a need for a genetic classification of PPK [1]. The most common type in the Japanese population is Nagashima-type PPK (NPPK) caused by loss-of-function mutations in *SERPINB7*, which encodes serpin B7, a member of the serine protease inhibitor superfamily [2]. Serpin B7 is found in the stratum corneum, and its main function is to regulate intercellular protease activity [3]. The clinical features of NPPK include diffuse hyperkeratosis and erythema on the palms and soles, and also on their dorsal aspects, known as transgrediens [4]. The skin lesions in NPPK show a whitish spongy appearance within 10 min of water exposure [3].

Epidermolytic PPK (EPPK) is an autosomal dominant genetic disorder caused by mutations in *KRT9*, encoding keratin 9 (KRT9) [5], or in *KRT1*. EPPK is characterized by diffuse yellowish thickening of the palmoplantar skin. Histologically, EPPK lesions show marked hyperkeratosis and thickening of epidermis, clumped keratohyalin granules and acantholysis (granular degeneration). KRT9 expression is restricted to the palmoplantar regions. Thus, the skin lesions do not spread around the palmoplantar area in EPPK patients. The present report suggests the possibility that the *SERPINB7* mutation is a disease modifying factor for EPPK.

We analyzed two cases of EPPK from two independent pedigrees. Case 1 was a 37-year-old male. Case 2 was a 23-year-old female. Both patients had had longstanding pain and hyperkeratosis on the palms and soles from soon after birth (Fig. 1a–d). Notably, generalized erythema was seen on the dorsal aspects of the fingers and toes only in case 2 (Fig. 1b and d). In addition, the hyperkeratosis was more severe in case 2 than in case 1 (Fig. 1a and c). Only case 2 showed a whitish spongy change on the hands after 10 min of water exposure, especially in the hyperkeratotic, reddish area including the dorsal aspects of the hands (Fig. 1e–h). A skin biopsy sample from the plantar lesion of case 2 showed marked hyperkeratosis and thickening of the epidermis. Keratohyalin granules of various sizes and acantholysis were seen at high magnification (Fig. 2). Inflammatory cell infiltration was obvious in the superficial dermis.

Ethical approval was obtained and all research was performed in accordance with the principles of *The Declaration of Helsinki*. To identify the pathogenetic mutation of the patients, whole-exome sequencing was performed using DNA extracted from peripheral blood from the patients by previously described methods with slight modifications [6]. Whole-exome sequencing showed that case 1 and case 2 had the previously reported heterozygous mutations c.488 G > A (p.Arg163Gln) and c.487C > T (p.Arg163Trp) in *KRT9*, respectively [5]. The two mutations are different substitution mutations in the identical amino acid Arg163. Interestingly, case 2 also had a previously reported heterozygous duplication mutation, c.522dupT (p.Val175Cysfs*46), in *SERPINB7* [7], but we found no other potentially pathogenic mutations in any genes associated with PPK. The identified mutations were verified by subsequent Sanger sequencing (data not shown).

Next, immunohistochemical staining using a rabbit polyclonal anti-serpin B7 antibody (HPA024200, Sigma-Aldrich, dilution 1:4000) was performed, as described elsewhere [8]. Immunohistochemically, plantar lesional skin biopsy samples from case 2 and our previously reported striate PPK patient (carrying a causative *DSG1* mutation and an additional heterozygous *SERPINB7* mutation [9]) showed weaker anti-serpin B7 antibody staining (Fig. 2e and f) than a control plantar skin sample (Fig. 2d). A plantar hyperkeratotic skin sample from an epidermolytic ichthyosis (EI) patient with a *KRT1* mutation but without any *SERPINB7* mutation showed insignificantly reduced anti-serpin B7 antibody staining, and the staining was obviously stronger than that seen in case 2 (data not shown). From these findings, we speculate that serpin B7 expression in typical EPPK patients without *SERPINB7* mutations might be normal or only very mildly reduced.

In previous reports, a lack of serpin B7 immunostaining was observed in the stratum corneum (SC) of hyperkeratotic plantar skin from NPPK patients with homozygous or compound heterozygous *SERPINB7* mutations [3,10]. In the present study, decreased serpin B7 expression was seen in the stratum granulosum and SC of the epidermis in case 2. There have been no reports of EPPK patients showing transgrediens. Furthermore, case 2 showed a unique whitish spongy appearance of the eruptions after water exposure.

Reddish hyperkeratotic lesions extending to the dorsal aspects of the hands and feet (transgrediens) and a whitish spongy appearance of the eruptions after water exposure are characteristic features of NPPK caused by *SERPINB7* mutations. Thus, there is the possibility that the present heterozygous *SERPINB7* duplication mutation modified the EPPK phenotype in case 2. A plantar hyperkeratotic skin sample from an EI patient with a *KRT1* mutation but without any *SERPINB7* mutation showed only minimal inflammatory cell infiltration in the superficial dermis (data not shown). Thus, we speculate that significant inflammatory cell infiltration in the superficial dermis of case 2 might be associated with the additional *SERPINB7* mutation. Our present findings suggest that the concurrent dysfunction of the two different molecules, KRT9 and serpin B7, may result in the complicated phenotype and mixed clinical features of EPPK and NPPK.

https://doi.org/10.1016/j.jdermsci.2020.05.001

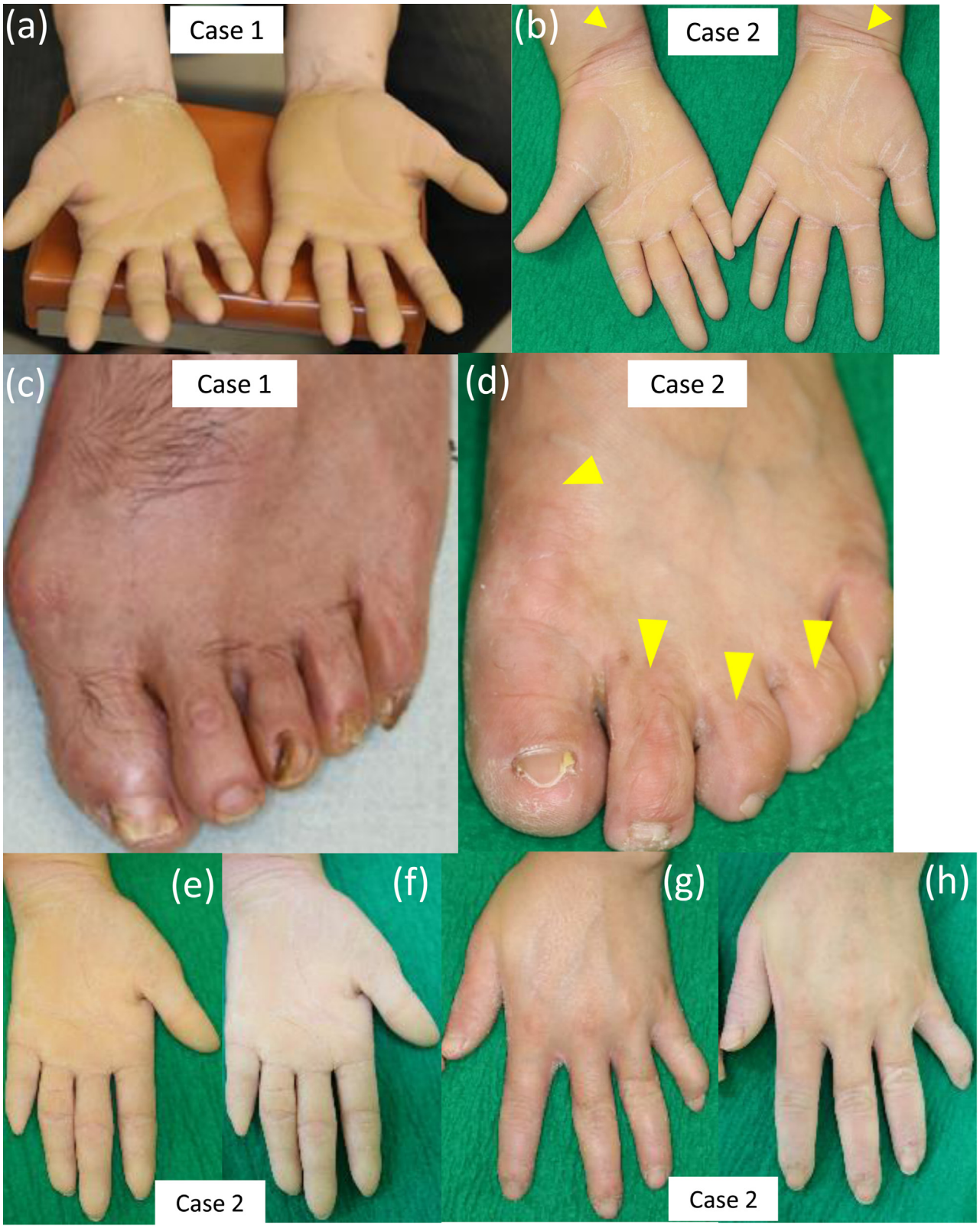

Fig. 1. Clinical features of the present two patients with EPPK.
(a–d) Although both case 1 (heterozygous *KRT9* mutation) and case 2 (heterozygous *KRT9* and *SERPINB7* mutations) have hyperkeratosis on the palms, the hyperkeratosis is more severe in case 2 (b) than in case 1 (a). Generalized erythema is seen on the wrists and dorsal aspects of the toes in case 2 (b and d, yellow arrowheads), but not in case 1 (a and c). (e–h) The left hand before water exposure (e and g) and after water exposure (f and h) in case 2. A whitish spongy appearance is observed on the hands after 10 min of water exposure, especially in the reddish hyperkeratotic areas including the wrist and dorsal aspects of the hands (f and h).

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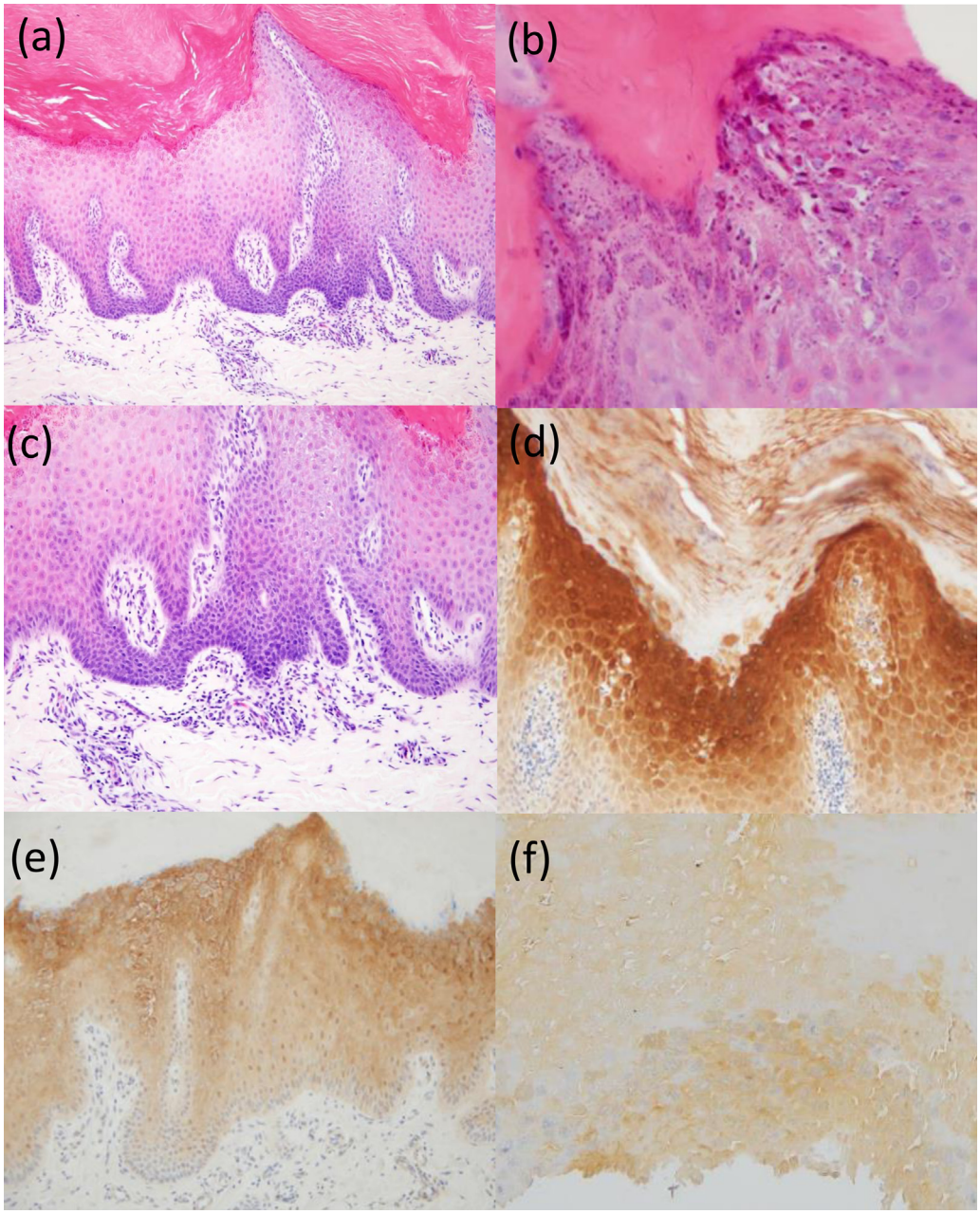

Fig. 2. Histological features and immunostaining for serpin B7 in the plantar lesions.
(a) The plantar lesion in case 2 shows severe hyperkeratosis and acanthosis with inflammatory cell infiltration in the superficial dermis (original magnification, ×100). (b) Keratohyalin granules of various sizes and acantholysis are seen in a high-magnification view of the plantar lesion in case 2 (original magnification, ×200). (c) Significant inflammatory cell infiltration is observed in the superficial dermis in case 2 (original magnification, ×200). (d–f) Immunohistochemical staining for serpin B7 reveals that serpin B7 immunoreactivity is lower in the patients' plantar skin lesions (e, case 2, the EPPK patient carrying the causative *KRT9* mutation and the heterozygous *SERPINB7* mutation; f, a previously reported striate PPK patient carrying a causative *DSG1* mutation and an additional heterozygous *SERPINB7* mutation [9]) than in a control skin sample (d).

We previously reported a patient with autosomal dominant striate PPK carrying a causative *DSG1* mutation and an additional *SERPINB7* mutation [9]. The striate PPK case also showed transgrediens [9]. Although heterozygous *SERPINB7* mutation carriers have never been symptomatic of NPPK, our findings suggest that heterozygous *SERPINB7* mutations might be a possible modifying factor for patients with not only striate PPK, but also EPPK. The carrier rate of the *SERPINB7* founder mutant allele c.796C > T is estimated as 0.9% from our screening data in the Japanese population and the Human Genome Variation Database (HGVD; http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html) [9]. The c.796C > T mutation in *SERPINB7* is also reported in approximately 2.8% of Asian individuals in the 1000 Genomes Database [3]. Thus, we cannot exclude the possibility that undetected, unsearched for, hidden *SERPINB7* mutations, such as the founder mutations in our cases, might modify clinical features of other cases with various types of PPK.

Funding statement

This work was supported by funding from the Advanced Research and Development Programs for Medical Innovation (AMED-CREST)19gm0910002h0105 to M.A. from the Japan Agency

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for Medical Research and Development (AMED). This work was also supported by Grant-in-Aid for Scientific Research (B) 18H02832 to M.A., and by Grant-in-Aid for Young Scientists18K16058 to T.T. from the Japan Society for the Promotion of Science (JSPS).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by funding from the Japan Agency for Medical Research and Development (AMED) JP18ek0109295h0002 and 18ek0109281h0002 to M.A. This work was also supported by Grant-in-Aid for Scientific Research (B) 18H02832 to M.A. and by Grant-in-Aid for Young Scientists 18K16058 to T.T. from the Japan Society for the Promotion of Science (JSPS).

References

[1] D. Hershkovits, J. Lugassy, M. Indelman, R. Bergman, E. Sprecher, Novel mutations in DSG1 causing striate palmoplantar keratoderma, Clin. Exp. Dermatol. 34 (2009) 224–228.
[2] A. Kubo, Nagashima-type palmoplantar keratosis: a common Asian type caused by SERPINB7 protease inhibitor deficiency, J. Invest. Dermatol. 134 (2014) 2076–2079.
[3] A. Kubo, A. Shiohama, T. Sasaki, K. Nakabayashi, H. Kawasaki, T. Atsugi, et al., Mutations in SERPINB7, encoding a member of the serine protease inhibitor superfamily, cause Nagashima-type palmoplantar keratosis, Am. J. Hum. Genet. 93 (2013) 945–956.
[4] A. Korekawa, E. Akasaka, D. Rokunohe, T. Fukui, T. Kaneko, H. Nakano, et al., Nagashima-type palmoplantar keratoderma and malignant melanoma in Japanese patients, Br. J. Dermatol. 180 (2) (2019) 415–416.
[5] A. Reis, H.C. Hennies, L. Langbein, M. Digweed, D. Mischke, M. Drechsler, et al., Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK), Nat. Genet. 6 (1994) 174–179.
[6] Y. Murase, T. Takeichi, A. Kawamoto, K. Tanahashi, Y. Okuno, H. Takama, et al., Reduced stratum corneum acylceramides in autosomal recessive congenital ichthyosis with a NIPAL4 mutation, J. Dermatol. Sci. 97 (1) (2020) 50–56.
[7] J. Yin, X. Guiwen, W. Huijun, J. Zhao, L. Duo, X. Cao, et al., New and recurrent SERPINB7 mutations in seven Chinese patients with Nagashima-type palmoplantar keratosis, J. Invest. Dermatol. 134 (2014) 2269–2272.
[8] T. Takeichi, Y. Okuno, A. Kawamoto, T. Inoue, E. Nagamoto, C. Murase, et al., Reduction of stratum corneum ceramides in Neu-Laxova syndrome caused by phosphoglycerate dehydrogenase deficiency, J. Lipid Res. 59 (12) (2018) 2413–2420.
[9] R. Fukaura, T. Takeichi, Y. Okuno, D. Kojima, M. Kono, K. Sugiura, et al., Striate palmoplantar keratoderma showing transgrediens in a patient harbouring heterozygous nonsense mutations in both DSG1 and SERPINB7, Acta Derm. Suppl. (Stockh) 97 (2017) 399–401.
[10] A. Shiohama, T. Sasaki, S. Sato, J. Sakabe, T. Ito, H. Isoda, et al., Identification and characterization of a recessive missense mutation p.P277L in SERPINB7 in Nagashima-type palmoplantar keratosis, J. Invest. Dermatol. 136 (1) (2016) 325–328.

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Received 20 February 2020