Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2016.01.011.

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Letter to the Editor

A founder deletion of corneodesmosin gene is prevalent in Japanese patients with peeling skin disease: Identification of 2 new cases

Keywords
Corneodesmosin
Gene deletion
Multiplex PCR
Peeling skin disease
Peeling skin syndrome

Peeling skin disease (PSD), also called generalized peeling skin syndrome (PSS) and inflammatory PSS type B (OMIM #270300), is a rare autosomal recessive type of ichthyosis characterized by lifelong pruritic or non-pruritic superficial peeling of the skin [1,2].

PSD was first reported to be caused by mutations in corneodesmosin gene (*CDSN*) on chromosome 6p21.3, which encodes *CDSN* [3]. *CDSN* is a specific component of corneodesmosomes, which play an important role in maintaining structural integrity of stratum corneum. To date, 6 different loss-of-function *CDSN* mutations have been reported in PSD worldwide [3–10] (Table S1).

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Our and other groups recently identified and characterized a homozygous 59-kb genomic deletion with loss of entire *CDSN* in 3 Japanese PSD patients [4–6]. The deletion, which is likely caused by *Alu*-mediated non-homologous end joining [4,6], extends from 40.6 kb upstream to 13.2 kb downstream of *CDSN* and affects 6 genes, including *TCF19* (partially), *CCHCR1*,*PSORS1C2*, *PSORS1C1*, *CDSN* and *C6orf15* [4]. Loss of the 5 genes did not produce additional phenotypes in either skin or other tissues [4–6], although histopathological psoriasis-like findings might be caused by deletion of *PSORS1* genes [4]. Having clearly demonstrated the deletion breakpoints, we previously developed a PCR method to screen for this deletion [4].

In this study, by our one-step multiplex PCR method, we identified the same 59-kb genomic deletion of *CDSN* in two additional Japanese patients.

Female patient 1 and male patient 2, who both first visited Juntendo University Urayasu Hospital at ages of 11 and 7, are now 19- and 14-year-old, respectively. Both patients developed skin symptoms soon after birth.

Physical examination for both patients revealed generalized erythematous and scaly skin lesions with peeling of skin, which resembled Netherton syndrome (NS) (Fig. 1A,B, Fig. S1A,B, Fig. S2A). Patient 2 also showed slight hair abnormality, including curly, frizzy and grizzled hair, although prominent hair loss was not seen (Fig. S2B). The skin lesions got worse with high temperature and humidity during summer season in both patients (Fig. S2C,D). Histopathology of skin biopsy from patient 1 showed extensive acanthosis and loss of cornified layer (Fig. S1C). None of the 4 parents showed any skin abnormality.

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In the present study, we performed both standard genomic DNA PCR for exon 1 of *CDSN* and our previously developed multiplex

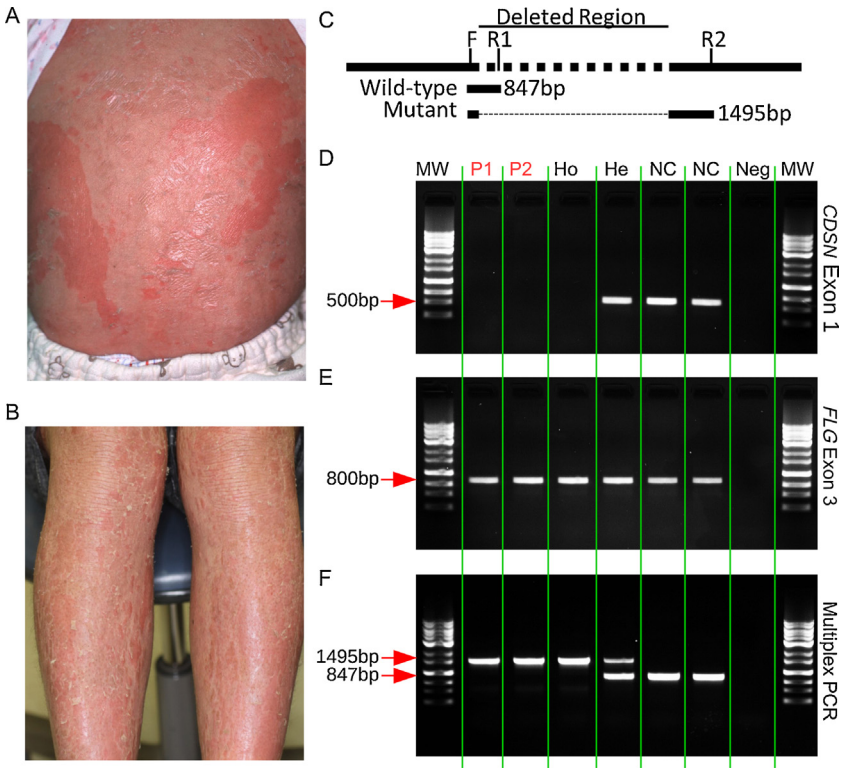

Fig. 1. Clinical features and PCR analysis of *CDSN* deletion in PSD patients.(A,B) Clinical features on the trunk of patient 1 (A) and on the legs of patient 2 (B). (C) Schematic presentation of multiplex PCR method for detection of 59-bp deletion on chromosome 6p21.3. Solid lines represent non-deleted region and dashed lines represent deleted region. The sizes of amplifiable PCR products are indicated. F: Common rorward primer. R1: Allele specific reverse primer 1. R2: Allele specific forward primer 2. (D,E) Standard genomic DNA PCR analysis of exon 1 of *CDSN* (D) and part of exon 3 of FLG (E) for 2 new PSD patients and disease and normal controls. (F) Our multiplex PCR analysis to detect *CDSN* deletion for PSD patients and disease and normal controls. MW: Molecular weight markers. P1: Patient 1. P2: Patient 2. Ho: Homozygous deletion control. He: Heterozygous deletion control. NC: Normal control. Neg: Negative control (no template).

PCR for identification of the 59-kb deletion [4]. In addition, we performed tag SNP analysis around the deleted region for all 4 PSD patients in our hands as described in Supplementary methods. We also determined deletion allele frequency in the general population by novel real-time PCR method as described in Supplementary methods. Genomic DNA was extracted from blood of the patients using QIAamp DNA Blood Kit (Qiagen, Hilden, Germany), according to manufacturer's protocol. All studies were performed in accordance with Declaration of Helsinki Principles. Medical Ethics Committee of Juntendo University Urayasu Hospital and Kurume University School of Medicine approved this study. Written informed consents were obtained from both patients and their parents.

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In patient 1, preliminary genetic studies for *SPINK5*, *KRT1/KRT10 and KER2e* showed negative results, excluding the diagnoses of NS, epidermolytic ichthyosis and superficial epidermolytic ichthyosis, respectively. Immunohistochemistry demonstrated positive staining for LEKTI but negative staining for *CDSN*. In patient 2, preliminary genetic study for *SPINK5* showed negative results, and immunohistochemistry demonstrated negative staining for *CDSN*. Genetic study for *TGM5* was not performed, because both patients showed generalized skin lesions, which did not indicate diagnosis of acral type PSS.

Our multiplex PCR for identification of the 59-kb deletion produces an 847-bp band in normal DNA samples and a 1495-bp band in DNA samples with the deletion, whereas heterozygotes produce both bands (Fig. 1C) [4].

By standard PCR for exon 1, the *CDSN* fragment was amplified in 3 control individuals, but not in both patients (Fig. 1D). Successful amplification of partial exon 3 of *FLG* in all subjects suggested that there was no gross degradation of genomic DNA preparations of the patients (Fig. 1E). The multiplex PCR to identify the 59-kb deletion revealed that the 2 patients carried the same homozygous deletion of *CDSN* (Fig. 1F). According to self-declarations, the marriages are non-consanguineous in these 2 patients.

We surveyed English literature for *CDSN* mutations in PSD in various geographic locations, and found 13 PSD cases with homozygous mutations in *CDSN* [3–10]. While one Moroccan, 2 Israeli and 5 European (one French and 4 German) cases showed early pre-mature termination codons due to non-sense, small insertion or deletion mutations, all 5 Japanese cases showed the same deletion of entire *CDSN* (Table S1). These results suggest that deletion of *CDSN* is prevalent in Japanese PSD patients.

To determine whether the deletion was caused sporadically or inherited from a single ancestor, we performed analysis of tag SNP having >0.4 minor allele frequencies around the deleted region for all 4 patients in our hands. The analysis showed the same SNP alleles at 29 out of 34 sites up to 457-kb and down to 946-kb away from the breakpoint (Table 1). However, one patient showed heterozygosity after 246-kb upstream of breakpoint suggesting a recombination event might have occurred at some point in time. Furthermore, by our newly developed real-time PCR method, we did not find the deletion in 7000 Kyushu area Japanese chromosomes (Fig. S3A-C). These findings suggested that the deletion originated recently from a single founder.

Identification of 2 new cases suggested that PSD is more prevalent in Japan than expected so far, probably because most cases are misdiagnosed as NS, particularly during young ages. Our multiplex PCR for *CDSN* should be useful to distinguish between NS and PSD. In addition, we found the same SNP haplotype around the deleted region in all 4 patients and we did not find the deletion in