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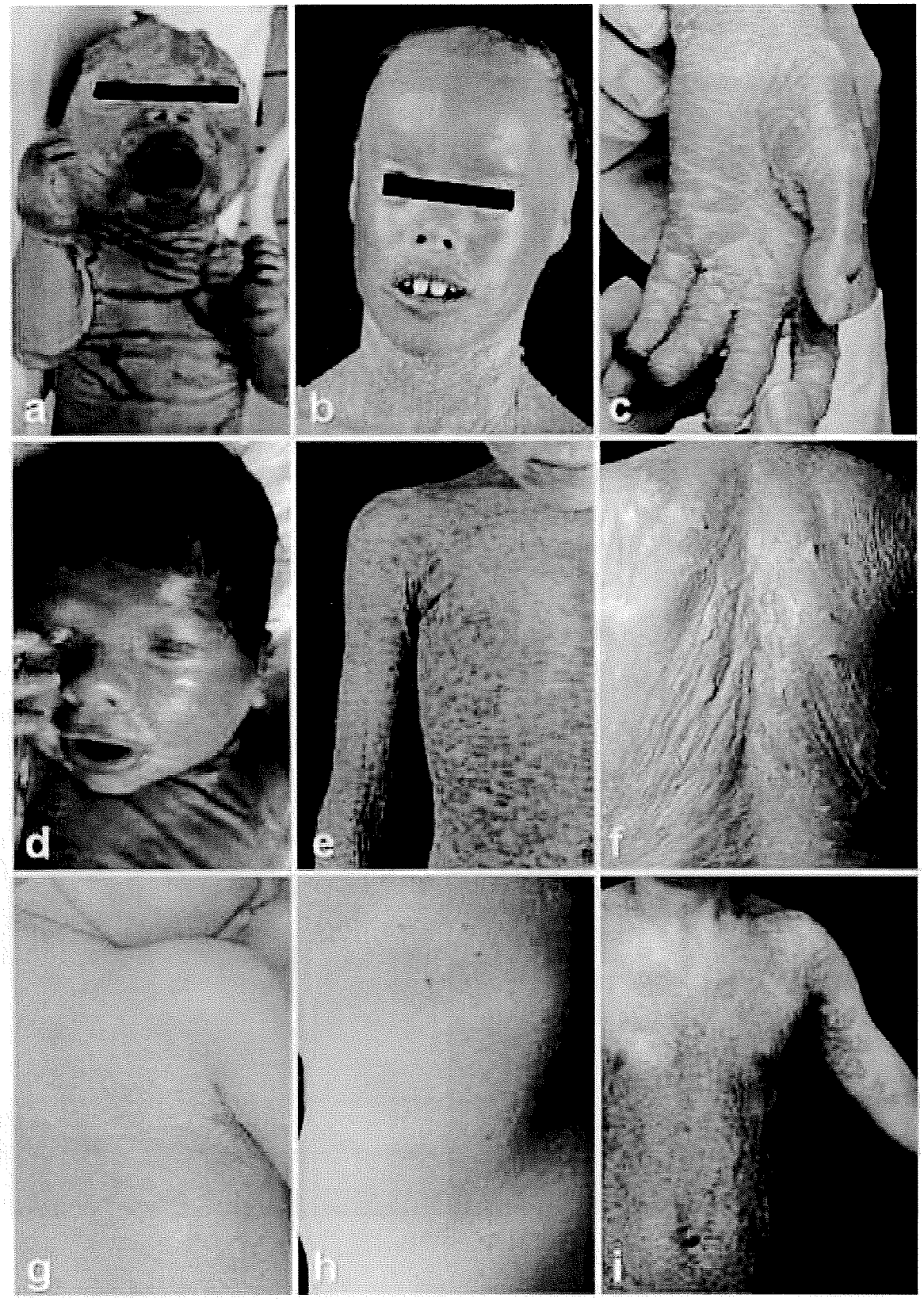

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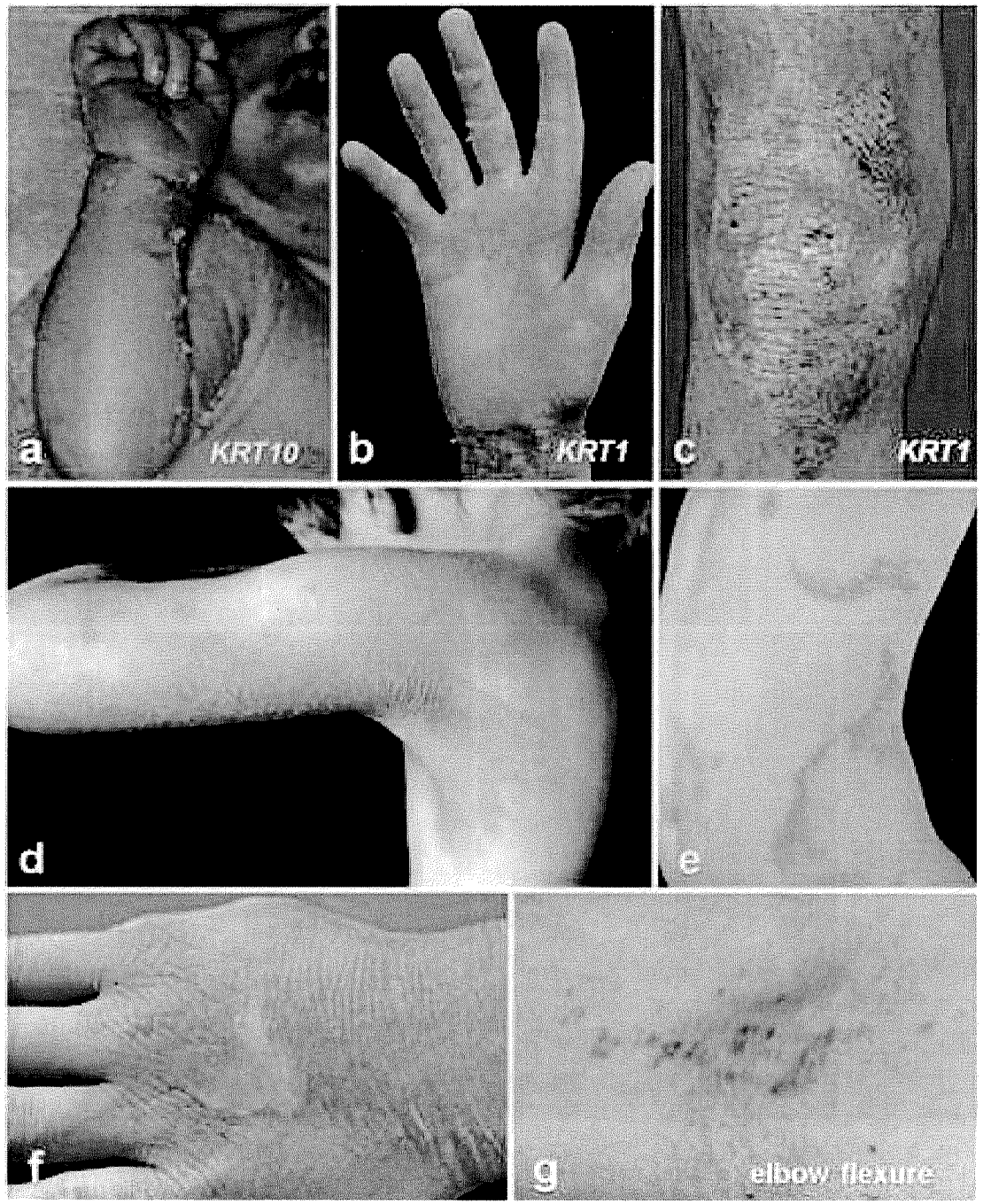

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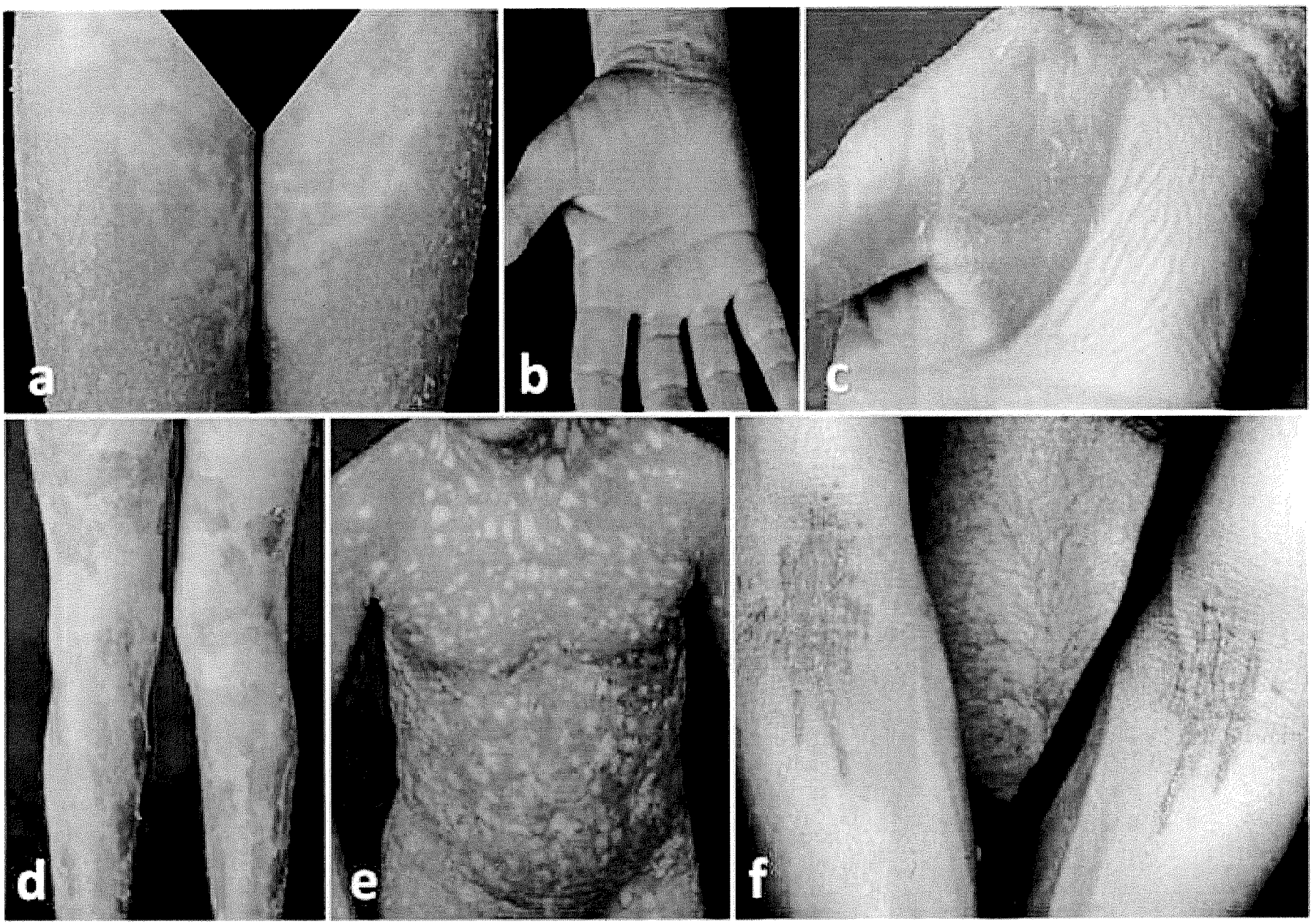

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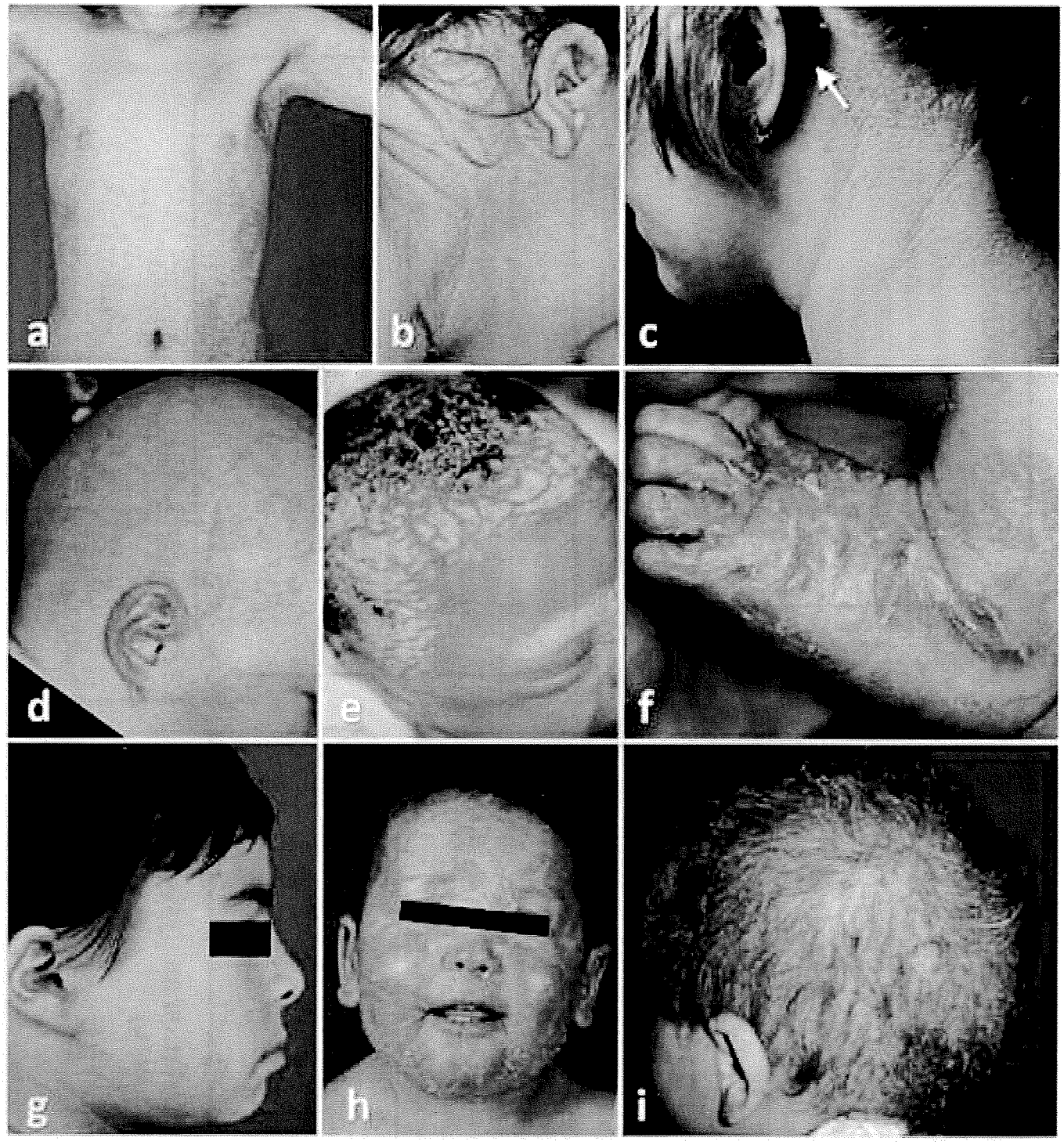

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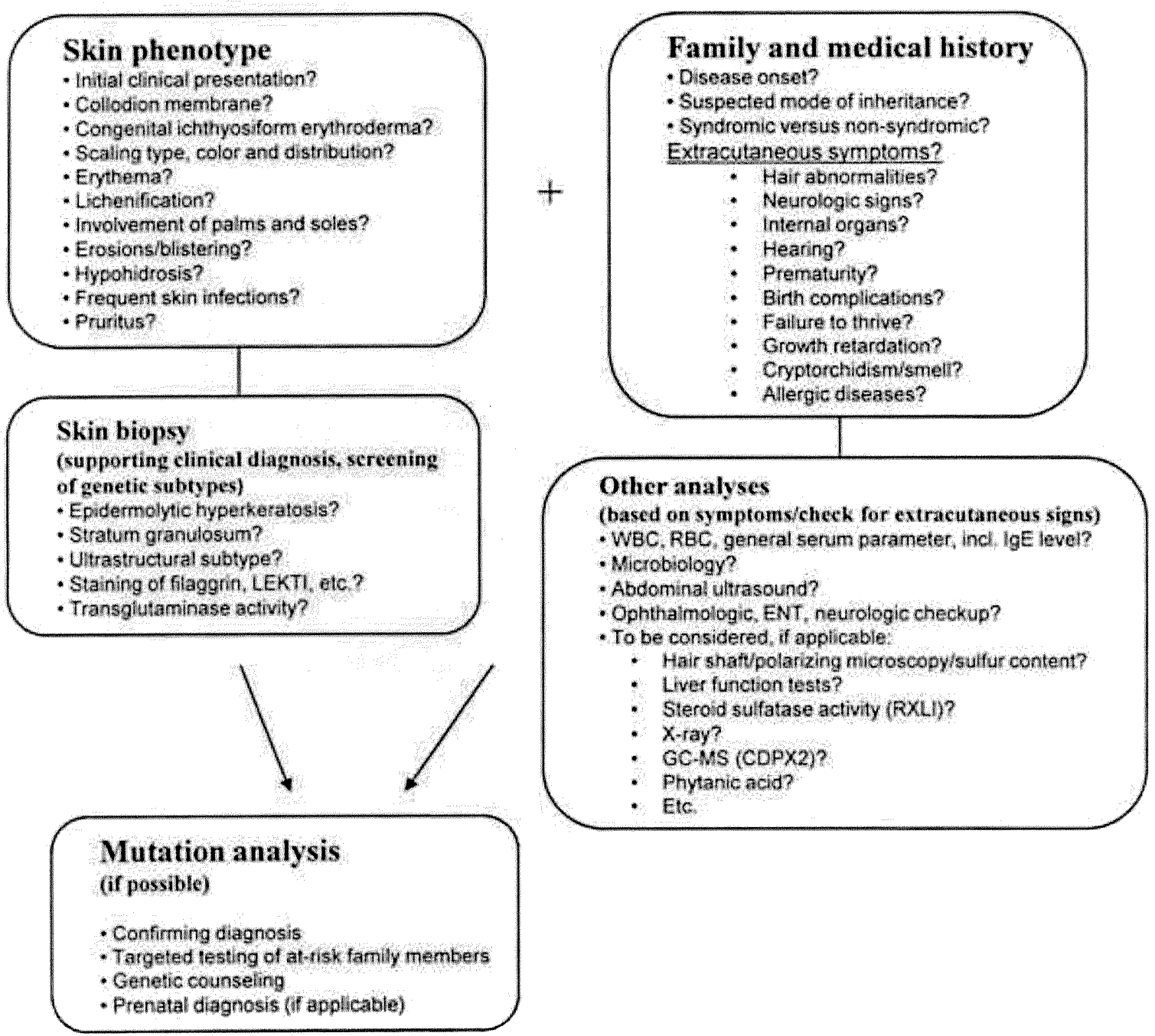

BJD
British Journal of Dermatology

Three-base deletion mutation c.120_122delGTT in *ATP2A2* leads to the unique phenotype of comedonal Darier disease

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calcium pump, comedone, hair follicle, SERCA2, small deletion

Conflicts of interest
None declared.

The first two authors contributed equally to this work.

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Darier disease (DD; Darier–White disease; OMIM 124200) is an autosomal dominant inherited disorder.[1] Clinically, it is characterized by recurrent and multiple hyperkeratotic papules or nodules affecting the trunk and flexural aspects of the extremities.[1] Characteristic histopathological features are dyskeratotic cells in the form of corps ronds and grains, suprabasal acantholysis forming suprabasal lacunae and irregular upward proliferation into the lacunae of papillae lined with a single layer of basal cells, the so-called villi.[2] The causative gene is *ATP2A2* (OMIM 108740) on chromosome 12, which encodes the sarco/endoplasmic reticulum calcium pump ATPase (SERCA2).[2]

Clinical variants include the hypertrophic, vesiculobullous, hypopigmented, cornifying, zosteriform and linear subtypes, and the rare subtype comedonal Darier disease (CDD).[1,3–6] CDD tends to appear in seborrhoeic areas. The characteristic morphological features are prominent follicular involvement, sometimes associated with keratotic plugs, and the presence of greatly elongated dermal villi and papillary projections.[4] There have been no conclusive reports on the aetiology of CDD and it is still controversial as to whether or not CDD is a variant of DD, and if it is caused by *ATP2A2* gene mutations, although a combination of CDD and classic DD was reported in one patient.[7] The present study identifies a previously unreported three-base deletion mutation in *ATP2A2* in a patient with CDD.

Patient and methods

A 22-year-old Japanese man presented with acne-like comedonal lesions on the face and chest, most densely distributed on the forehead, cheeks, back, axillae and chest. The comedonal lesions had first appeared in his teens and had gradually increased in number. Physical examination showed open comedones, closed comedones, red papules, nodules, cysts and ice-pick scars (Fig. 1a,b). His parents were clinically healthy, without any skin problems. He had been treated with oral biotin, Korean ginseng, an antihistamine, topical bufexamac ointment, calcipotriol ointment and betamethasone butyrate propionate ointment without any improvement. Histopathological observations revealed suprabasal acantholytic clefts and numerous dyskeratotic cells (corps ronds) in the outer root sheath in the affected follicular infundibulum, which was surrounded by plasma cells and lymphocytes (Fig. 1c,d). We made a diagnosis of CDD. Oral etretinate 10 mg daily combined with adapalene gel remarkably improved most of the skin lesions, except those on the forehead.

Polymerase chain reaction (PCR) amplification and direct sequencing of the entire coding region and exon/intron boundaries of *ATP2A2* were performed using the proband's and his parents' genomic DNA samples and genomic DNA samples from 50 healthy Japanese individuals as controls. A detected mutation was verified by mutant allele-specific amplification analysis[8] with mutant allele-specific primers carrying the substitution of two bases at the 3′ end, a PCR product band derived from the mutant allele.

This study was approved by the Hokkaido University Medical Ethics Committee and conducted according to the principles of the Declaration of Helsinki. All clinical samples were obtained with informed consent.

Results and discussion

Direct sequencing of *ATP2A2* in the proband's genomic DNA revealed a heterozygous three-base deletion c.120_122delGTT in exon 2, which causes deletion of leucine at the 41st amino acid residue from the amino terminus (p.Leu41del). This mutation was not detected in his parents nor in 100 normal unrelated alleles from 50 healthy individuals (Fig. 2). No other pathogenic mutations were detected within *ATP2A2* in the patient's DNA. By mutant allele-specific amplification ana-

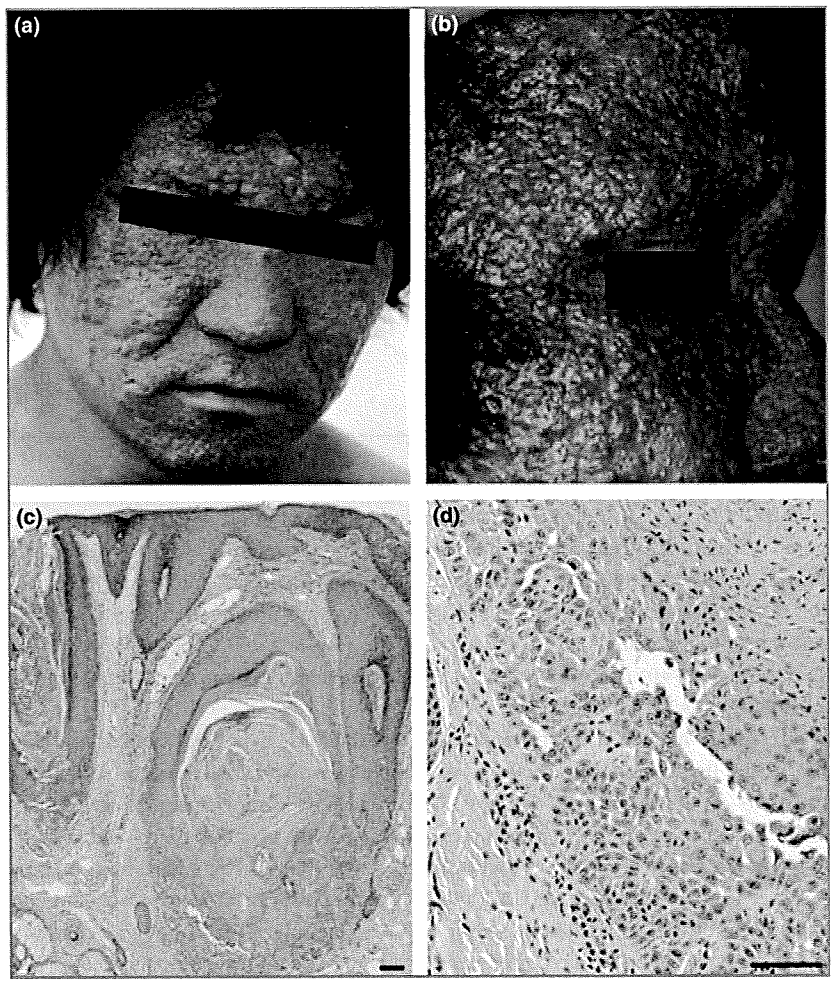

Fig 1. Unique clinical and histological features of comedonal Darier disease in the patient. (a, b) Open comedones, closed comedones, red papules, nodules, cysts and ice-pick scars are present on the face. (c, d) Histology of the comedonal lesions. Dilated, cystic hair follicles with keratin plugs are seen in the dermis (c). Suprabasal acantholytic clefts and numerous dyskeratotic cells in the form of corps ronds are apparent in the outer root sheath in the affected follicular infundibulum (d). Bars = 100 mm.

lysis,[8] a PCR product band derived from the mutant allele was amplified from the patient's genomic DNA, but not from either parent nor from the control DNA samples.

Since the first description of CDD by Derrick *et al.*,[4] only seven cases including the present one have been reported. The present case is the first in which a causative mutation has been identified. *ATP2A2* gene mutations in DD have been reported to result in alterations in calcium signalling during keratinocyte differentiation, causing acantholytic dyskeratosis.[2,9,10] The function of SERCA2 is to pump calcium from the cytosol to the endoplasmic reticulum and to excite oscillation of calcium spikes in the cytosol.[11–13] The mutation site in our patient localized to the first stalk (S1) of SERCA 2. The S1 region adjacent to transmembrane helices is considered to be highly conserved at the amino acid level by many species.[2] p.Leu41-del in S1 of SERCA2 is considered to impair calcium-binding sites in the α-helix of the region that contains a signal for sarco/endoplasmic reticulum localization, and to change the conserved alignment of five glutamic acid residues.[11,14] Several mutations in the S1 region of *ATP2A2* in DD have been reported.[2,10,14–16] In addition, the dephosphorylation process of SERCA2 is thought to be important for Ca^{2+} ion release into the lumen by SERCA2 and, recently, Miyauchi *et al.*[17] reported that both p.Leu41del and p.Pro42del mutations inhibit the dephosphorylation process. The present c.120_122delGTT mutation has not been reported, although a heterozygous deletion of the identical leucine residue, c.121_123delTTA (p.Leu41del), was reported in one patient.[14] The patient showed severe hypertrophic scar formation in addition to common DD skin manifestations and had severe emotional problems and a family history of suicide.[14] Our patient with CDD had a quite different skin phenotype and showed no mental problems. We do not know exactly why the phenotype differs between our case and the previously reported cases. There is a possibility that a silent mutation or allelic variant of *ATP2A2* may have affected the phenotypic expression in our patient and/or the previously reported cases. Certain environmental factors, such as mechanical trauma, sun exposure, heat and sweating often define a phenotype of DD,[2] and such factors may be related to the formation of the CDD phenotype in our patient, because the face is more frequently affected than other body sites by environmental factors. Further functional studies are required to elucidate the pathomechanisms of CDD, a unique phenotype of DD. Interestingly, an *ATP2A2* mutation was reported to underlie two cases from one British family with another unique phenotype, acrokeratosis verruciformis (AKV), providing evidence that AKV and DD are allelic disorders.[18] On the other

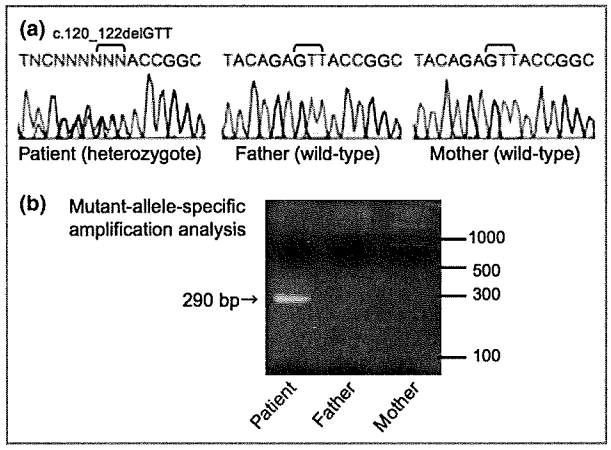

Fig 2. A heterozygous in-frame three-nucleotide deletion mutation c.120_122delGTT in *ATP2A2* was detected. (a) Direct sequencing of *ATP2A2* exon 2 polymerase chain reaction products by a reverse primer revealed that the patient was heterozygous for the three-nucleotide deletion mutation c.120_122delGTT. This mutation was not detected in genomic DNA samples from the patient's parents. (b) Mutant allele-specific amplification analysis shows the amplification band from the mutant allele as a 290-bp fragment only from the DNA sample of the patient, confirming the mutation c.120_122delGTT in the patient.

hand, mutational analysis showed no mutation in *ATP2A2* and genotyping and linkage analysis results revealed no linkage evidence to the locus including *ATP2A2* in a large Chinese family with AKV.[19] Thus, AKV might be a genetically heterogeneous disorder. In any case, further accumulation of cases with molecular genetic assessment is needed to improve understanding of the pathogenesis of variant phenotypes of DD such as CDD and AKV.

Acknowledgments

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References

1 Burge SM, Wilkinson JD. Darier–White disease: a review of the clinical features in 163 patients. *J Am Acad Dermatol* 1992; **27**:40–50.
2 Sakuntabhai A, Ruiz-Perez V, Carter S *et al.* Mutations in *ATP2A2*, encoding a Ca^{2+} pump, cause Darier disease. *Nat Genet* 1999; **21**:271–7.
3 Berth-Jones J, Hutchinson PE. Darier's disease with peri-follicular depigmentation. *Br J Dermatol* 1989; **120**:827–30.
4 Derrick EK, Darley CR, Burge S. Comedonal Darier's disease. *Br J Dermatol* 1995; **132**:453–5.
5 Hayakawa K, Nagashima M. A rare presentation of acantholytic dyskeratosis. *Br J Dermatol* 1995; **133**:487–9.
6 Peck GL, Kraemer KH, Wetzel B *et al.* Cornifying Darier disease – a unique variant. I. Report of a case. *Arch Dermatol* 1976; **112**:495–503.
7 Aliağaoğlu C, Atasoy M, Anadolu R *et al.* Comedonal, cornifying and hypertrophic Darier's disease in the same patient: a Darier combination. *J Dermatol* 2006; **33**:477–80.
8 Hasegawa Y, Takeda S, Ichii S *et al.* Detection of K-ras mutations in DNAs isolated from feces of patients with colorectal tumors by mutant-allele-specific amplification (MASA). *Oncogene* 1995; **10**:1441–5.
9 Cho JK, Bikle DD. Decrease of Ca(2+)-ATPase activity in human keratinocytes during calcium-induced differentiation. *J Cell Physiol* 1997; **172**:146–54.
10 Sakuntabhai A, Burge S, Monk S *et al.* Spectrum of novel *ATP2A2* mutations in patients with Darier's disease. *Hum Mol Genet* 1999; **8**:1611–19.
11 MacLennan DH, Brandl CJ, Korczak B *et al.* Amino-acid sequence of a Ca^{2+} + Mg^{2+}-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. *Nature* 1985; **316**:696–700.
12 Petersen CC, Petersen OH, Berridge MJ. The role of endoplasmic reticulum calcium pumps during cytosolic calcium spiking in pancreatic acinar cells. *J Biol Chem* 1993; **268**:22262–4.
13 Pozzan T, Rizzuto R, Volpe P *et al.* Molecular and cellular physiology of intracellular calcium stores. *Physiol Rev* 1994; **74**:595–636.
14 Ringpfeil F, Raus A, DiGiovanna JJ *et al.* Darier disease – novel mutations in *ATP2A2* and genotype–phenotype correlation. *Exp Dermatol* 2001; **10**:19–27.
15 Jacobsen NJ, Lyons I, Hoogendoorn B *et al.* *ATP2A2* mutations in Darier's disease and their relationship to neuropsychiatric phenotypes. *Hum Mol Genet* 1999; **8**:1631–6.
16 Ruiz-Perez VL, Carter SA, Healy E *et al.* *ATP2A2* mutations in Darier's disease: variant cutaneous phenotypes are associated with missense mutations, but neuropsychiatric features are independent of mutation class. *Hum Mol Genet* 1999; **8**:1621–30.
17 Miyauchi Y, Daiho T, Yamasaki K *et al.* Comprehensive analysis of expression and function of 51 sarco(endo)plasmic reticulum Ca^{2+}-ATPase mutants associated with Darier disease. *J Biol Chem* 2006; **281**:22882–95.
18 Dhitavat J, Macfarlane S, Dode L *et al.* Acrokeratosis verruciformis of Hopf is caused by mutation in *ATP2A2*: evidence that it is allelic to Darier's disease. *J Invest Dermatol* 2003; **120**:229–32.
19 Wang PG, Gao M, Lin GS *et al.* Genetic heterogeneity in acrokeratosis verruciformis of Hopf. *Clin Exp Dermatol* 2006; **31**:558–63.

International Journal of Dermatology

Case report

An Indian family with Sjögren-Larsson syndrome caused by a novel *ALDH3A2* mutation

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Running head: ALDH3A2 mutation in Indian SLS patients

Key words: genodermatosis, ichthyosis, pediatric dermatology

Abbreviations: ALDH, aldehyde dehydrogenase; FALDH, fatty aldehyde dehydrogenase; MASA, mutant allele-specific amplification; SLS, Sjögren-Larsson syndrome

Abstract

Sjögren-Larsson syndrome is an autosomal-recessive hereditary disorder characterized by congenital ichthyosis, mental retardation and spastic diplegia or tetraplegia. It is known that mutations in the fatty aldehyde dehydrogenase (FALDH) gene (*ALDH3A2*) underlie SLS. We report two Indian sisters showing typical clinical features of SLS. Direct sequencing of the entire coding region of *ALDH3A2* revealed a novel homozygous mutation, c.142G>T (p.Asp48Tyr) in exon 1, in both patients. Their parents harbored the mutation heterozygously. Mutant-allele-specific amplification analysis using PCR products as a template verified the mutation in the patients. The aspartic acid residue at the mutation site is located in the C-terminal portion of the second α-helix strand, α2, of N-terminal four helices of FALDH and the FALDH amino-acid sequence alignment shows that this aspartic acid residue is conserved among several diverse species. Until now, a number of mutations in *ALDH3A2* have been shown to be responsible for SLS in Europe, the Middle East, Africa, and North and South America. However, in Asian populations, *ALDH3A2* mutations have been identified only in Japanese SLS patients. Here we report an *ALDH3A2* mutation for the first time in SLS patients in the Asian country other than Japan. The present results suggest that *ALDH3A2* is a gene responsible for SLS in Asian populations. We hope *ALDH3A2* mutation search will be globally available including many Asian countries in the future.

Case

Two sisters were born in an Indian non-consanguineous family. The proband was a 1.5-year-old girl. She had had severe ichthyosis on the whole body since birth, especially prominent on the bilateral lower limbs (Fig. 1a, b, c). She showed mental retardation and spastic tetraplegia. Ocular fundus evaluation revealed white dots in the maculae. The elder sister also had icthyotic lesions all over the body at birth and had global developmental delay. She had had seizures since 2.5 years of age that had been controlled with multiple antiepileptic medications. At the age of four, severe hyperkeratosis appeared on the chest, back, axillae and predominantly over the limbs (Fig. 1d, e). She has hypertelorism, dolichocephalic head, large low-set ears, long eyelashes and short 3rd, 4th and 5th metatarsals. Neurological evaluations revealed severe spastic tetraplegia with persistent ankle clonus and complete head lag. She showed serious mental retardation. She had severe photophobia, and ocular fundus evaluation showed white glistening dots in the maculae bilaterally. Severe auditory startle reaction was a characteristic feature. Magnetic resonance imaging of the brain showed bilateral symmetrical diffuse white matter at high intensity in T2-weighted images in the frontal, temporal and parietal regions. Both sisters were diagnosed with Sjögren-Larsson syndrome (SLS) from these clinical features and laboratory data.

Fatty aldehyde dehydrogenase (FALDH) gene (*ALDH3A2*) mutational analysis was performed on the affected girls and their parents, as previously described.[1, 2] In the patients, a novel homozygous mutation, c.142G>T (p.Asp48Tyr) in exon 1, was identified. Their parents harbored the mutation heterozygously (Fig. 2a). This mutation was not found in 200 normal unrelated alleles (100 individuals) by direct sequence analysis. Mutant-allele-specific amplification (MASA) analysis verified the mutation

in this family (Fig. 2b).

Discussion

SLS (MIM# 270200) is an autosomal-recessive hereditary disorder characterized by congenital ichthyosis, mental retardation and spastic diplegia or tetraplegia.[3] In 1996, De Laurenzi *et al.*[4] reported that mutations in *ALDH3A2* underlie SLS. The present study reports a novel homozygous mutation in *ALDH3A2* in an Indian family with SLS.

The FALDH amino-acid sequence alignment shows that this aspartic acid residue at codon 48 is conserved among several diverse species. Compared with other aldehyde dehydrogenase (ALDH)-related sequences identified by Perozich *et al.*,[5] this aspartic acid is highly conserved among many members of the ALDH family (Fig. 2c). Analysis of the crystallized 3-D structure of the related class 3 rat cytosolic ALDH revealed that this aspartic acid is located in the C-terminal portion of the second α-helix strand, α2, of N-terminal four helices (Fig. 2c).[6] These findings strongly suggest that this aspartic acid residue is essential for the normal function of the FALDH. In the literature, missense mutation p.Ile45Phe in the α2 helix, three codons upstream of the present mutation site, was reported and the mutant enzyme was revealed to have only 9% residual enzyme activity compared with the wild-type enzyme.[7]

Until now, a number of mutations in *ALDH3A2* have been shown to be responsible for SLS in Europe, the Middle East, Africa, and North and South America.[1, 7] However, in Asian populations, *ALDH3A2* mutations have been identified only in Japanese SLS patients.[1, 2, 8-10] Here we report an *ALDH3A2* mutation for the first time in SLS patients in the Asian country other than Japan. The present results suggest that *ALDH3A2* is a

gene responsible for SLS in Asian populations. Mutation analysis of the *ALDH3A2* gene is a highly sensitive method of confirming a diagnosis of SLS. It does not require a skin biopsy or FALDH enzymatic assays. We hope *ALDH3A2* mutation search will be globally available including many Asian countries in the future.

Acknowledgments

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References

1 Rizzo WB, Carney G, Lin Z. The molecular basis of Sjögren-Larsson syndrome: mutation analysis of the fatty aldehyde dehydrogenase gene. *Am J Hum Genet* 1999; **65:** 1547-1560.

2 Shibaki A, Akiyama M, Shimizu H. Novel ALDH3A2 heterozygous mutations are associated with defective lamellar granule formation in a Japanese family of Sjögren-Larsson syndrome. *J Invest Dermatol* 2004; **123:** 1197-1199.

3 Sjögren T, Larsson T. Oligophrenia in combination with congenital ichthyosis and spastic disorders; a clinical and genetic study. *Acta Psychiatr Neurol Scand Suppl* 1957; **113:** 1-112.

4 De Laurenzi V, Rogers GR, Hamrock DJ, *et al.* Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. *Nature Genet* 1996; **12:** 52-57.

5 Perozich J, Nicholas H, Wang B-C, *et al.* Relationships within the aldehyde dehydrogenase extended family. *Protein Sci* 1999; **8:** 137-146.

6 Liu Z-J, Sun Y-J, Rose J, *et al.* The first structure of an aldehyde dehydrogenase reveals novel interactions between NAD and the Rossmann fold. *Nat Struct Biol* 1997; **4:** 317-26.

7 Rizzo WB, Carney G. Sjögren-Larsson syndrome: diversity of mutations and polymorphisms in the fatty aldehyde dehydrogenase gene (*ALDH3A2*). *Hum Mutat* 2005; **26:** 1-10.

8 Tsukamoto N, Chang C, Yoshida A. Mutations associated with Sjögren-Larsson syndrome. *Ann Hum Genet* 1997; **61:** 235-242.

9 Aoki N, Suzuki H, Ito K, Ito M. A novel point mutation of the FALDH gene in a Japanese family with Sjögren-Larsson syndrome. *J Invest Dermatol* 2000; **114:** 1065-1066.

10 Sakai K, Akiyama M, Watanabe T, *et al.* Novel ALDH3A2 heterozygous mutations in a Japanese family of Sjögren-Larsson syndrome. *J Invest Dermatol* 2006; **126:** 2545-2547.

Figure Legends

Figure 1 Clinical features of the Indian sisters with SLS. (a-c) The younger sister. Hyperkeratosis and scales cover whole body surface at 1.5 years of age (a). Dark brown scales are seen on the bilateral legs (b), the arms and the trunk (c). (d, e) The elder sister shows hyperkeratosis and brown scales on the bilateral arms at 4 years of age.

Figure 2 *ALDH3A2* mutation in the present SLS patients, and sequence alignments around the missense mutation.

(a) Sequence analysis of *ALDH3A2*. In both patients, the younger sister (Child 1) and the elder sister (Child 2), a homozygous missense mutation c.142G>T (p.Asp48Tyr) in exon 1 derived from their parents was detected. The parents were heterozygous for the mutation.

(b) Mutant-allele-specific amplification (MASA) analysis. With normal allele-specific primers, no amplification band is seen in the PCR products from the patients' DNA samples, suggesting that they have no normal allele. With mutant allele specific primers, the amplification band from the mutant alleles is detected as a 434-bp fragment only in the PCR products from the DNA samples from the patients, and not in the PCR products from control DNA samples. This confirms the presence of the mutation c.142G>T in the patients.

(c) Top: a sequence alignment between FALDH, rat class 3 and human class 2 ALDHs. Aspartic acid residue at codon 48 of FALDH is conserved. Secondary structure components found in the class 3 rat ALDH structure by Liu *et al.* [6] are presented with bars representing α-helices.

Bottom: FALDH amino acid sequence alignment shows the level of conservation in diverse species of aspartic acid residue at codon 48 (D48) (red characters), which was altered by the missense mutation in the present family.

Journal of the American Academy of Dermatology
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CASE LETTER

Extremely severe palmoplantar hyperkeratosis in an generalized epidermolytic hyperkeratosis patient with a keratin 1 gene mutation

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Word count: 572 words in the main text

Key words
bullous congenital ichthyosiform erythroderma, epidermolytic ichthyosis, keratinopathic ichthyosis, KRT1, palmoplantar keratoderma

To the Editor:

Epidermolytic hyperkeratosis (EHK) (OMIM#113800), also termed as bullous congenital ichthyosiform erythroderma, is a rare genetic disorder of keratinization. We report a generalized EHK case showing extremely severe palmoplantar hyperkeratosis with digital contractures.

A 45-year-old Japanese man visiting our hospital reported that he had been born with erythroderma. He had exhibited skin blistering, erosions and hyperkeratosis on the erythrodermic skin since infancy. The blistering and erosion gradually diminished with age. He had developed severe palmoplantar hyperkeratosis and digital contractures at the age of 7 years. At the age of 24 years, surgical operation was performed to improve the contraction of his toes. Physical examination revealed hyperkeratosis on the whole body, especially at the ankles, elbows and knees, and erosions were observed on the inner side of the elbows and knees (Fig. 1a-d). Palmoplantar hyperkeratosis was severe with digital contracture. The morphology of his hair, nails and teeth were normal. There was no known family history of skin disease. Skin biopsy from the left upper arm showed severe granular degeneration in all the suprabasal layers (Fig. 1e). Ultrastructural analysis revealed clumping of the intermediate filaments within keratinocytes of the suprabasal layers (Fig. 1f).

Direct sequencing of the whole coding regions of *KRT1* and *KRT10* (GenBank accession numbers: NT029419.11, NT010755.15) was performed as previously described[1] and a novel heterozygous *KRT1* missense mutation c.1457T>G (p.Leu486Arg) was identified in exon 7. This mutation was verified by restriction enzyme *Msp*I digestion. The mutation p.Leu486Arg was not found in 100 normal, unrelated Japanese

alleles (50 healthy unrelated individuals) by sequence analysis (data not shown).

The present novel *KRT1* mutation p.Leu486Arg is in the 2B segment of keratin 1 (Fig. 2a, b). This mutation occurred within the highly conserved helix termination motif (HTM) of the K1 protein. The palmoplantar hyperkeratosis was extremely severe. It is noteworthy that another mutation at the identical position of K1, p.Leu486Pro, was reported in EHK with severe palmoplantar hyperkeratosis (Fig. 2c) and digital contracture, and the affected individuals exhibited clinical features similar to our patient's.[2] Thus, our data further suggest that a non-conservative amino-acid change at codon 486 of K1 results in a severe form of generalized EHK.

The rod domains consist of four alpha-helical segments that possess a repeating heptad amino acid residue peptide motif ***(a-b-c-d-e-f-g)n*** that has the potential to form a two-chain coiled coil with a corresponding sequence (Fig. 2d).[3-5] The residues at positions ***a,d,e,g*** are considered to be highly sensitive to mutations.[6]

The present patient with generalized EHK had some of the most severe palmoplantar hyperkeratosis of previously reported cases with mutations in *KRT1*. The leucine residue at codon 486 is located in the ***a*** position of the heptad repeat at the C-terminal end of the 2B helix and the substitution of arginine for leucine alters the character of amino acid seriously. Thus, it is reasonable to say that this mutation caused generalized EHK with severe palmoplantar hyperkeratosis, compared with that seen in patients harbouring mutations in the other residues.

26 EHK cases including the present case with point mutations at the helix initiation motif (HIM) and HTM of *KRT1* have been reported to date (Fig. 2c) (Human Intermediate Filament Database, http://www.interfil.org/). Only 9 cases including the present case were diagnosed as generalized EHK with severe palmoplantar hyperkeratosis, and 7 cases out of 9 harboured missense mutations in the heptad repeat position ***a***, ***d***, ***e*** and ***g***. These facts indicate that the mutation site and the nature of amino acid alterations in K1 may determine the level of severity of palmoplantar hyperkeratosis.

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