or IgE, but not IgG, class of anti-Dsg antibodies may be pathogenic (8). This possibility can be confirmed by IF, IB or ELISA using antibodies specific to each immunoglobulin subtype as a second antibody.

As the fifth possibility, we speculate that pathogenic autoantibodies may react with p38 mitogen-activated protein kinases (MAPK) activating domain within the Dsg molecule, because p38 MAPK-related signal transduction has been reported to play an important role in induction of keratinocyte detachment (18). This possibility can be confirmed by detection of phosphorylated p38 MAPK or addition of p38 MAPK-inhibitor in the study of addition of autoantibodies into cultured keratinocytes (18).

In addition, the difference in avidity (affinity) of autoantibodies suggested by Muro et al. is an interesting possibility for pathogenicity of anti-Dsg autoantibodies, which can be confirmed by autoantibodies-stripping ELISA by urea treatment or inhibition by self-antigen in a liquid phase (9).

Further research is needed in order to elucidate the reasons for non-pathogenicity of a high Dsg index in pemphigus vulgaris patients in remission.

The authors declare no conflicts of interest.

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The corresponding author of the paper by Li et al. has been contacted but abstained from replying.

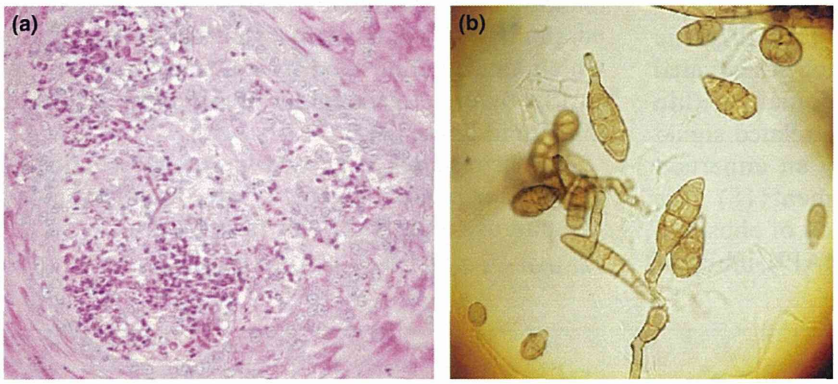

Figure 2 (a) Biopsy of a verrucous nodule of patient 2, evolving for 2 months. HXE staining, 40×, showing a branched, septated hyphae surrounded by a mixed lymphocytic and neutrophilic infiltrate within a granuloma in the dermis. (b) *Alternaria alternata*, with medium-brown slender, multiseptated conidia.

sessions of cryotherapy, with clinical resolution. Nevertheless, within 5 months she developed verrucous papules and nodules on the nose, forearms and legs bilaterally. These were treated with terbinafine (250 mg id, 12 weeks) and subsequently voriconazole (400 mg id, 8 weeks) along with excision and cryotherapy.

Six years later, despite no identifiable factor, she continues to develop lesions. Apart from this, we did not observe relapses or new lesions during a mean follow-up of 32 months.

Cutaneous alternariosis represents 74.3%[1] of overall *alternaria* cases, mostly by *A. alternata,* and is observed in Mediterranean countries in patients with predisposing factors (immunosuppression, local wound or systemic disease).[1] Over 50%[1] occur in transplanted patients as evidenced in our series.

Rural surroundings are predominant[1] as we observed, and local trauma facilitates the penetration of the pathogen, justifying why reported lesions are mainly solitary.[1] In our experience, however, multiple lesions were more frequent.

Moreover, we can speculate that the recurrent picture of our 'healthy' patient may be due to a specific host susceptibility to *Alternaria* infection.

In transplanted patients, immunosuppressant reduction is obligatory to improve immune status and because of the interaction of antifungals (namely itraconazole) at tacrolimus' common CYP3A4 pathway.[4] Close track of tacrolinemia and renal function is mandatory.

Itraconazole,[2] voriconazole,[2] posaconazole,[2] amphotericine B[3] and terbinafine[3] can all be used. In our series, itraconazole was efficacious when combined with cryotherapy, surgical excision or both.

Therefore, we suggest a 12-week course of itraconazole, 200-mg bid, associated with physical intervention and follow-up for at least 24 months.

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Successful treatment with infliximab of sibling cases with generalized pustular psoriasis caused by deficiency of interleukin-36 receptor antagonist

Editor

Tumour necrosis factor-α (TNF-α) inhibitors often lead to rapid resolution of generalized pustular psoriasis (GPP).[1] However, the agents' mechanism of action against GPP remains to be elucidated, because the aetiology of the disease had been unknown. Recently, we reported that the majority of GPP cases that are not preceded by psoriasis vulgaris (PV; GPP alone) are caused by deficiency of interleukin-36 receptor antagonist (DITRA) due to homozygous or compound heterozygous *IL36RN* mutations.[2]

The patients were three Japanese siblings and their mother: a 39-year-old woman (Patient 1), a 36-year-old man (Patient 2), a

29-year-old man (Patient 3) and a 65-year-old woman (Patient 4). The parents are non-consanguineous. Patient 4 had been suffering from GPP preceded by PV since she was 53-years-old (Fig. 1a). The disease onset was at 10 years of age for Patients 1 and 2 and at 6 years of age for Patient 3. Patients 1, 2 and 3 had not had any previous PV lesions. They had recurrent erythema with pustules on the whole body and a fever of over 38°C. At exacerbation of the disease, blood examinations revealed elevated white blood cell count and C-reactive protein concentration. Bacterial cultures of the pustules were negative. They had pathological findings of spongiform pustules of Kogoj by skin biopsies from pustular eruptions. The siblings were diagnosed with GPP alone.

Following ethical approval, informed consent was obtained from the patients in compliance with the Declaration of Helsinki principles. All the coding regions of *IL36RN*, including the exon/intron boundaries, were sequenced using genomic DNA samples from the patients.[2] Patients 1, 2 and 3 were found to have the homozygous mutation c.115 + T > C (p.Arg10ArgfsX1) in *IL36RN*, which is one of the GPP-causing founder mutations in Japanese[2] (Fig 2). Patient 4 had heterozygous mutation c.115 + T > C (Fig. 2). The siblings were diagnosed with GPP caused by DITRA.

Satisfactory treatment results had not been obtained with various drugs, including oral cyclosporine A. Then, Patients 1, 3 and 4 were treated with 5 mg/kg of infliximab on the first treatment day, 2 weeks later and 4 weeks later as the initial treatment, and thereafter once every 8 weeks for maintenance therapy. The GPP lesions of Patients 1 and 3 rapidly resolved during the initial treatment period and have not relapsed for 3 years (Fig. 1b–e). The GPP lesions of Patient 4 have largely resolved. There are no apparent adverse effects. Infliximab therapy was recently started also in Patient 2.

Interleukin-36 (IL-36) is considered to play a major role in the immunopathogenesis of GPP caused by DITRA.[3,4] Carrier *et al.* reported that IL-36 expression in keratinocytes is enhanced by IL-1α, TNF-α and IL-17 *in vitro*.[5] Thus, we consider that the infliximab down-regulated IL-36 production and resolved the GPP lesions in Patients 1 and 3.

Viguier *et al.*[1] reported infliximab to be effective for two cases of GPP caused by DITRA, but both patients had severe adverse effects, including vomiting, fever and culture-negative pneumonia. Both patients were successfully treated by switching them from infliximab to adalimumab or etanercept, which are alternative TNF-α inhibitors.[1] Herein, we clearly demonstrated that infliximab was effective without any serious side-effects for two sibling cases of GPP caused by DITRA. Given that the majority of GPP alone cases are caused by DITRA, we think that most cases of GPP alone could be successfully treated with TNF-α

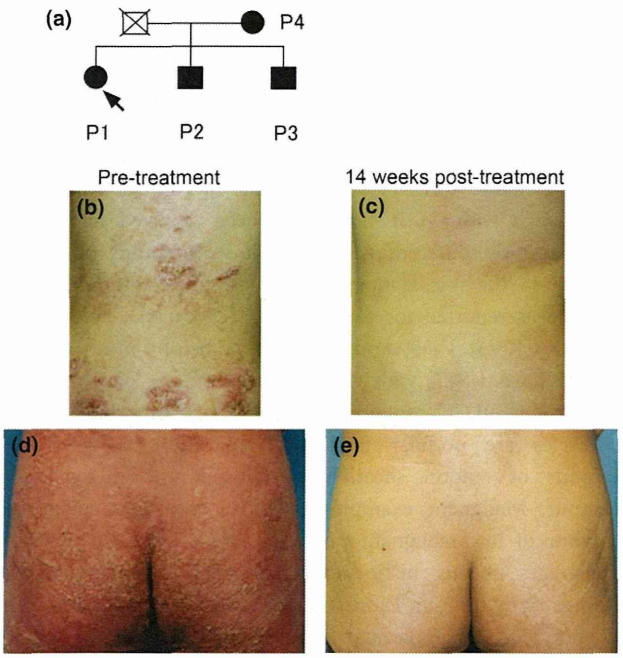

Figure 1 Pedigree of the patients' family and skin manifestations of Patients 1 and 3 before and after treatment with infliximab (a) Pedigree of the patients' family. The mother (Patient 4) was suffering from GPP preceded by PV. (b) Pustules in the background of erythema were observed on trunk in Patient 1 before treatment with infliximab. (c) The skin eruptions largely resolved by the 14th week of treatment with infliximab. Skin eruptions on the buttocks of Patient 3 before (d) and after 14 weeks (e) of treatment with infliximab.

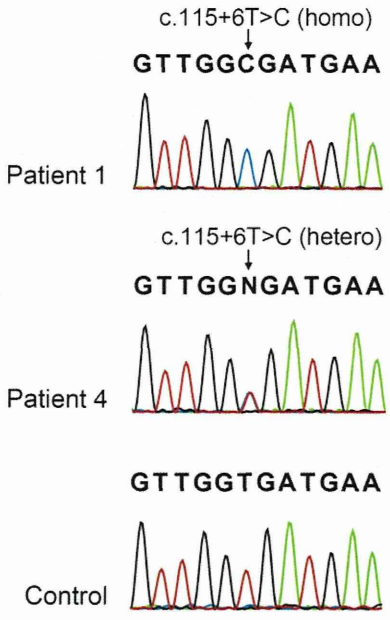

Figure 2 Sequence data of *IL36RN*. Sequence data of *IL36RN* are shown for Patient 1, Patient 4 and a control.

inhibitors such as infliximab, because TNF-α plays a major role in the immunopathogenesis of GPP caused by DITRA.

In conclusion, Viguier's cases and our cases suggest that TNF-α inhibitors are powerful tools for treating GPP caused by DITRA.

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Cutaneous squamous cell carcinoma with osteoclast-like giant cells: a very rare variant of cutaneous squamous cell carcinoma

Editor

An 89 year-old female presented to us with an asymptomatic ulcerated multinodular tumour on the left temple which had grown over the past 6 months. On clinical examination, a 3 cm-sized erythematous, ulcerated, multinodular tumour was seen on the left temple. With the clinical suspicion of squamous cell carcinoma (SCC) complete excision for histologic examination was performed.

On H&E staining, a deeply infiltrating multinodular tumour consisting of two components was seen. One component was a moderately-differentiated, keratinizing, cutaneous SCC with deep dermal infiltration (Fig. 1a,b). Surrounding the infiltrating strands of carcinoma cells, there was a mixed inflammatory infiltration consisting of lymphocytes, several eosinophils and numerous, partly bizarre osteoclast-like giant cells (OLGCs) (Fig. 1c). Most OLGCs were located in close proximity to the epithelial strands and contained up to 40 nuclei. Some OLGCs showed eosinophilic inclusions. On immunohistochemistry (IHC), the SCC was positive for Pan-Cytokeratin (AE1/3) and p63. OLGCs were negative for p63 and Pan-Cytokeratin except for some intracellular inclusions which were positive for Pan-Cytokeratin (Fig. 2c,d). In contrast, CD68 was strongly expressed by OLGCs, but not by SCC cells (Fig. 2a,b). Thus, the diagnosis of cutaneous SCC with OLGC was made.

After complete excision, wound closure was achieved with a split-skin graft. On clinical examination and sonography of cervical lymph nodes, there was no evidence of metastatic spread. Thus, regular follow-up according to the guidelines for cutaneous SCC was recommended.

OLGCs have been described to occur in rare variants of diverse extraosseous, visceral, usually moderately- to poorly differentiated malignant tumours. Cutaneous malignant tumours with infiltration of OLGCs are extremely rare. In the literature, OLGCs were first reported in a malignant melanoma in 2005[1] and in a cutaneous SCC in 2007.[2] Overall, up to now only six cases of cutaneous SCC and three cases of malignant melanoma with OLGCs have been reported.[3–6] In the majority of cases, it is assumed that OLGCs are rather a reactive component as they show a markedly different pattern of marker expression as compared to the neoplastic component. However, it cannot be excluded that OLGCs derive from the neoplastic cells in certain cases of cutaneous malignant tumours. In contrast to our case most SCCs with OLGCs that have been reported so far were rather poorly differentiated or even sarcomatoid potentially leading to the misdiagnosis of atypical fibroxanthoma (AFX) which was reported for two cases.[4] IHC for p63, cytokeratins (AE1/3, CK5/6), CD99 and CD10 can be helpful in distinguishing AFX from SCC with OLGCs. Furthermore, discrimination from other OLGC-containing conditions is important. Benign examples are nodular fasciitis, dermatofibroma, giant cell tumour of tendon sheath and phosphaturic mesenchymal tumour. Malignant examples comprise soft tissue giant cell tumour of low malignant potential (STGCT-LMP), giant cell malignant fibrous histiocytoma (GC-MFH), plexiform fibriohistiocytiy tumour, leiomyosarcoma with giant cells and AFX. Phenotypic and molecular studies suggest that OLGCs in STGCT-LMP and GC-MFH indeed derive from the neoplastic component and in these tumours the cells demonstrate osteoclast differentiation.[7] The mechanisms underlying OLGC infiltration in malignant melanoma and SCC are unknown. It may be hypothesized that cytokines or growth factors that are secreted by tumour cells are involved. As a heavy inflammatory infiltration of lymphocytes and eosinophils was seen besides the OLGCs in our case a pathogenic relationship with lympoepithelioma-like carcinoma might exist. In this tumour, a combination

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SHORT COMMUNICATION

Lamellar Ichthyosis Caused by a Previously Unreported Homozygous *ALOXE3* Mutation in East Asia

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Autosomal recessive congenital ichthyosis (ARCI) includes a wide range of ichthyosis phenotypes, including harlequin ichthyosis, lamellar ichthyosis (LI), congenital ichthyosiform erythroderma (CIE), and self-improving collodion ichthyosis (SICI) (1, 2). To date, 9 causative genes for ARCI have been identified (1, 2). *ALOXE3* is a causative gene in LI as well as CIE, and it encodes the eLOX-3 lipoxygenase, which is predominantly synthesised in the epidermis. ARCI caused by an *ALOXE3* mutation is very rare, with less than 30 families with the mutation reported in the literature. The previously reported cases with homozygous or compound heterozygous *ALOXE3* mutations were from Europe, North Africa, the Middle East, and South Asia (3–8). Here, we describe an LI patient with a previously unreported homozygous *ALOXE3* mutation in a consanguineous family from Japan and review ARCI cases with *ALOXE3* mutations.

CASE REPORT

The patient is a 58-year-old Japanese woman who presented with symptoms of ichthyosis since birth. Her parents were first cousins. She has 3 siblings, of which one has a similar ichthyosis phenotype (Fig. 1a). Ectropion was not reported at birth. She showed brown-to-gray scaling without erythroderma on her trunk and extremities (Fig. 1b). She did not show pal-

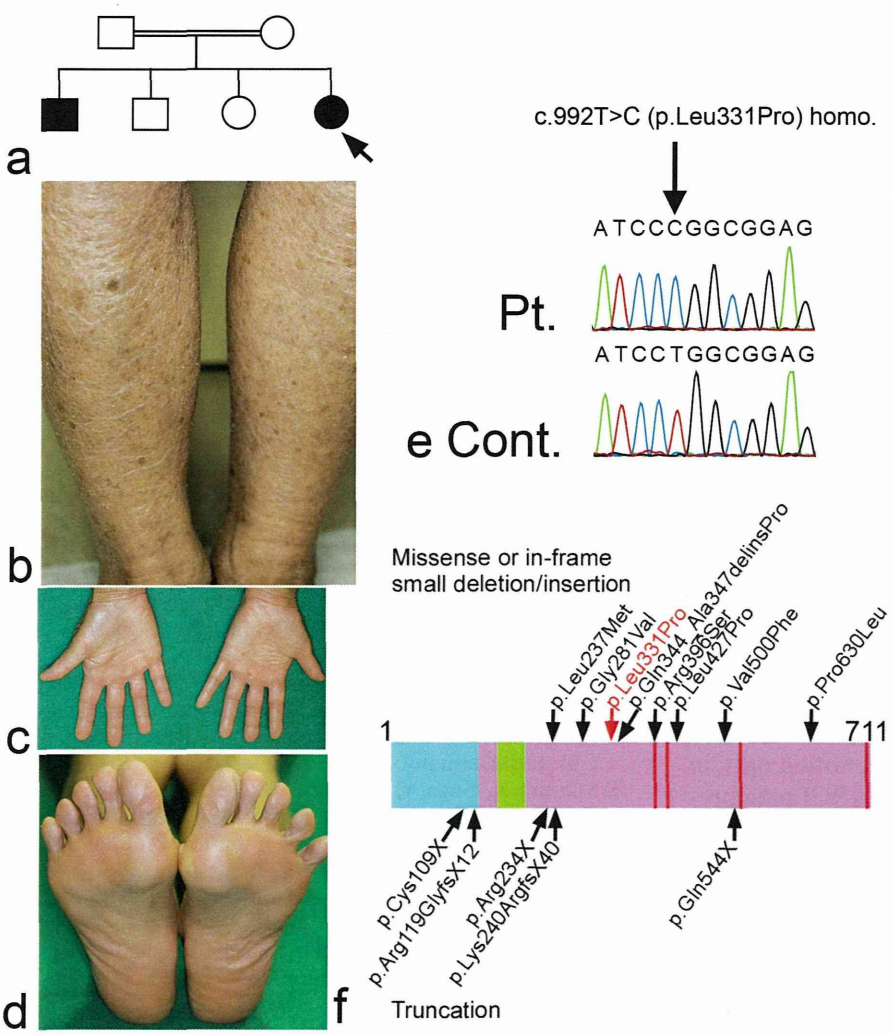

Fig. 1. Pedigree, clinical features, and *ALOXE3* sequence data of the patient; sequence alignments around the missense mutation; and a summary of known *ALOXE3* mutations. (a) Pedigree of the patient. (b) The patient showed brown-to-gray scaling bilaterally on the lower legs. (c) The patient did not show palmar keratosis. (d) The patient showed mild plantar hyperkeratosis. (e) Sequence data of *ALOXE3* in the patient with the mutation and a control without the mutation. The arrow indicates c.992T>C (homozygous). (f) The eLOX-3 protein domain structure and *ALOXE3* mutations from this study and the literature. The previously unreported missense mutation identified in this study, p.Leu331Pro, is shown in red. A blue box and a green box indicate the N-terminal β-barrel LH2 domain and an inserted specific extra domain, respectively. Pink boxes indicate C-terminal catalytic lipoxygenase domain from amino acid position 126. Putative iron ligands of the active sites are in red.

mar keratosis or alopecia, but did show mild plantar keratosis during middle age (Fig. 1c, d).

Following ethical approval, informed written consent was obtained in compliance with the Declaration of Helsinki guidelines. The coding regions, including the exon-intron boundaries of *TGM1, ABCA12, ALOX12B*, and *ALOXE3*, were amplified from genomic DNA by PCR as described elsewhere (3). Direct sequencing of the patient's PCR products revealed that the patient had a homozygous *ALOXE3* mutation, c.992T>C (p.Leu331Pro) (gene accession number: NM_021628.2) (Fig. 1e). p.Leu331Pro was analysed using SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). The SIFT score was 0.000 and PolyPhen-2 score was 1.000; both scores predicted that p.Leu331Pro had damaging effects. We found no mutation in the other 3 genes tested. c.992T>C was not detected in the 200 control alleles (100 control individuals, data not shown). Thus, the patient was diagnosed as having LI caused by the homozygous *ALOXE3* mutation.

DISCUSSION

All previously reported ARCI cases with *ALOXE3* mutations have been in families from Europe, North Africa, the Middle East, and South Asia (Fig. 1f, Table SI[1]). To our knowledge, the present patient is the first case with *ALOXE3* mutations in a family from East Asia. Our case suggests that *ALOXE3* mutations are possibly found in families worldwide. We reported more than 50 Japanese cases of ARCI that had *TGM1, ABCA12, ALOX12B*, or *CYP4F22* mutations (2, 9, 10). Although we do not have data indicating how often patients with ichthyosis are offered genetic testing in Japan, no other patients with *ALOXE3* mutations have been found to date. We hypothesise that the carrier rate of ichthyosis-causing *ALOXE3* mutations may be very low in Japan.

We reviewed 39 cases of ARCI from 29 families that had *ALOXE3* mutations, including the case described here (3–8) (Table SI[1]). Thirteen *ALOXE3* mutations have been reported (Fig. 1f). Truncation mutations, missense mutations, and an in-frame small deletion/insertion mutation have been reported. The truncation mutations include nonsense mutations, a deletion mutation resulting in a frame shift, and a splice site mutation. In 2 cases, *ALOXE3* mutations were identified only in one allele. In the literature (Table SI[1]), ARCI phenotypes caused by *ALOXE3* mutations were categorised as CIE, LI, and SICI. In 3 cases, clinical features were not described, and their ARCI phenotypes were unknown.

In conclusion, the present case clearly indicates that *ALOXE3* is a possible causative gene in East Asian ARCI patients.

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The authors declare no conflicts of interest.

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Letter to the Editor

Porokeratotic eccrine ostial and dermal duct nevus with a somatic homozygous or monoallelic variant of connexin 26

Keywords
GJB2
monoallelic
mutation analysis
porokeratotic eccrine ostial and dermal duct nevus

Porokeratotic eccrine ostial and dermal duct nevus (PEODDN) is an uncommon, benign dermatosis characterized by asymptomatic keratotic papules and plaques with a linear distribution mainly on the extremities. Histopathologically, PEODDN is characterized by the presence of a cornoid lamella exclusively overlying eccrine acrosyringia or overlying both eccrine acrosyringia and hair follicles. The lesions usually appear at birth or in childhood [1] and are localized at the extremity of a single limb, although wider distribution has occasionally been documented. The molecular cause of PEODDN has not been completely clarified. Recently, somatic mutations in the gene (*GJB2*) encoding the gap junction protein connexion 26 (Cx26) were presented to be a cause of PEODDN [2,3]. Here, we report a patient with PEODDN in whom a somatic homozygous or monoallelic variant of Cx26 was confirmed in the lesional skin.

A 10-year-old male presented to our hospital with unilateral dominant linear skin lesions. He was the son of healthy, nonconsanguineous parents, and was born at full term after an uneventful pregnancy. He was generally in good health, and had achieved normal development. His familial history was negative for similar cutaneous disorders. His parents first recognized a change in skin colour on his right armpit at the age of 2. The skin lesions began to spread to his right upper arm and forearm when he was 5 years old. He was treated at another hospital with topical steroids, but the condition did not resolve.

Upon first visiting our hospital, the dermatosis consisted of hyperkeratotic streaks with bilateral, asymmetrical distribution following Blaschko's lines (Fig. 1a–c). Physical examination revealed red-coloured macules and white hyperkeratotic papules, localized to the right side of the body involving the fifth finger, forearm, upper arm, armpit, thigh, lower leg, and popliteal fossa, strictly respecting the midline.

Histopathology from a representative skin lesion showed hyperkeratosis, acanthosis, and papillomatosis under low power magnification. Keratotic invaginations with prominent parakeratosis overlying a hair follicle were observed. The granular layer was absent below the parakeratotic column (Fig. 1d). These histopathologic features are characteristic of PEODDN.

Following ethical approval, informed written consent was obtained from the patient in compliance with the Declaration of Helsinki guidelines. The coding regions of *GJB2*, including the exon–intron boundaries, were amplified by PCR from genomic DNA obtained from the lesional epidermis and peripheral blood of the patient as described elsewhere [4]. Later, the exon 1 and intron 1–2 of *GJB2* were also amplified by PCR from genomic DNA obtained from the peripheral blood of the patient. The mutation analysis of *GJB2* revealed that the patient's blood was heterozygous for the missense variant c.608T > C (p.Iso203Thr), and was homozygous or monoallelic for the variant in the lesional epidermis (Fig. 2). No other heterozygous *GJB2* variant was found in the patient's blood. According to the 1000 genomes project (http://www.1000genomes.org/), 9 out of 89 Japanese subjects had the heterozygous variant p.Iso203Thr, but none was homozygous for the variant. The Sorting Intolerant from Tolerant (SIFT) score (http://sift.jcvi.org/) of the variant was 0.000, which predicted that p.Iso203Thr is deleterious. The PolyPhen-2 score (http://genetics.bwh.harvard.edu/pph2/) was 0.541, which indicated that the variant has possibly damaging effects.

It is currently thought that PEODDN is a mosaic form of keratitis-ichthyosis-deafness (KID) syndrome caused by a mutation in *GJB2* [2,3]. By using whole-exome sequencing, Levinsohn et al. showed that somatic GJB2 mutation alone is sufficient to cause PEODDN [3]. In Easton et al. and Levinsohn et al. causative *GJB2* mutations appeared to induce a protein gain-of-function [2,3]. Heterozygous mutations caused aberrant opening of gap junctions in epidermal keratinocytes, resulting in PEODDN. The *GJB2* mutation/variant in the present case seems to not be pathogenic when heterozygous since, according to the 1000 genomes project, heterozygous p.Iso203Thr Cx26 was found

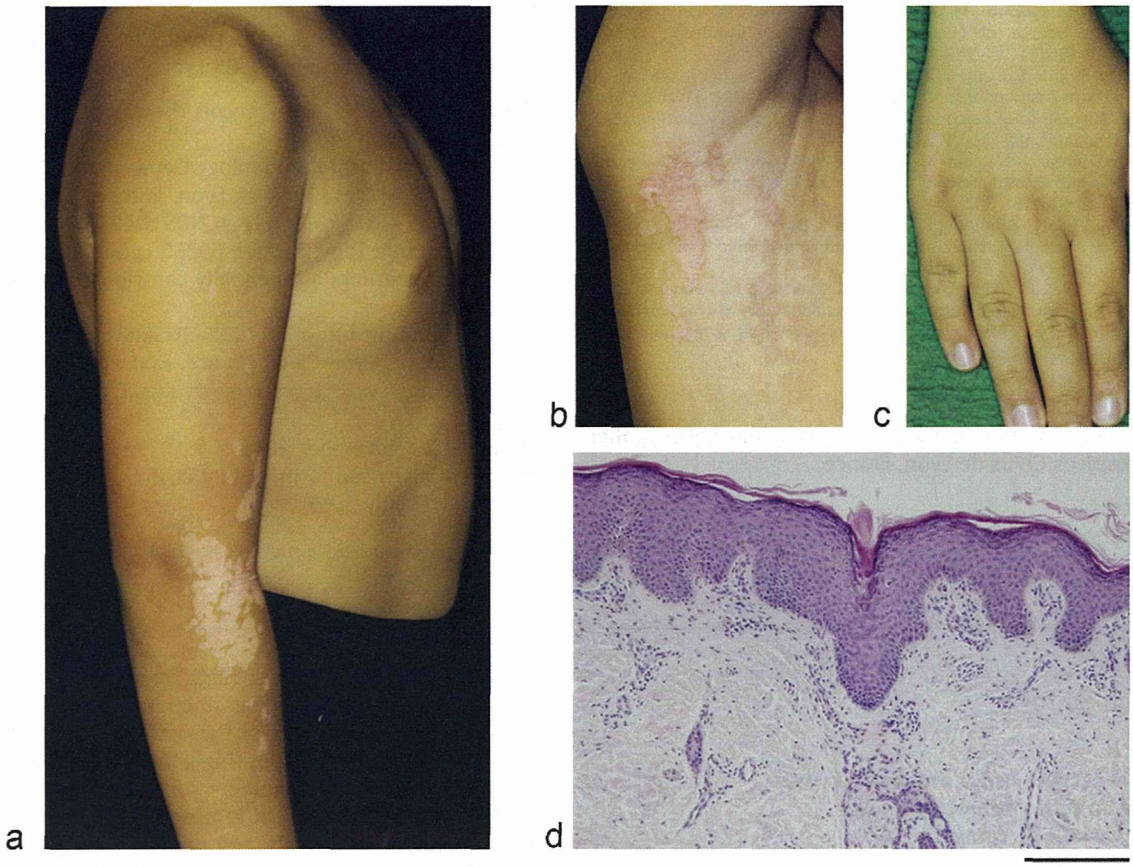

Fig. 1. (a–c) The dermatosis consisted of hyperkeratotic streaks with a bilateral, asymmetrical distribution following Blaschko's lines. (d) A cornoid lamella overlying a hair follicle was present. Bar: 150 μm

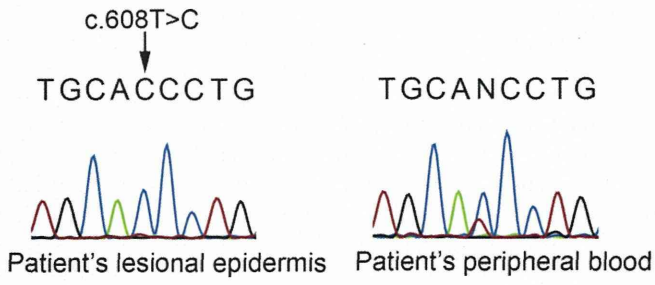

Fig. 2. Mutation analysis of *GJB2* in the patient's epidermis and peripheral blood. Direct sequencing of *GJB2* derived from the patient's lesional epidermis revealed a homozygous or monoallelic variant c.608T > C, although the PCR product from the patient's peripheral blood showed both wild type and variant alleles.

in 32 individuals without KID syndrome out of 286 individuals, exclusively in Asian (Chinese and Japanese) populations. No homozygous p.Iso203Thr Cx26 individuals were reported, suggesting that homozygous p.Iso203Thr Cx26 may be pathogenic and possibly lethal in humans. Both the SIFT score and the PolyPhen-2 score predicted the variant to be deleterious or damaging. We therefore consider the present case of epidermal somatic homozygous or monoallelic variant of Cx26 as a possible cause of PEODDN. The present patient is heterozygous for the variant p.Iso203Thr, but keratinocytes within the lesional epidermis are homozygous or monoallelic for the *GJB2* variant. Hence, we believe *GJB2* mutations and variants can induce PEODDN.

In conclusion, we show for the first time, to our knowledge, a somatic homozygous or monoallelic variant of Cx26 in the lesional epidermis of PEODDN. This finding supports the theory that somatic *GJB2* mutations cause PEODDN [2,3].

Funding sources

None.

Conflict of interest

We have no conflict of interest to declare.

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A novel mutation in *SLURP1* in patients with mal de Meleda from the Indian subcontinent

Keywords
Mal de Meleda
Diffuse palmoplantar keratoderma
SLURP1
Mutation

Mal de Meleda, (MDM, OMIM #248300) is an autosomal recessive palmoplantar keratoderma. Its major features are progressive transgredient hyperkeratosis of the palms and soles, hyperhydrosis, malodourous scent due to microbial superinfection, and minor symptoms such as perioral erythema, hyperkeratosis on elbows and knees, pseudo-ainhum and nail abnormalities. Onset of the disease is usually in infancy, with a slowly progressive course during adult life [1]. Penetrance is complete, although symptoms are highly variable, probably caused by environmental factors such as mechanical trauma or heat [1,2]. In 2001, mutations in the *SLURP1* gene, encoding the secreted Ly-6/uPar related protein-1 (SLURP1), were identified as the cause of MDM [1]. SLURP1 enhances the function of the nicotinic acetylcholine receptor α7 (nAChRα7) in epidermal keratinocytes, thereby promoting keratinocyte differentiation in the stratum granulosum [3]. Mutations in *SLURP1* show a remarkable geographic distribution, with most mutations reported in Europe and the Middle East. Certain mutations are highly prevalent in societies due to a founder effect [4]. The p.Gly86Arg mutation is most often found in sporadic patients with MDM of Asian origin [5,6]. To date, no mutations in Indian patients have been described. We describe an Indian family with MDM caused by a novel splice site mutation in intron 1 and provide functional consequences of this *SLURP1* mutation.

A fifteen year old girl developed a diffuse palmoplantar keratoderma in the first year of life. The hyperkeratosis slowly advanced to the dorsal side of her wrists (transgrediens), hands and feet, led to loss of dermatoglyphics and sclerodactyly and developed a malodorous scent. The distal phalanges acquired a conical shape without development of pseudo-ainhum, most prominently seen in the 5th digit of both hands (Fig. 1a and b). Nail changes other than a pinkish-pale hue, perioral erythema or skin changes elsewhere were not noted. Her 6 year old brother developed similar but less severe symptoms. There was no evidence of hearing loss, eye abnormalities or dental caries in both the siblings. Their performance in school was above average. Parents were consanguineous (Fig. 1c). No other family members had similar complaints. Histopathology of palmar skin showed hyperkeratosis, acanthosis with minimal superficial dermal perivascular infiltrate, without evidence of epidermolytic hyperkeratosis. Probands and their parents gave written informed consent to further molecular analysis. Genomic DNA was extracted from whole blood (QIAamp DNA Blood Mini Kit, Qiagen). Mutation analysis of *SLURP1* revealed a homozygous c.58+5G>T splice donor variation in intron 1 in both index patients (Fig. 2a and b). The parents were heterozygous for the variant. Splice site prediction software tools (integrated in Alamut 2.4) showed reduced scores of the variant splice donor site, putatively leading to aberrant RNA splicing. To test the consequences of this variant, whole blood RNA was isolated from the patient and analyzed with reverse transcriptase-PCR. The SLURP1 transcript was not detected in patient or control blood (data not shown) suggesting SLURP1 is not expressed in blood. Since a skin biopsy was not available, we used a *SLURP1* minigene construct to show if cryptic splicing occurs by the variant. Patient and control genomic DNA was PCR amplified using primers SLURP1F1 5′-GAACAGTGAGTTCCC-CAGTG-3′ and SLURP1R3 5′-GTCATGTCCACTCTTGGCTT-3′ and cloned in pJet1.2/blunt (Fermentas). Cloned products (GRCh37/hg19Chr8:143824033-143822375) were cut from pJet1.2/blunt with *Xba*I and *Not*I (New England Biolabs) and cloned in the forward orientation in the pEGFP-N1 backbone (EGFP removed) digested with *Nhe*I and *Not*I (New England Biolabs). HEK293 cells were transfected with either a plasmid containing the wild type genomic DNA sequence of *SLURP1* or the sequence with the c.58 +5G>T variation using GENIUS Transfection Reagent (Westburg) according to the manufacturer's instructions (sequence shown in Fig. 2c and d). RNA was isolated from the HEK293 cells (RNeasy Kit, Qiagen) 24 h after transfection with either construct and cDNA was synthesized (super-script first-strand synthesis system, Invitrogen). SLURP1 cDNA was amplified using primers SLURP1cF1 5′-CCTCTCGCTGGGCTGTGCAG-3 and SLURP1cR3 5′-AGGTCTCG-GAAGCAGCAGAAG-3′. PCR amplification of wildtype cDNA yielded a correctly spliced product of 289 bp, and cDNA with the c.58+5G>T variant yielded an aberrant 694 bp product retaining intron 1 (Supplemental Fig. 1). Sanger sequencing in both directions of the mutant cDNA PCR product showed retention of the full sequence of intron 1, excluding the occurrence of a new splicing acceptor site in the intronic sequence (Supplemental Fig. 2). The consequence of the c.58+5G>T mutation is aberrant splicing of mRNA (r.58_59ins58+1_59-1) putatively leading to a frameshift and premature protein truncation (p. (Val17Metfs*16)).

Up to 17 different mutations in *SLURP1* associated with MDM have been previously reported in the literature, three of which were splice site mutations in the introns (c.58+1G>A, c.58+1G>C, c.178+1G>A) [1,2,7]. The c.58+5G>T mutation found in our patients

Letter to the Editor

Novel indel mutation of *STS* underlies a new phenotype of self-healing recessive X-linked ichthyosis

Dear Editor,

Recessive X-linked ichthyosis (RXLI, OMIM 308100) is clinically characterized by widespread dark brown, polygonal scales and generalized dryness. RXLI is an inherited disorder caused by deficiency of the enzyme steroid sulfatase (STS) due to *STS* gene mutations [1]. Measurement of substrate accumulation in the skin (cholesterol sulfate) or in the peripheral blood (cholesterol sulfate or other sulfated steroid hormones) is diagnostic, as is the assay of STS activity in the epidermis, cultured fibroblasts and blood leukocytes. However, some patients who are diagnosed with other types of ichthyosis may also show low STS activity. In addition, measuring STS activity in RXLI carriers lacks diagnostic accuracy [2].

Except for sporadic cases and patients showing clinical features mimicking other forms of ichthyosis, such as ichthyosis vulgaris and lamellar ichthyosis (LI), the diagnosis of RXLI is not usually difficult, and can be established from the family history and clinical features, although some milder phenotypes may be clinically challenging to diagnose [3].

Since ~90% of RXLI patients have large deletions involving *STS* and adjacent DNA, in some instances with contiguous gene loss, fluorescence *in situ* hybridization (FISH) analysis is a useful technique to identify patients and carriers of RXLI who have such deletions [4]. Nevertheless, although FISH in these cases is helpful, this is not the situation for other individuals with partial deletions or point mutations [5–7]. For those subjects, other DNA sequencing approaches may be preferable. Here, we used whole-exome sequencing (WES) to identify a new indel mutation in *STS* in a RXLI patient in whom FISH was unable to detect a large deletion mutation. With regard to genotype-phenotype correlation, our patient with the small indel mutation in *STS* showed a unique "self-healing" phenotype of RXLI.

A Japanese boy was born by Caesarean section. At birth, he had large white scales with deep fissuring skin over the whole body (Fig. 1a–c). Hair or nail abnormalities were not observed. Echocardiography demonstrated a mild ventricular septal defect. He had no sign of Kallmann syndrome or X-linked recessive chondrodysplasia punctata. Furthermore, he had no family history of consanguinity or skin disorders. He was treated with a heparinoid-containing moisturizer and the large scales desquamated gradually over the first 2 months of life. A skin biopsy specimen obtained one month after birth showed compact hyperkeratosis with a normal granular layer (Fig. 1d). Ultrastructurally, mild hypoplasia of the cornified cell envelope was seen (Fig. 1e). There was no increased melanogenesis. His skin manifestations spontaneously healed by 5 months. These clinicopathologic features suggested either LI or RXLI.

The ethics committee of Nagoya University Graduate School of Medicine approved the present studies, which were conducted according to the principles of the Declaration of Helsinki. The participants/guardians gave written informed consent. Initially, we performed chromosome analysis using a specific probe for Xp22.3, which includes the region of *STS* in chromosome X, to detect a large deletion, including *STS*. FISH analysis for Xp22.3 revealed no deletion of Xp22.3 in the patient (Fig. 2a).

Next, genomic DNA from the patient was used for WES analysis, using methodology described elsewhere [8]. In total, 350 novel variants were identified by WES. Within these variants, there was a previously unreported indel mutation (c.529_532del4insAG) in exon 5 of *STS*, which was then confirmed by Sanger sequencing; his mother was shown to be a heterozygous carrier (Fig. 2b). The mutation was not identified in genomic DNA from the unaffected father or 674 normal control individuals. This indel mutation leads to a frame-shift, causing a premature stop codon 81 codons downstream from the substitution site (p.Val 177 Serfs × 81).

With respect to the phenotypic spectrum of RXLI, Hand et al. [3] suggested *STS* gross deletions might cause milder skin abnormalities than most classic forms of RXLI, in that those cases incidentally found to have an *STS* deletion by whole genome chromosomal microarray typically lacked the polygonal or "dirty" scales considered a hallmark of RXLI. In such cases, the milder findings comprised dry or peeling skin and eczema [3]. Our case has a small indel mutation and an initial LI-like skin phenotype. Thus far, 21 pathogenic mutations in *STS* other than gross deletion/insertions have been reported in RXLI (www.hgmd.cf.ac.uk). These findings comprise 14 missense/nonsense, 1 splice-site, 4 regulatory, 1 small deletion and 1 small insertion mutations. To our knowledge, our patient is the first case of RXLI due to a small *STS* indel mutation.

Interestingly, our patient showed clinical features of typical LI at birth and as a neonate (Fig. 1a–c), although his skin spontaneously healed by 5 months with the clinical course resembling that of a self-healing (self-improving) collodion baby. This clinical outcome is atypical as RXLI does not tend to improve with age in childhood. On the contrary, the onset of typical RXLI most commonly only appears after 2–6 months of age. Thus far, there has been no reported case of self-healing RXLI. The present *STS* variant leads to frame-shift (p.Val 177 Serfs × 81), and the altered reading frame is generated from Val 177. Thus, the reported active site, His^{136}, within the encoded transcript [9] may be spared if some truncated mutant protein is synthesized (Fig. 2c).

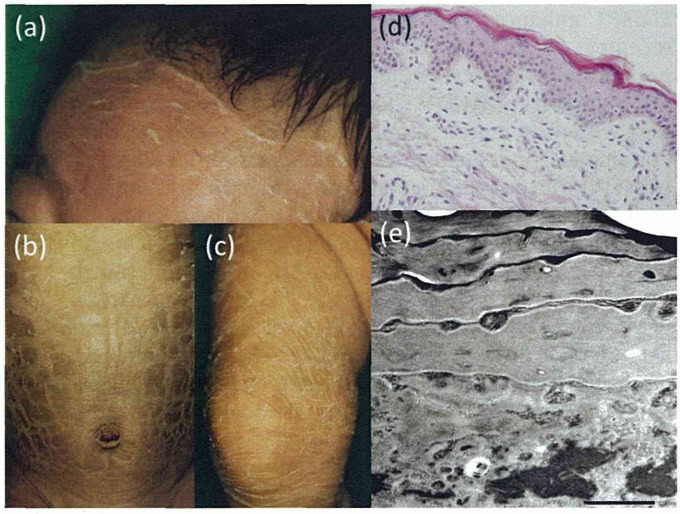

Fig. 1. Clinical features of RXLI mimicking LI.
(a–c) At birth, the entire body surface is covered with scales. On the trunk and extremities, the scales are dark, large thick; forehead (a), abdomen (b) and right leg (c). (d) Hematoxylin–eosin staining shows compact hyperkeratosis with normal granular layers. (e) Electron microscopy shows mild hypoplasia in cornified cell envelopes. Scale bar: 1.0 μm.

Abbreviations: RXLI, recessive X-linked ichthyosis; STS, steroid sulfatase; LI, lamellar ichthyosis; FISH, fluorescence *in situ* hybridization; WES, whole-exome sequencing.