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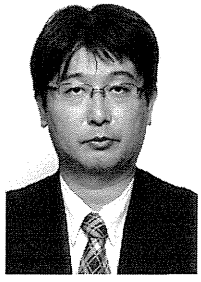

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Topical minoxidil improves congenital hypotrichosis caused by *LIPH* mutations

DOI: 10.1111/bjd.13790

Dear Editor, Mutations in *LIPH* are one cause of autosomal recessive woolly hair/hypotrichosis (ARWH).[1] *LIPH* mutations are not uncommon and are found all over the world. In this report, we present four patients with ARWH with *LIPH* mutations who showed hair growth after application of topical minoxidil.

Four nonconsanguineous Japanese patients with ARWH who used 1% or 5% topical minoxidil were observed and followed up. Topical minoxidil is used for androgenic alopecia and is available over the counter in Japan. The present patients used it on their own initiative. Direct sequencing of exon 6 of the *LIPH* gene revealed homozygous c.736T>A (p.Cys246Ser) mutations in patients A and B. The homozygous c.742C>A (p.His248Asn) mutation was found in patient C, and compound heterozygous c.736T>A and c.742C>A mutations were

Table 1 Mutations in the *LIPH* gene in four patients and application of topical minoxidil

Patient	Age (years)	Sex	*LIPH* mutation	Concentration of topical minoxidil (%)	Application period until observation (months)
A	3	Male	c.736 T>A homozygous	1 and 5	31
B	7	Female	c.736 T>A homozygous	1	17
C	70	Female	c.742C>A homozygous	1	6
D	5	Female	c.736 T>A, c.742C>A compound heterozygous	1	55

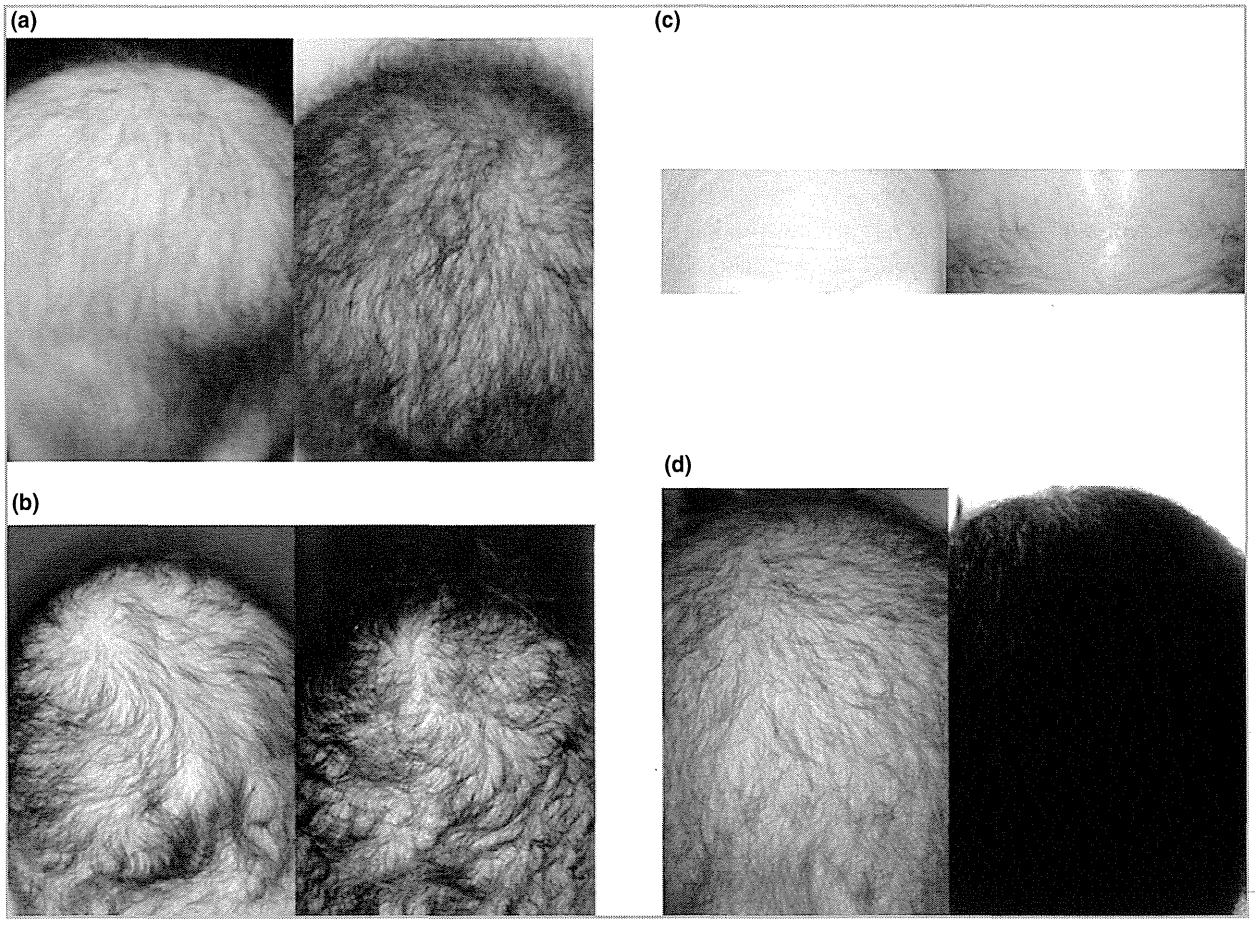

Fig 1. Clinical features of the present patients before and after topical minoxidil application. (a) Patient A, (b) Patient B, (c) Patient C and (d) Patient D. Left, photos before topical minoxidil application; right, photos after topical minoxidil application. Patient A after 2·5 years' treatment (1% topical minoxidil in the first year, 5% topical minoxidil during the second year); Patient B after 1·5 years' treatment (1% topical minoxidil); Patient C after 6 months of treatment (1% topical minoxidil application); Patient D after 4·5 years of topical minoxidil application.

found in patient D (Table 1). Patients A and C were included in previous reports, but their use of topical minoxidil was unknown at the time.[2,3] The mutation search was approved by the Medical Ethics Committee of Nagoya University Graduate School of Medicine and was conducted according to the Declaration of Helsinki principles. The patients each provided written informed consent. The patients had used 1% or 5% topical minoxidil of their own will for 6 months to 3 years. Amazingly, the hair of all these patients grew after use of topical minoxidil, and all patients felt that they benefited from the treatment (Table 1, Fig. 1). In particular, patient C had little hair growth from childhood to the age of 70, but after topical minoxidil application, her hair grew visibly for the first time in her life. No patient claimed adverse effects, such as hypertrichosis of other body parts or cardiovascular problems, during the periods of use.

Mutations in *LIPH* are one cause of ARWH. *LIPH* encodes a membrane-associated phosphatidic acid-preferring phospholipase $A_1\alpha$, which produces lysophosphatidic acid from phosphatidic acid and plays a crucial role in hair growth in humans.[1]

The two missense mutations, c.736T>A and c.742C>A, are considered the prevalent founder mutations in Japanese ARWH patients, and the combined carrier rate of the mutations is about 2·1% in the Japanese population.[3] Hence, as many as 10 000 ARWH patients are estimated to exist in Japan. Unfortunately, no effective treatment has yet been established, and the hypotrichosis significantly decreases quality of life of the patients for almost their entire lifetime.

As well as the four patients with ARWH and *LIPH* mutations described here, we recently reported another case of ARWH that improved with topical minoxidil.[2] The mechanism by which topical minoxidil affects hair growth is not fully understood, although it is thought to have an effect on cell growth and duration of the anagen hair growth phase and to enlarge miniaturized follicles. Topical minoxidil has generally been used for androgenic alopecia but has also been reported to be effective for other hair loss, including congenital hypotrichosis, such as ectodermal dysplasia.[4] The present cases suggest minoxidil could improve congenital hypotrichosis due to *LIPH* mutations, and it may also potentially improve congenital hypotrichosis due to mutations in other causative genes.

The most frequent adverse effect of minoxidil is hypertrichosis, usually localized to the head. Additionally, adverse cardiovascular effects, such as sinus tachycardia, palpitation and dizziness, have been reported with 2% topical minoxidil use in three patients aged from 10 to 14 years, all of whom recovered from these adverse effects after discontinuance of minoxidil.[5] Although these adverse effects are rare, topical minoxidil must be used carefully, especially in children.

In conclusion, combined with a previous report, improvement of hypotrichosis was observed after topical minoxidil application in five cases of ARWH due to *LIPH* mutations. These cases suggest that topical minoxidil could be useful for treating congenital hypotrichosis caused by LIPH mutations.

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Conflicts of interest: none declared.

White piedra caused by *Trichosporon inkin*: a report of two cases in a northern climate

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Dear Editor, We recently diagnosed two patients attending a specialty hair clinic in Boston, with white piedra, a superficial fungal infection caused by several species of *Trichosporon* that affects the terminal hair shaft. This is notable because white piedra is said to be rare in the U.S.A., particularly in northern climates. The causative species and the laboratory techniques used to identify the organism are of interest, as the organism is difficult to culture and taxonomy has recently changed.

Acta Derm Venereol 2015; 95: 872–874

LETTER TO THE EDITOR

What are the "True" Pathogenic Anti-desmoglein Antibodies?

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Sir,

We read with interest the reports by Li et al. (1) and Demitsu et al. (2). The former showed that, in some populations of pemphigus vulgaris, there is a significant gap in anti-desmoglein (Dsg) 3 antibody enzyme-linked immunosorbent assay (ELISA) index as measured by conventional ELISA vs. that measured by ethylenediaminetetraacetic acid (EDTA)-treated ELISA. The latter report demonstrated that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor, but not the mature, forms of Dsg1 and Dsg3. Both studies suggest that anti-Dsg1 and Dsg3 antibodies are heterogeneous and can be categorized into pathogenic and non-pathogenic. However, even these recent methods for evaluating pathogenic anti-Dsg1/3 antibodies have not completely clarified the discrepancy between the disease activity and the ELISA index.

The usefulness of anti-Dsg1/3 ELISAs and indirect immunofluorescence (IIF) for the monitoring of pemphigus, based on a possible correlation between autoantibody levels and disease activity, remains controversial (3, 4). A recent study showed that ELISA index values can be a valuable tool for monitoring the disease, whereas IIF titres insufficiently reflect clinical activity (5). However, an interesting report was published recently in which a patient with pemphigus vulgaris in remission had a high anti-Dsg3 antibody ELISA index, but negative IIF results (6). The case presented a discrepancy between the disease activity, the ELISA index for Dsg3 and the IIF findings. The authors tried to resolve this discrepancy by devising a conformational ELISA index in which the EDTA-ELISA index is subtracted from the conventional ELISA index, which is a better indicator of disease activity than the conventional ELISA index. However, the patient's antibodies were found to be directed against $Ca2^{+}$-dependent epitopes even during remission, because the conformational ELISA index showed high levels even during remission. If the patient's antibodies were recognizing mainly precursor Dsg3, which is synthesized in the endoplasmic reticulum, and not recognizing mature Dsg3, then this could explain the negative findings in IIF. Using recombinant proteins, the patient's antibodies were shown to react preferentially with mature Dsg3. Thus, the mechanisms behind the inconsistency between the ELISA and IIF findings remain uncertain.

Some reports suggest that levels of the IgG_4 subclass and IgE class of anti-Dsg3 antibody are associated with active disease and that the IgG_1 levels are associated with remission (7, 8). Nagel et al. (8) demonstrated that, in addition to IgG_4, IgE autoantibodies to Dsg3 were present in a significant number of patients with acute-onset and active pemphigus vulgaris and that tissue-bound and serum IgE autoantibodies were detected in acute-onset pemphigus vulgaris by direct and indirect IF microscopy. This concept of class switch to IgG_1 during remission is also difficult to apply in the case described by Nakahara et al. (6), because there is almost no possibility that secondary antibodies against the human immunoglobulins used in IIF contain no anti-human IgG_1 antibodies.

Here, we propose a possible explanation of the discrepancy. We recently showed that the avidity of autoantibodies differs between a disease group and healthy individuals (HI) (9). Anti-dense fine speckles 70-kDa (DFS70) antibodies are anti-nuclear antibodies that are found more frequently in patients with atopic dermatitis (AD) than in HI, although the pathogenicity of these antibodies remains obscure. By comparing conventional anti-DFS70 ELISA values and antibody-stripping ELISA values by urea, we measured the avidity of anti-DFS70 antibodies in the AD and HI groups. The avidity of the antibodies was significantly higher in the patients with AD than in the HI, even though there was no difference in the avidity of anti-diphtheria toxoid between the 2 groups. A few reports have addressed the association between autoantibody avidity and disease activity. Regarding rheumatoid arthritis, Suwannalai et al. (10) reported that higher-avidity anti-citrullinated protein antibodies were observed only in symptomatic patients, whereas low-avidity anti-citrullinated protein antibodies were observed in both healthy subjects and patients with rheumatoid arthritis. Another study showed that, in some cases of vasculitis, myeloperoxidase anti-neutrophil cytoplasmic antibody had a high affinity that correlated with disease activity, irrespective of antibody titre (11). As far as we know, there are no reports investigating the avidity of anti-Dsg1/3 antibodies.

Autoantibody-defined epitopes are often conformation-dependent. For example, centromere protein-C (CENP-C), the main target of anti-centromere antibodies, has multiple epitopes formed from the C-terminal protein (12). Affinity-purified antibody against the dimer formation of the C-terminus in a liquid phase was found to be reactive only in IIF. We also demonstrated that a synthetic compound peptide composed of

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discontinuous sequences mimicked the conformation-dependent epitope found in patients with systemic lupus erythematosus (13). However, the polyclonal antibody against this peptide had weaker avidity than that of human autoantibody. The following are thought to cause affinity changes: a point mutation in an amino acid, post-transcriptional modifications and interactions with other molecules within epitopes.

Table I summarizes the reported heterogeneity of anti-Dsg1/3 antibodies. Further research into the avidities of anti-Dsg1/3 antibodies is required in order to elucidate the pathogenic mechanisms of pemphigus toward establishing a measurement for "true" pathogenic anti-Dsg1/3 antibodies.

The authors declare no conflicts of interest.

Table I. *Heterogeneity of anti-desmoglein antibodies*

Conventional enzyme-linked immunoassay (ELISA) vs. conformational ELISA
Autoantibodies to precursor desmogleins vs. mature desmogleins
Autoantibody subtypes: IgG1, IgG2, IgG4, IgA and IgE

Response to the Letter to the Editor by Muro et al.

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We read with much interest the Letter to the Editor on pathogenic (true) anti-desmoglein (Dsg) antibodies by Muro et al., which commented on our article (2) and another article by Li et al. (1). We reported that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor form, but not the mature form, of Dsg1 and Dsg3 (2).

It is occasionally found that the results of Dsg ELISAs and indirect immunofluorescence (IF) do not correlate with disease activity (5). In addition, individuals with no clinical features of pemphigus may show positive results in Dsg ELISA (2). In particular, we have encountered several patients with pemphigus vulgaris, who showed very high index values of Dsg3 ELISA even in the remission stage of the disease. To confirm the causes of the loss of pathogenicity of the serum autoantibodies, we considered 5 possible mechanisms, as described below, and summarized in Table I.

The first possibility is that pathogenic autoantibodies react with Ca^{++}-dependent conformational epitopes on Dsgs, because various autoantibodies have been shown to react with such conformation-dependent epitopes (13). This possibility can be confirmed by EDTA-treated Dsg ELISA or conformational ELISA index in which the conventional ELISA index is subtracted by EDTA-treated ELISA index (14).

The second possibility is that non-pathogenic autoantibodies react with precursor fragment on immature Dsg, which is present in the endoplasmic reticulum. This possibility can be confirmed by immunoprecipitation (IP)-immunoblotting (IB) or ELISA of recombinant proteins (RPs) of immature and mature Dsg (15).

The third possibility is that pathogenicity depends on extracellular domains of Dsg. Several studies have suggested that pathogenic autoantibodies react with N-terminal domains of mature Dsg (EC1 or EC2 domain), and autoantibodies to EC3-EC5 are non-pathogenic. The best method to confirm this possibility is IP-IB of Dsg3/Dsg2 or Dsg1/Dsg2 domain swapping molecule RPs (16, 17).

The fourth possibility is that pathogenicity depends on IgG subclasses (IgG1-IgG4) (7). Alternatively, IgA

Table I. *Possible mechanisms for pathogenicity of anti-desmoglein (Dsg) antibodies along with methods to confirm each possibility*

No.	Possible mechanisms	Methods for confirmation
1	Ca^{2+}-dependent conformation	EDTA-treated ELISA Conformational ELISA (subtraction of conventional ELISA by EDTA-ELISA)
2	Autoantibodies to precursor fragment in immature Dsgs	IB or ELISA of immature Dsg RP
3	Dependency to extracellular Dsg domains, EC1–EC5	IP-IB or ELISA of Dsg1/Dsg2 and Dsg3/Dsg2 swapping molecule RPs
4	Dependency to immunoglobulin subtypes (IgG1–IgG4, IgA and IgE)	IF, IB or ELISA using subtype-specific antibodies
5	Autoantibodies to p38 MAPK activating domain within Dsg	p38 MAPK phosphorylation study p38 MAPK inhibitor study
6	Avidity (affinity) of anti-Dsg antibodies	Autoantibody-stripping ELISA by urea treatment or inhibition by self-antigen in a liquid phase

IP: immunoprecipitation; IB: immunoblotting; RP: recombinant protein; ELISA: enzyme-linked immunoassay; EDTA: ethylenediaminetetraacetic acid; MAPK; mitogen-activated protein kinases.

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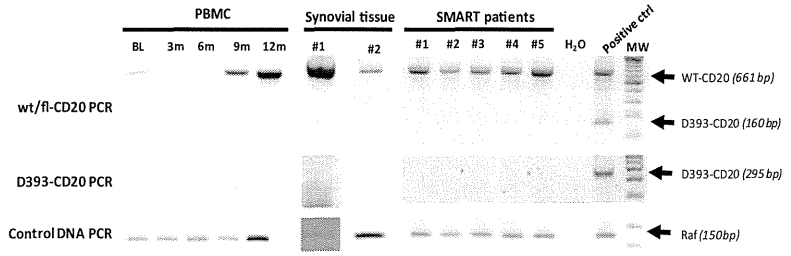
The corresponding author of the paper by Li et al. has been contacted but abstained from replying.

FIG. 1 Alternative CD20 transcript variant expression in PBMCs and synovial tissue from patients with RA



Representative qualitative RT-PCR analysis of *wt/fl cd20* and *d393-cd20* transcripts performed on cDNA from PBMCs of a RA patient, sampled at baseline (BL) and 3, 6, 9 and 12 months after RTX treatment, from two synovial tissues sampled during arthroplasty and from five PBMC samples representative of the SMART cohort (non-responder RTX-treated patients). *fl/wt-cd20* PCR allowed amplification of both *fl/wt-cd20* and *d393-cd20* transcripts, whereas *d393-cd20* PCR amplified specifically the *d393-cd20* transcripts, using a primer spanning the splicing junction. H_2O was used as negative control and cDNA from a B-cell line (positive ctrl) was used as positive control. MW: 100 bp molecular marker. PBMC: peripheral blood mononuclear cells.

Rheumatology key message

- The alternative CD20 transcript is not a marker for resistance to rituximab in RA.

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High incidence of cancer in anti-small ubiquitin-like modifier activating enzyme antibody-positive dermatomyositis

SIR, The idiopathic inflammatory myopathies (IIMs) are a group of systemic autoimmune diseases that include PM and DM [1]. Several myositis-specific autoantibodies, which have been regarded as mutually exclusive, are associated with certain clinical forms of IIM.

Since autoantibodies to small ubiquitin-like modifier activating enzyme (SAE) in patients with DM were described [2, 3], a few studies on anti-SAE antibodies in DM have been published from Italy [4], Japan [5] and Hungary [6]. We analysed serum samples from 110 DM patients and 2 were found to be anti-SAE positive [7]. The frequency of anti-SAE antibodies in DM overall was 1.5–5.7%. Nearly all patients with anti-SAE antibodies had skin and muscle symptoms, and most of them had skin disease before the muscle disease; however, the clinical features of the patients with anti-SAE antibodies are

not conclusive. We aimed to establish a quantitative assay for measuring anti-SAE antibodies and to clarify the clinical features of DM patients with these antibodies.

We screened 134 consecutive Japanese patients with DM (12 children, 122 adults) followed at Nagoya University Hospital, Nagoya, Japan. The serum samples were from 85 patients with DM and the remaining 49 samples were from patients with clinically amyopathic DM (CADM). An additional 16 adult patients with DM, including 11 with CADM, were also screened because their doctors introduced them for investigation of DM-marker autoantibodies. Of these 150 patients (male:female ratio 41:109), 67 patients were complicated with interstitial lung disease as diagnosed by chest radiograph or chest CT scan and 22 patients were diagnosed with cancer-associated DM. The definitions of DM, CADM and cancer-associated DM are as defined in our previous study [7]. This study was approved by the ethics committee of Nagoya University. All the patients and healthy individuals provided written informed consent according to the Declaration of Helsinki.

The full-length cDNA clones of SAE1 and SAE2 were purchased from Thermo Scientific Open Biosystems (Waltham, MA, USA). Biotinylated recombinant proteins were produced from the cDNA, using the SP6 Quick Coupled Transcription/Translation System (Promega, Madison, WI, USA). Antibodies against SAE1 and SAE2 were tested by antigen-capture ELISA according to our published protocols [8]. Cut-off values were determined as the mean (+ 5 S.D.) of the units obtained from 36 control serum samples from healthy individuals. Anti-MDA5, anti-Mi-2, anti-NXP-2 and anti-TIF1γ antibodies were also measured.

Serum samples that were positive for anti-SAE by ELISA were analysed with IIF using the Fluoro HEPANA Test (MBL, Nagoya, Japan). The samples were also screened by ELISA kits for antibodies against SS-A/Ro60, SS-B, U1-RNP, Sm, CENP-B, ds-DNA and aminoacyl tRNA synthetases consisting of a mixture of EJ, Jo-1, KS, PL-7 and PL-12 (MBL). Anti-SS-A/Ro52 antibodies were measured by using the ELISA kit of Orgentec (Mainz, Germany). Fisher's exact probability tests were used for comparison of frequencies. Correlations between two parameters were analysed by Spearman's correlation coefficients.

In the first cohort, consisting of 134 serum samples from consecutive patients, 4 (3.0%) patients were positive for both anti-SAE1 and anti-SAE2 antibodies (supplementary Fig. S1A, available at *Rheumatology* Online). Serum samples from two patients had been shown to be positive for anti-SAE antibodies by immunoprecipitation and western blotting [7]. In an additional cohort of 16 patients, 3 had anti-SAE1 antibodies, and 2 of these also had anti-SAE2 antibodies. Anti-SAE1 and anti-SAE2 titres had mutually significant positive correlations ($R = 0.807$, $P < 0.0284$). After the initial screening by ELISA, we investigated anti-SAE antibodies in the serum of five new anti-SAE-positive candidate patients for their ability to immunoprecipitate biotinylated recombinant SAE1 and SAE2. All of the candidates immunoprecipitated recombinant SAE1 and SAE2 (supplementary Fig. S1B, available at *Rheumatology* Online). According to these results, we concluded that we found five new serum samples that were positive for anti-SAE antibodies. All seven of the anti-SAE antibody-positive serum samples exhibited nuclear speckled patterns by IIF analysis (Table 1). Surprisingly, ELISA and immunoprecipitation using

TABLE 1 Clinical characteristics of DM patients with anti-small ubiquitin-like modifier activating enzyme antibody

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Total ($n = 7$), %
Age at onset, years	57	70	65	55	65	77	66	Mean, 65
Sex	Female	Male	Female	Male	Male	Male	Female	4 male:3 female
Heliotrope	+	+	–	+	–	–	–	43
Gottron's sign	+	+	+	+	+	+	+	100
Periungual lesions	+	–	+	+	+	+	NA	83
Mechanic's hands	–	–	–	+	+	+	–	43
V-neck sign	+	–	+	+	–	+	+	71
Shawl sign	+	–	+	+	–	–	+	43
Dysphagia	–	–	–	–	+	+	+	43
Muscle weakness	+	+	+	+	+	–	+	86
Creatine kinase, IU/l	542	429	662	6133	311	5187	1084	Elevated, 100
Interstitial lung disease	+	–	–	+	+	+	–	57
Arthritis	–	–	–	–	NA	–	–	0
Malignancy[a]	–	Rectum	Uterus	–	Oesophagus	Colon	–	57
RP	–	–	–	–	NA	–	–	0
Calcinosis	–	–	–	–	NA	NA	–	0
Other features	PH	–	–	Dysphonia	–	–	–	
Presentation (months)	S (2)	S/M	S (2)	S/M	S (7)	S/M	S (2)	S (mean, 1.9)
Other autoantibodies	Ro52	–	NXP-2, Ro60	Ro60	–	–	–	Ro60 (2), Ro52 (1), NXP-2 (1)

[a]Malignancy associated with DM was defined as that occurring within 3 years of the DM diagnosis. NA: not available; PH: pulmonary hypertension; S: skin disease presented first; S/M: skin and muscle disease presented together.

recombinant NXP-2 protein clarified that one patient also had anti-NXP-2 antibody (data not shown).

Seven anti-SAE-positive patients were diagnosed with adult DM, and all had internal involvement, such as interstitial lung disease, cancer and/or dysphagia, except for patient 7. The frequency of cancer in the anti-SAE-positive patients was significantly higher than in the anti-SAE-negative patients (4/7 *vs* 18/143, $P < 0.0093$). Since anti-NXP-2 antibodies in adult patients with DM are associated with cancer [1], we recalculated the association between anti-SAE antibodies and cancer when the patient with both anti-SAE and anti-NXP-2 antibodies was excluded. The significant association was still confirmed (3/6 *vs* 18/143, $P < 0.0369$).

Previous studies reported the frequency of cancer in anti-SAE-positive patients as 14–25% [3–6]. Interestingly, the cumulative results including our data showed that there were significantly more male patients in the anti-SAE-positive adult cancer-associated myositis group than in the myositis group without cancer (supplementary Table S1, available at *Rheumatology* Online). Multivariate analysis using a large cohort will be needed to clarify whether anti-SAE antibodies independently contribute to the specific clinical characteristics.

Rheumatology key message

- Risk of malignancy should be considered in anti-small ubiquitin-like modifier activating enzyme antibody-positive adult DM patients.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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Tocilizumab in the treatment of a polyostotic variant of fibrous dysplasia of bone

SIR, Fibrous dysplasia of bone (FDB) is a benign disease leading to the slow replacement of normal bone by fibrous tissue, without osteoblastic rimming [1]. Three-quarters of FDB cases are monostotic and occur mainly in craniofacial bones, ribs, femurs and tibias. Polyostotic forms involve, in decreasing order of frequency, femurs, tibias, skull and facial bones, humerus and cervical spine. When associated with café-au-lait macules and hyperfunctioning endocrinopathies, the disease is identified as McCune–Albright syndrome [2]. Bone homeostasis is regulated by the balance between osteoblasts, which build up bone, and osteoclasts, which degrade bone. Pathophysiology of FDB is secondary to an activating mutation in the gene *GNAS* that leads to undifferentiated bone marrow stromal cell

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).

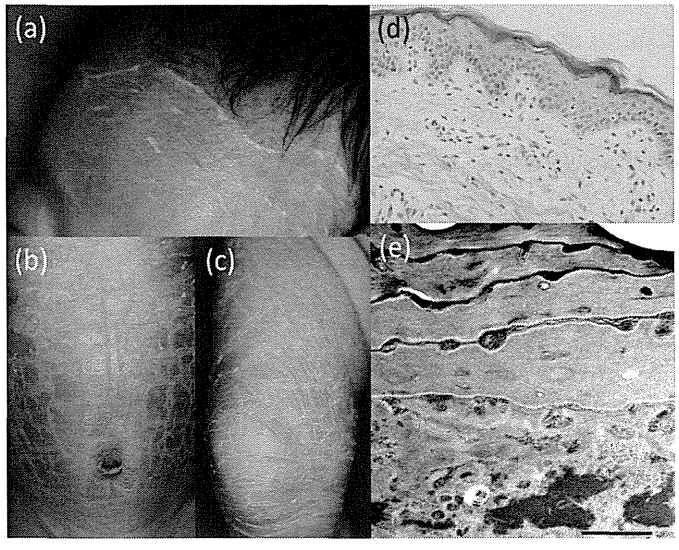

Fig. 1. Clinical features of RXLI mimicking LI.
(a–c) At birth, the entire body surface is covered with scales. On the trunk and extremities, the scales are dark, large thick; forehead (a), abdomen (b) and right leg (c). (d) Hematoxylin–eosin staining shows compact hyperkeratosis with normal granular 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.

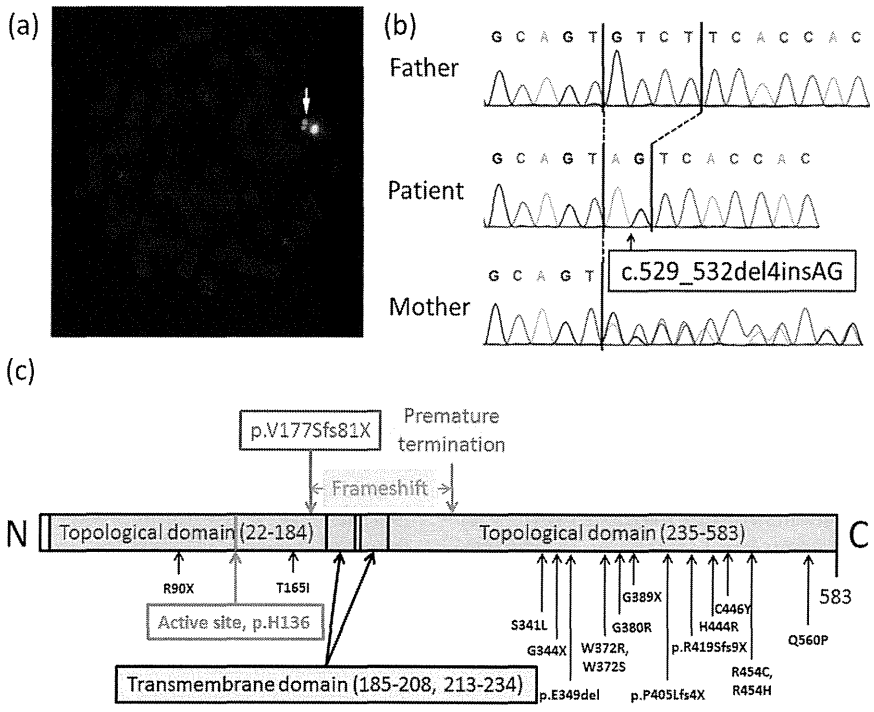

Fig. 2. FISH of Xp22.3, hemizygous mutations of STS of the patient, and the scheme of STS domain structure.
(a) Fluorescence *in situ* hybridization test for Xp22.3 indicates the existence of Xp22.3 (white arrow) in the patient. Red probe: region of *STS*, Xp22.3; green probe: a control for chromosome X. (b) Sanger sequencing reveals a hemizygous c.529_532del4insAG mutation of STS in the patient. His mother is a heterozygous carrier whereas his father shows wild-type sequence only. (c) The scheme of domain structure of STS. STS has two topological domains (green areas, amino acids 22–184, 235–583) and two transmembrane domains (gray areas, amino acids 185–208, 213–234). The N-terminal topological domain contains the reported active site, p.136 His (red arrow). Sites of the present novel indel mutation p.Val 177 Serfs × 81 in exon 5 and the premature termination are shown with blue arrows (top). Sites of the previously described mutations are indicated with black arrows (bottom).

Therefore, we hypothesize that there may be some residual STS enzyme activity in this case that contributed to the skin healing.

In summary, our case expands the phenotypic diversity and outcomes in RXLI, and highlights the value of WES in accurately identifying a pathogenic mutation in this subtype of ichthyosis, as well as providing a database for the future elucidation of other genetic modifiers contributing to the phenotypic variability.

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None.

Conflict of interest

The authors have no conflict of interest to declare.

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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.2015.07.001.

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Letter to the Editor

Impetigo herpetiformis with *IL36RN* mutations in a Chinese patient: A founder haplotype of c.115+6T>C in East Asia

Keywords:
Founder haplotype
Generalized pustular psoriasis
IL36RN
Impetigo herpetiformis
Interleukin-36 receptor antagonist

Impetigo herpetiformis (IH) is a rare pustular dermatosis of pregnancy [1]. Most patients with IH do not have personal and family histories of psoriasis. Early diagnosis is essential, as IH occasionally leads to maternal or foetal death. Despite the clinical importance of IH, its aetiology has not been clarified sufficiently. Recently, we reported two Japanese cases of IH with homozygous and heterozygous mutations of *IL36RN*, which encodes the interleukin-36 receptor antagonist (IL-36RN) [2]. However, the incidence of IH cases with *IL36RN* mutations is unknown. To our knowledge, no subsequent case of IH with or without an *IL36RN* mutation has been reported thus far.

After a long-standing controversy over whether IH is an independent disease entity from generalized pustular psoriasis (GPP), today there is a tentative consensus that IH is GPP occurring during pregnancy [3]. We reported that most GPP cases that are not accompanied by psoriasis vulgaris (PV; GPP alone) are caused by *IL36RN* mutations, although only a small number of cases with GPP preceding or accompanied by PV were found to have *IL36RN* mutations [4].

Here, we report a case of IH in a Chinese patient with a homozygous *IL36RN* mutation c.115+6T>C, the most frequent GPP-causing mutation in the Chinese population. We also found a novel haplotype of *IL36RN* c.115+6T>C, which is a probable founder haplotype, both in the present patient and in 2 Japanese families.

The patient was a 25-year-old Chinese woman who was admitted to our hospital for pustular lesions after her first normal vaginal delivery (Fig. 1a). She had neither a family history of GPP and IH nor consanguinity in her family. She had no history of GPP. Her pustular lesions began to develop at the 29th week of her first pregnancy, and she had been treated in a maternity hospital. Oral betamethasone of 3 mg/day was administrated, although the eruptions persisted. The skin biopsy of a specimen from a pustular eruption on the trunk revealed a spongiform pustule of Kogoj in the epidermis, consistent with IH (Fig. 1b). She had erythema with pustules all over her body and fever of a body temperature higher than 38 °C. Blood examinations revealed white blood cell counts of 31,590/μL and C-reactive protein concentrations of 2.45 mg/dL (reference range: <0.3 mg/dL). Bacterial culture of the pustules yielded negative results. Thus, she was diagnosed as having IH.

After ethical approval, informed consent was obtained in compliance with the guidelines of the Declaration of Helsinki. The entire coding regions of *IL36RN*, including the exon/intron boundaries, were sequenced by using a genomic DNA sample from the patient. The patient had the homozygous mutation c.115+6T>C (p.Arg10ArgfsX1), which is a GPP-causing mutation that was found in both Chinese and Japanese cohorts [4–6] (Fig. 1c).

We previously reported *IL36RN* c.115+6T>C as a founder mutation (haplotype; ACTACACC) in a Japanese GPP cohort [4]. Later, in Japanese GPP and IH cases [2,7], we found another haplotype (ACCGAGCC) of c.115+6T>C and herein report the haplotype for the first time. The analysis method for the haplotype of *IL36RN* was described previously [4]. The present Chinese patient also had the haplotype (ACCGAGCC). Thus, the haplotype seems to be shared by the Chinese and Japanese populations.

The prevalence of the *IL36RN* mutation c.115+6T>C is 0.90% (10/1,114 individuals) in the Japanese population and 4.1% (15/365 individuals) in the Chinese population [6,8]. However, the prevalence of the *IL36RN* mutation c.115+6T>C of the specific haplotype (haplotype: ACCGAGCC) in both populations is not known. The *IL36RN* mutation c.115+6T>C (haplotype: ACTACACC) has not been reported in the Chinese population. However, independent from the haplotype, it might be an IH-causing mutation in the Chinese population.

Several twin or sibling cases of IH have been reported [9,10]. Therefore, IH has been thought to be a genetic disease, although the genetic background had been unknown. To date, we have sequenced *IL36RN* in 3 IH cases, including the present case, and found that all of the 3 cases had *IL36RN* mutations [2]. Only a small number of IH patients have been studied genetically, including the present case; thus, further studies of a large number of IH patients are needed in the future.

Abbreviations: GPP, Generalized pustular psoriasis; IH, Impetigo herpetiformis; IL-36, RNInterleukin-36 receptor antagonist; PV, psoriasis vulgaris.

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CASE REPORT

Magnetic resonance imaging findings are useful for evaluating the three-dimensional development and follow-up of linear lupus erythematosus profundus

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Lupus erythematosus profundus (LEP), which is a variant of chronic cutaneous lupus erythematosus (CLE), is seen in approximately 2~3% of CLE patients, and only 10% to 20% of LEP patients present with systemic LE (SLE). LEP shows subcutaneous nodules with or without discoid LE (DLE). Linear LEP, a very rare variant of LEP, was first reported in 1991 in Japanese and in 1998 in English. Since LEP sometimes leaves skin depressions or scars as a result of atrophy of adipose tissue, early and adequate treatments are necessary. Here, we introduce an LEP case in which magnetic resonance imaging (MRI) was quite effective in evaluating a lesion that had been considered to be linear DLE. *Lupus* (2015) **24,** 1214–1216.

Key words: Lupus erythematosus profundus; magnetic resonance imaging

Introduction

Lupus erythematosus profundus (LEP), which is a variant of chronic cutaneous lupus erythematosus (CLE), is seen in approximately 2~3% of CLE patients, and only 10% to 20% of LEP patients present with systemic LE (SLE). LEP shows subcutaneous nodules with or without discoid LE (DLE). Linear LEP, a very rare variant of LEP, was first reported in 1991 in Japanese and in 1998 in English.[1] Since LEP sometimes leaves skin depressions or scars as a result of atrophy of adipose tissue, early and adequate treatments are necessary.[2] Here, we introduce an LEP case in which magnetic resonance imaging (MRI) was quite effective in evaluating a lesion that had been considered to be linear DLE.

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Case history

A 42-year-old Japanese female presented with linear erythematous plaques extending around the left arm. Initially, one small plaque had appeared on the left upper arm six years before her first visit and had gradually expanded. A subcutaneous nodule had started to grow under the plaque. Skin biopsy by the previous doctor led to the diagnosis of pseudo-lymphoma. The patient also had an episode of swelling on the right cervical lymph node during the disease course. For detailed examination, she was referred to our hospital. During a year of follow-up in our department, the plaque and nodule were observed to grow in size, and a second biopsy was performed. The specimen from the erythematous plaques on the extensor side of the upper arm showed dense perivascular and periappendicular inflammatory infiltration in the entire dermis, and liquefaction degeneration was seen in the basal layer of the epidermis. In the subcutaneous fat tissue, panniculitis and lymphoid follicle formation were observed (Figure 1). In light of these clinicopathological features, including the history of gradual growth of the subcutaneous nodule, linear DLE with LEP was suspected. Anti-single-stranded DNA antibody (120.9 AU/ml) and

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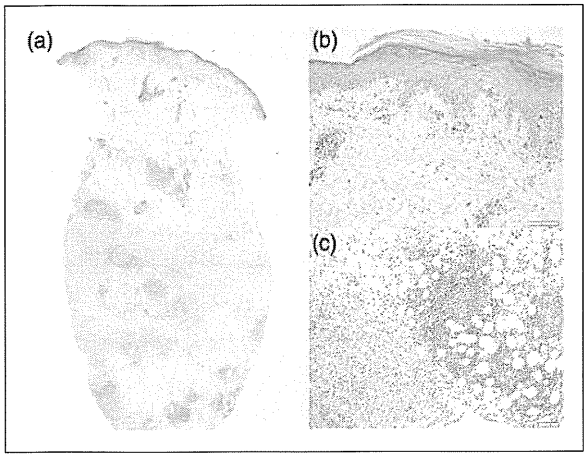

Figure 1 Histopathological findings.
Dense perivascular and periappendicular inflammatory infiltration throughout the dermis. (a) In the subcutaneous fat, dense inflammatory infiltration and lymphatic follicle-like lymphocytic accumulation are observed (original magnification ×2). (b) Liquefaction degeneration of the basal layer is observed in the covering epidermis (original magnification ×10). (c) Severe inflammatory infiltration in the fat tissue (original magnification ×10).

anti-SS-A antibody (85.0 U/ml) were positive. C3 was slightly low, at 80.5 mg/dl (normal: 86~160), but antinuclear antibody and anti-double-stranded DNA were negative. Due to abnormal findings in urine blood and protein, renal biopsy was performed. Hematoxylin and eosin staining, indirect immunofluorescence and electron microscope studies of biopsy samples confirmed class V pure membranous lupus nephritis without nephrosis.

MRI of the left limb was taken for evaluation of panniculitis. The MRI revealed the panniculitis to be linear rather than nodular, and to have widely expanded beyond the erythematosus area (Figure 2(a)). Under the diagnosis of linear LEP, systemic corticosteroid therapy was started at 20 mg/day of prednisolone (PSL), targeting the severe panniculitis. This PSL dosage was also sufficient for class V lupus nephritis treatment.[3] The systemic PSL was tapered with improvements in the panniculitis as evaluated by MRI during the three-year disease course. The panniculitis remained at the primary site but slowly shrank with a maintenance dose of PSL at 5 mg/day. There has been no regrowth of the lesion (Figure 2(b)).

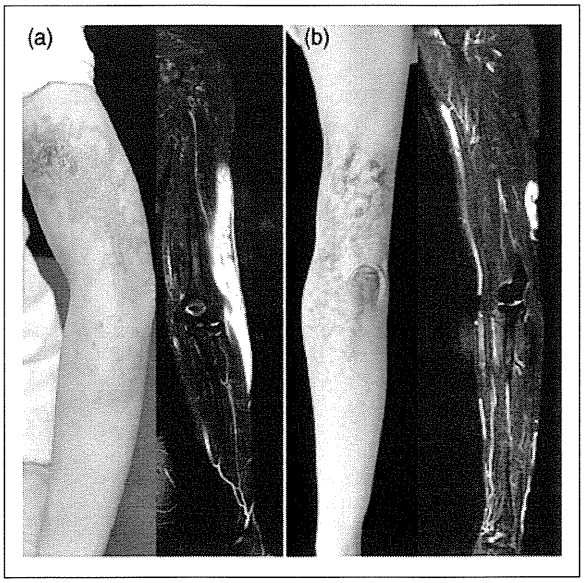

Figure 2 Clinical features and magnetic resonance imaging (MRI) findings of the left arm.
A short tau inversion recovery (STIR) image reveals that the panniculitis is not a nodule, but is a linear mass that has expanded beyond the erythematous plaques (a). After three years, atrophy of adipose tissue is seen to correspond to the linear panniculitis previously detected in (a). Small remaining active panniculitis is observed at the primary lesion (b).

Discussion

Generally, LEP follows Blaschko's lines,[4] as it did in our case. McNallan et al.[5] hypothesized that microchimerism involving immunocompetent maternal cells mediates tissue damage solely in areas with skin mosaicism, thus giving rise to inflammatory lesions with a Blaschko-like pattern.

LEP can be difficult to diagnose because it can develop in isolation from SLE and because there have been no effective tools for verifying panniculitis other than biopsy. Considering that biopsy evaluates only a spot of the lesion, it is possible to miss panniculitis when it underlies DLE. There are only a few case reports[6,7] in which MRI was used to evaluate LEP that appeared independently from DLE. It is difficult to find LEP-specific MRI signs; however, inflammation or hypertrophy of the fat layer in images should be considered for evaluation of LEP. Although the extent of panniculitis was difficult to evaluate from the skin surface in our case, MRI enabled us to evaluate the size and severity of the panniculitis and to decide on an effective therapeutic regimen. The present case suggests that MRI can be a very effective tool for evaluating the disease activity of subcutaneous panniculitis including LEP, toward accurate diagnosis and proper follow-up.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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LETTER TO THE EDITOR

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.

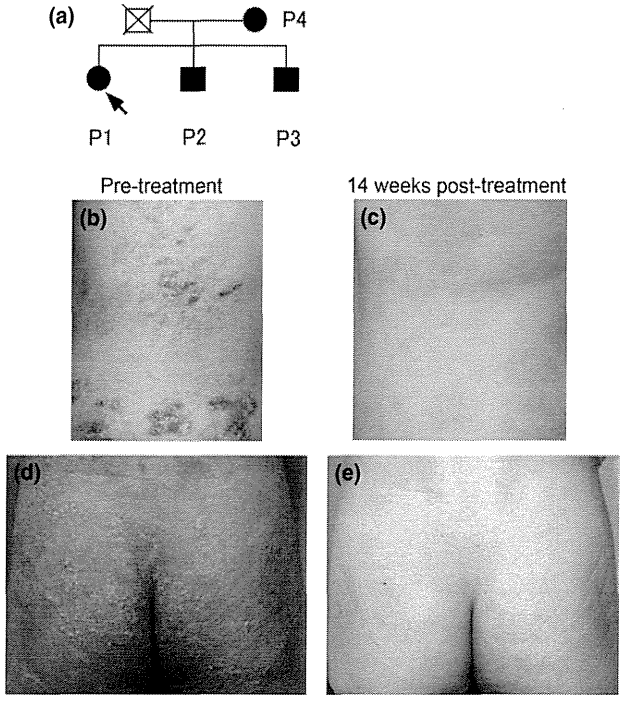

Figure 1 Pedigree of the patients' family and skin manifestations of Patients 1 and 3 before and after treatment with infliximab (a) Pedigree of the patients' family. The mother (Patient 4) was suffering from GPP preceded by PV. (b) Pustules in the background of erythema were observed on trunk in Patient 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.

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

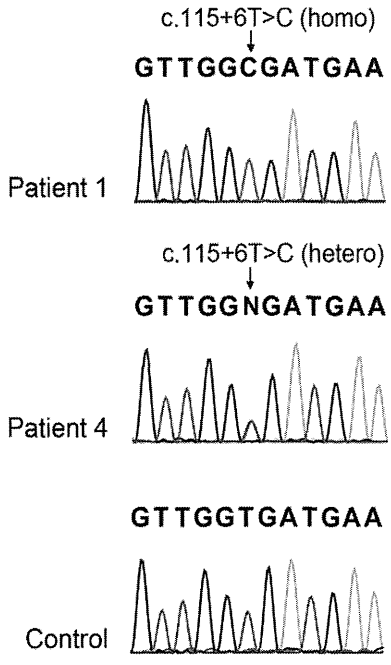

Figure 2 Sequence data of *IL36RN*. Sequence data of *IL36RN* are shown for Patient 1, Patient 4 and a control.

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 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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SHORT COMMUNICATION

Familial Primary Localized Cutaneous Amyloidosis Results from Either Dominant or Recessive Mutations in *OSMR*

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Primary localized cutaneous amyloidosis (PLCA; MIM 105250) is a chronic itchy skin disorder associated with amyloid deposits in the superficial dermis (1). Clinically, most skin lesions comprise small, flat-topped papules (lichen amyloidosis) or brown-grey macules (macular amyloidosis). Recently, proteins containing a considerable amount of β-sheet structures, such as galectin-7 and actin, have been reported as amyloidogenic in PLCA (2, 3).

Most cases of PLCA are sporadic, but familial cases (FPLCA) with autosomal dominant inheritance also exist (4–6). Pathogenic mutations in *OSMR* and *IL31RA* have been reported as the major cause of FPLCA (5, 7); both of these genes belong to the family of interleukin (IL)-6 family cytokine receptors. *OSMR* encodes oncostatin M receptor-beta (OSMRβ), a component of both the OSM type II receptor and the interleukin (IL)-31 receptor (8, 9), whereas *IL31RA* encodes the IL-31 receptor alpha, which combines with OSMRβ to form the IL-31 receptor (7). To date, 10 heterozygous missense mutations in *OSMR* and 1 heterozygous missense mutation in *IL31RA* have been reported in FPLCA, with all cases showing autosomal dominant inheritance (5–7, 10).

In this study, we examined 2 large pedigrees with FPLCA originating from Pakistan (family A) and Malaysia (family B) (Fig. 1a, b). Unusually, however, the occurrence of FPLCA in both families is consistent with autosomal recessive, rather than dominant, inheritance.

MATERIALS, METHODS AND RESULTS

All individuals provided written informed consent according to a protocol approved by local ethics committees in adherence with the guidelines of the Declaration of Helsinki. The diagnosis of FPLCA was made by dermatologists based on typical clinical skin features. Blood samples were collected from 4 affected and 4 unaffected individuals of family A and 4 affected individuals of family B marked in the respective pedigrees (Fig. 1a, b; "DNA"). Genomic DNA was extracted from peripheral blood leukocytes by standard procedures.

Genome-wide linkage-scan in family A was performed using the Illumina HumanOmniExpress BeadChips (Illumina Inc., San Diego, CA, USA). Analysis of genotype data was carried out using easyLinkage (11). In family A, whole-exome sequencing was performed using DNA samples of 1 affected individual (III-1) (Appendix S1[1]). In family B, whole-exome sequencing

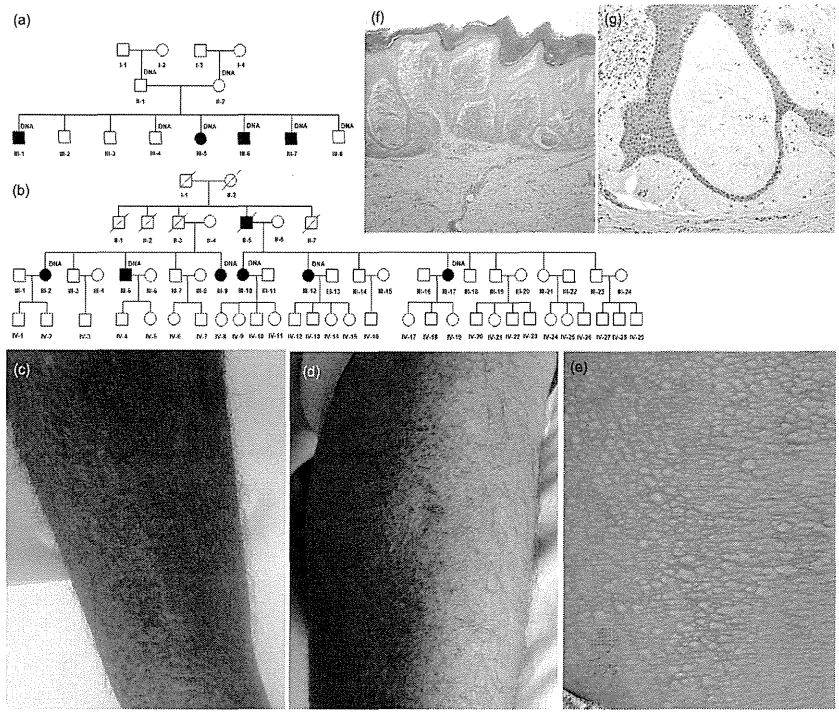

Fig. 1. Pedigree structure, Clinical and histopathological features of familial primary localized cutaneous amyloidosis (FPLCA). Pedigree of (a) family A and (b) family B, showing segregation of the FPLCA phenotype. Affected males and females are indicated by filled squares and circles, respectively. Deceased individuals are indicated by crossed symbols. Symbols with "DNA" represent the samples available for the study. Clinical appearances of FPLCA in an affected individual (III-1) of family A showing hyperpigmented flat papules on (c) the right arm and (d) the lower leg. (e) Clinical features of an affected individual (III-2) of family B showing skin-coloured papules on the lower back. Histopathological examination of skin from an affected individual (III-2) in family B showing amyloid deposition immediately below the epidermis using Congo red staining (f) 40× image resolution, (g) 100× image resolution.

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was performed using DNA samples of 2 affected individuals (III-2, and III-9) using previously reported methods (12).

In family A, all affected siblings showed the first symptoms at the age of 13–14 years. They presented with marked skin lichenification. The hyperpigmented flat papular lesions were itchy and present above the ankles, extending to the shins, thighs and abdominal regions (Fig. 1c, d). There was no history of FPLCA in previous generations. In family B, the age of onset ranged from 18 to 70 years. Symptoms started on the legs and arms with pruritus, followed by brown papules and patches. Later, the papules spread to other areas, involving trunk, limbs, neck and back (Fig. 1e). Histopathology of lesional skin showed amorphous eosinophilic material in the papillary dermis by use of Congo red (Fig. 1f, g). In this pedigree, the inheritance pattern was more complex; part of the pedigree showed probable recessive inheritance in 3 siblings (Fig. 1b, individuals III-2, III-5, and III-9), while autosomal dominant transmission was more likely in other relatives (II-5, III-10, III-12, and III-17).

Thus far, not a single gene/gene locus had been reported for autosomal recessive FPLCA. Therefore, we performed a genome-wide linkage scan with 8 individuals from family A. Analysis of genotype data identified 3 chromosomal regions segregating with the FPLCA phenotype: 1q23.3–q24.2; 5p14.2–q11.2; 14q32.33. The linkage region on chromosome 5 harbours *OSMR* and *IL31RA*, and therefore these genes were sequenced. Sequencing of *IL31RA* did not reveal any pathogenic variant(s), but a homozygous single nucleotide substitution, c.1385A>G; p.Asn462Ser (NM_003999), was detected in exon 11 of *OSMR* (Fig. S1a[1]). Sequencing of all available DNA samples of family A revealed co-segregation of the mutation with disease phenotype. Heterozygous carriers (II-1, II-2, III-4, and III-8) did not show any clinical signs of FPLCA or report symptoms of pruritus, arguing against semi-dominant inheritance. This mutation has not been reported in dbSNP, the 1,000 genomes project or the ESP6500 data-set. To demonstrate that there were no other potentially pathogenic mutations in genes located in regions of linkage, we performed whole-exome sequencing of one additional family member (III-1). Our filtering strategy (Appendix S1[1]) retained only the variant described above in *OSMR*.

Analysis of the exome data in family B revealed a homozygous missense mutation in exon 11 of *OSMR* (c.1538G>A; p.Gly513Asp) in both individuals (Fig. S1b[1]), with the amino acid change predicted to be damaging by bioinformatic analysis with PolyPhen-2 (score 1.000) and SIFT (score 0). Sanger sequencing showed homozygosity for the mutation in individuals III-2, III-5 and III-9, but heterozygosity in subject III-10. Clinically, individual III-10, heterozygous for p.Gly513Asp, had very similar features of FPLCA to the other affected cousins who were homozygous for this mutation, with no differences in age of onset, pattern or severity of the disease. Intriguingly, further history revealed no symptoms in individuals II-3 (deceased) or II-4, who, as parents of 3 homozygous offspring, were likely to be heterozygotes for the mutation. Furthermore, none of the offspring of III-2 or III-5, obligate heterozygotes for p.Gly513Asp, had any features of FPLCA.

DISCUSSION

All the dominant mutations reported for *OSMR* are located within the 2 extracellular fibronectin III-like domains (FNIII domains) that are closest to the transmembranous region of OSMRβ (10). In contrast, both new mutations are located in a more distal FNIII domain (Fig. S1c[1]). Our data further suggest that p.Gly513Asp in family B may act as both a dominant and a recessive mutation, but, at present, it is not known what factors influence the presence or absence of FPLCA in heterozygotes.

The cutaneous amyloid deposits comprise collections of keratins (from basal keratinocytes), serum amyloid P component, apolipoprotein E, galectin-7 and actin (2), with galectin-7 peptides contributing to amyloidogenesis (3), although other amino acid motifs may also be implicated in forming the β-sheets that are an essential part of the pathophysiology of cutaneous amyloidosis (13).

The link between mutations in *OSMR* and the pathogenesis of cutaneous amyloidosis is not fully known. Previous studies have demonstrated that the pathogenic missense mutations in *OSMR* result in aberrant IL-31 signalling (5). Abnormalities in IL-31 signalling may be directly relevant to the key clinical symptom of itch (14), although an additional consequence of alterations in the IL-31 pathway demonstrated for mutations in *OSMR* is a failure to induce expression of monocyte-chemotactic protein-1 (MCP-1) (15). The implications for the pathogenesis of cutaneous amyloidosis could be that a lack of inducible MCP-1 results in less monocyte chemotaxis in patient skin, which leads to altered innate immunity, with reduced scavenger function and accumulation of cellular debris (15). Transcriptomic analysis of RNA from lesional cutaneous amyloidosis skin has also revealed upregulation of keratinocyte proliferation and differentiation markers, downregulation of keratinocyte stem cell markers, and downregulation of anti-apoptotic factors (6). The latter observations are helpful in explaining the clinicopathological features (dyschromia or lichenification with keratinocyte apoptosis) and may be relevant to the pathophysiology of the disease, although the precise mechanisms leading to changes in keratinocyte gene expression are not fully known. In conclusion, this study offers new findings on the molecular genetics and disease relevance of mutations in *OSMR* in FPLCA.

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