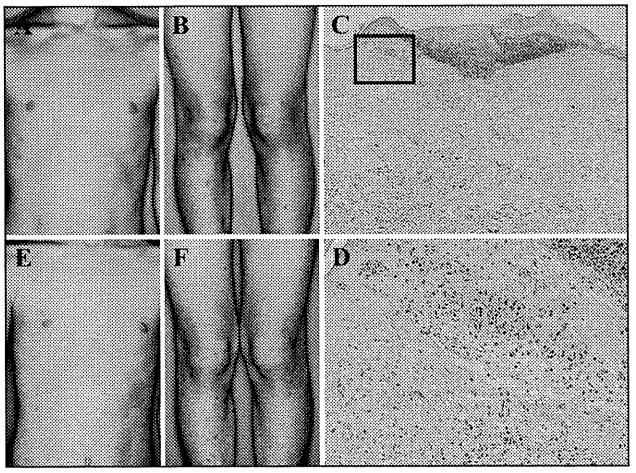

Figure 1. A) Before the tonsillectomy, several erythematous macules with vesicles on the trunk. **B)** Brownish macules and plaques with vesicles on the lower extremities. **C, D)** Skin biopsy showed spongiosis, focal epidermal necrosis, crust formation, lymphocyte exocytosis, and liquefaction degeneration. Extravasation of erythrocytes, and heavy lymphocytic infiltrates are found in the superficial dermis (hematoxylin-eosin staining, original magnification: C, ×80; D, ×400). The squared area in *figure 1*C corresponds with the area shown in *figure 1*D. **E)** After the tonsillectomy, multiple erythematous macules and papules with vesicles and crusts are scattered all over his trunk. **F)** Widespread brownish plaques with crusts on the lower extremities.

minocycline, and roxithromycin and dapsone were used; however, the lesions were quite refractory. Although we proposed further treatment with ultraviolet light and oral prednisone, no consent was obtained.

As far as we know, 2 cases of pityriasis lichenoides, one with PLEVA [2] and the other with pityriasis lichenoides chronica [3], resolved by tonsillectomy have been reported thus far. Both patients were approximately 10-year-old Japanese boys. Although their age and geographical characteristics were similar to our case, tonsillectomy modified PLEVA in the opposite direction in the present case. Given that the exacerbation occurred shortly after the operation and persisted for 5 years without any other triggers, tonsillectomy potentially modified the balance of the immune system, such as the proportion of $CD8^+$ and regulatory T cells in the skin, as well as stopping the hypersensitivity reaction to infectious agents. Immunohistochemistry has revealed that $CD8^+$ T cells predominate in the epidermal and dermal infiltrates of PLEVA lesions [1]. Since both regulatory T cells and conventional $CD8^+$ T cells reside in tonsils [4], tonsillectomy may result in a decrease of these cells in the skin lesions. The proportion of regulatory T cells and $CD8^+$ T cells in tonsils varies among individuals, which may be linked to the varied effects of tonsillectomy in PLEVA.

This kind of paradoxical therapeutic effect is also reported in TNF-α blockers. While TNF-α blockers are efficacious for pityriasis lichenoides, they also paradoxically induce the disease [5, 6]. The modulation of complex underlying immunological abnormalities in pityriasis lichenoides appears to cause the dual effects of tonsillectomy and TNF-α blockers.

To our best knowledge, this is the first report demonstrating the exacerbation of PLEVA after tonsillectomy. Although the efficacy of tonsillectomy in pityriasis lichenoides is still controversial and further studies are required, the present case may give us some clue to further understand the mechanism responsible for the development of this complicated disorder. ■

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Dyschromatosis symmetrica hereditaria with acral hypertrophy

Dyschromatosis symmetrica hereditaria (DSH) is a hereditary disorder caused by a mutation in the RNA-specific adenosine deaminase gene (*ADAR1*) and characterized by hyper- and hypopigmented macules on the backs of the hands and feet, sometimes with small freckle-like macules on the face. To date, no complications other than a few cases of neural symptoms have been reported. We describe a case of DSH with a known *ADAR1* mutation (C3247T) where the fingers and hands exhibited symmetrical hypertrophy in association with typical acral dyschromatosis. Camouflage techniques successfully treated the dyschromatosis.

A 30-year-old Chinese woman had had small hyper- and hypopigmented macules on the dorsa of her fingers, hands, and feet *(figure 1A)* and small freckle-like macules on her face since the age of three. Her father and son had similar macules. These characteristic clinical features led to a diagnosis of DSH. *ADAR1* gene analysis revealed a known heterogeneous misssense mutation C3247T. Curiously, the fingers but not the tips, and the dorsa of the hands were swollen and elastically soft *(figure 1A)*, which the patient recognized having had since childhood. X-ray

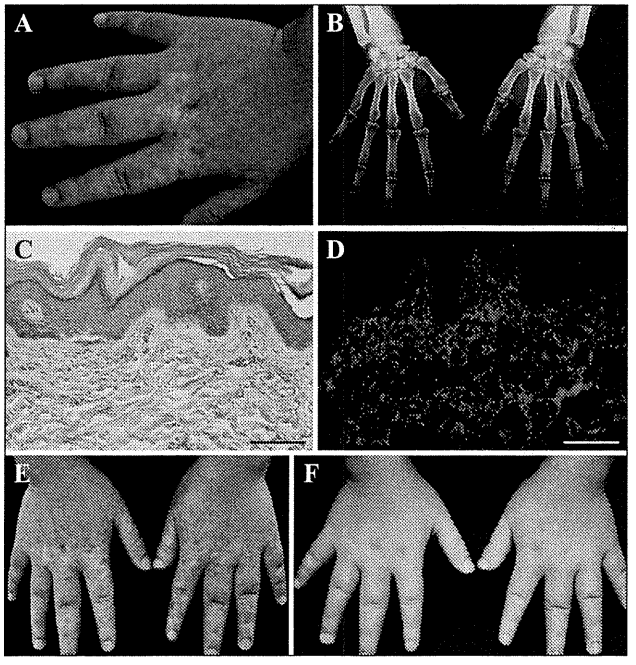

Figure 1. A) The fingers and hands of the patient exhibited moderate hyperkeratosis and small hyper- and hypopigmented macules and hypertrophy and were soft to the touch. **B)** X-ray examination exhibited no bone changes. **C)** Analysis of a biopsy specimen taken from the lesion on the hand by hematoxyline-eosin staining showed prominent spaces between the collagen bundles, **(D)** and staining with biotin-conjugated hyaluronan binding protein demonstrated more hyaluronan in the lesion than in the control dermis. ×200, Bar: 100 μm. The lesions were successfully managed by cosmetic camouflage using Covermark™, **(E)** before and **(F)** after treatment.

examination exhibited no bone changes *(figure 1B)*. Thyroid hormone and thyroid-stimulating hormone were within normal limits and she had no signs of autoimmune diseases, such as systemic lupus erythematosus. A biopsy from the lesion on the left hand metaphalangeal joint revealed prominent spaces between the collagen bundles *(figure 1C)*. Staining with biotin-conjugated hyaluronan binding protein (1 μg/mL Seikagaku Corporation, Tokyo, Japan) demonstrated more hyaluronan in the lesion than in the control dermis *(figure 1D)*. Since the patient seriously complained about pigmentary changes, she was treated with Covermark™ (Covermark, Northvale, NJ) which we use for patients with vitiligo. She was satisfied with the result *(figures 1E, F)*.

ADAR1 protein catalyzes the deamination of adenosine in double-stranded RNA substrates to inosine. To date, only three known target genes for ADAR1 have been identified, namely, ionotropic glutamate receptors, the serotonin receptor 2C subtype in the brain, and hepatitis delta virus antigen in the liver [1]. Glutamate receptors are expressed at high levels in the brain. This may explain three cases of DSH with neural disorders [2], which are the only symptoms speculated to be associated with DSH among 70 or more mutations previously reported [3]. Hypertrophy of acral soft tissues with excessive mucin deposition has not been previously reported in patients with DSH. Although the target genes through which the mutation in *ADAR1* induces the abnormal pigmentation remain unknown, it is thought that hyper- and hypopigmentation in DSH reflect the quantity of melanin in the basal cell layer [4]. Because the present case exhibited both hypertrophy and abnormal pigmentation at the same acral region, the C3247T *ADAR1* mutation leading to abnormal pigmentation might also lead to mucin overproduction in the dermal fibroblasts. However, an identical mutation has been reported in two reports, and acral soft tissue hypertrophy was not mentioned [5]. The acral hypertrophy was only seen in our patient, not in her affected family members. Although there is no evidence to attribute the hypertrophy of acral soft tissues to the mutation in *ADAR1* mutation, it seems that co-localization of the two different disease phenotypes suggests a possible linkage rather than mere coincidence. An identical mutation in the *ADAR1* gene has different phenotypes even within the same family [6]. This may explain why the patient's son and father did not exhibit hypertrophy. This might merely be the result of sex differences, or additional gene mutations which occurred only in the present case. Further accumulation of detailed case reports is required to elucidate the mechanisms by which the mutation in *ADAR1* leads to dyschromatosis in the acral skin. ■

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Oculocutaneous albinism type 3: A Japanese girl with novel mutations in *TYRP1* gene

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ABSTRACT

Background: Oculocutaneous albinism (OCA) type 3 caused by mutations of the *TYRP1* gene is an autosomal recessive disorder of pigmentation characterized by reduced biosynthesis of melanin pigment in the skin, hair, and eye. The clinical phenotype has been reported as mild in Caucasian OCA3 patients.
Objective: We had the opportunity to examine a Japanese girl with OCA3 and investigated activity of TYRP1 protein derived from the mutant allele detected in the patient.
Methods: Mutation search for OCA responsible genes was done. A mutant allele with a missense mutation was analyzed using melanocyte cultures (b cells) established from a mouse model of OCA3.
Results: Compound heterozygous mutations, p.C30R and p.367fsX384, were detected in the Japanese girl. Then we revealed that the missense mutation, p.C30R, was functionally incapable of melanin synthesis with in vitro experiments.
Conclusion: This is the first report of the occurrence of OCA3 in Japanese population.

1. Introduction

Oculocutaneous albinism (OCA) is a heterogeneous genetic disease with much clinical heterogeneity [1]. Four different types of non-syndromic OCA have been reported at present. OCA type 3 (OCA3) (MIM 203290) is an autosomal recessive hypopigmentary disorder caused by mutations in the tyrosinase-related protein 1 gene (*TYRP1*). *TYRP1* spans 17 kb on chromosome 9p23 and is composed of 8 exons. TYRP1 protein, which is one of melanosomal glycoproteins, has the activity of a catalase (catalase B). During melanin synthesis, hydroperoxides are produced during autooxidation of melanin precursor indoles by oxygen, and addition of catalase to tyrosinase reaction mixtures in vitro increases the yield of melanin. TYRP1 is one of essential members for melanogenesis, and indirectly controls the melanogenesis in melanosomes.

In 1996, Boissy et al. identified homozygosity for a 1-bp deletion in the *TYRP1* (c.1103delA, p.K368fs) resulting in premature truncation at codon 384 in an African American male with brown oculocutaneous albinism (BOCA) [2]. Then, Manga et al. [3] analyzed the *TYRP1* in 19 unrelated southern African blacks with rufous OCA (ROCA) and identified compound heterozygosity for c.1103delA, p.K368fs and a nonsense mutation (c.497C > G, p.S166X) in 17 of the 19 patients. Thus, OCA3 has been characterized in African origin people with albinism.

Meanwhile, OCA3 is very rare in Caucasian and Chinese [4], and has not been reported in the Japanese [5]. Recently, some non-African patients with OCA3 have been reported from a large consanguineous Pakistani family [6], a Caucasian German [7], an Asian [8], an Asian Indian [9], and very recently two Chinese [10], although the number is a few. The clinical phenotype of OCA3 has been reported as mild in Caucasian OCA3 patient, and the mild phenotype might be the reason why Caucasian OCA3 patients may be underdiagnosed.

We report here the first case of OCA3 in Japanese patients with an apparent clinical tyrosinase-positive OCA.

2. Patient and methods

2.1. Clinical report

The patient was an one-year-old girl of Japanese ethnicity. The pregnancy was uneventful and she was born at term. The girl was in good health, had a normal psychomotor development and had never been hospitalized. Physical examination showed a girl with blond hair, brown eyebrows, dark brown eyelashes, and irides, and lighter skin than those of parents (Fig. 1A). A small Mongolian spot

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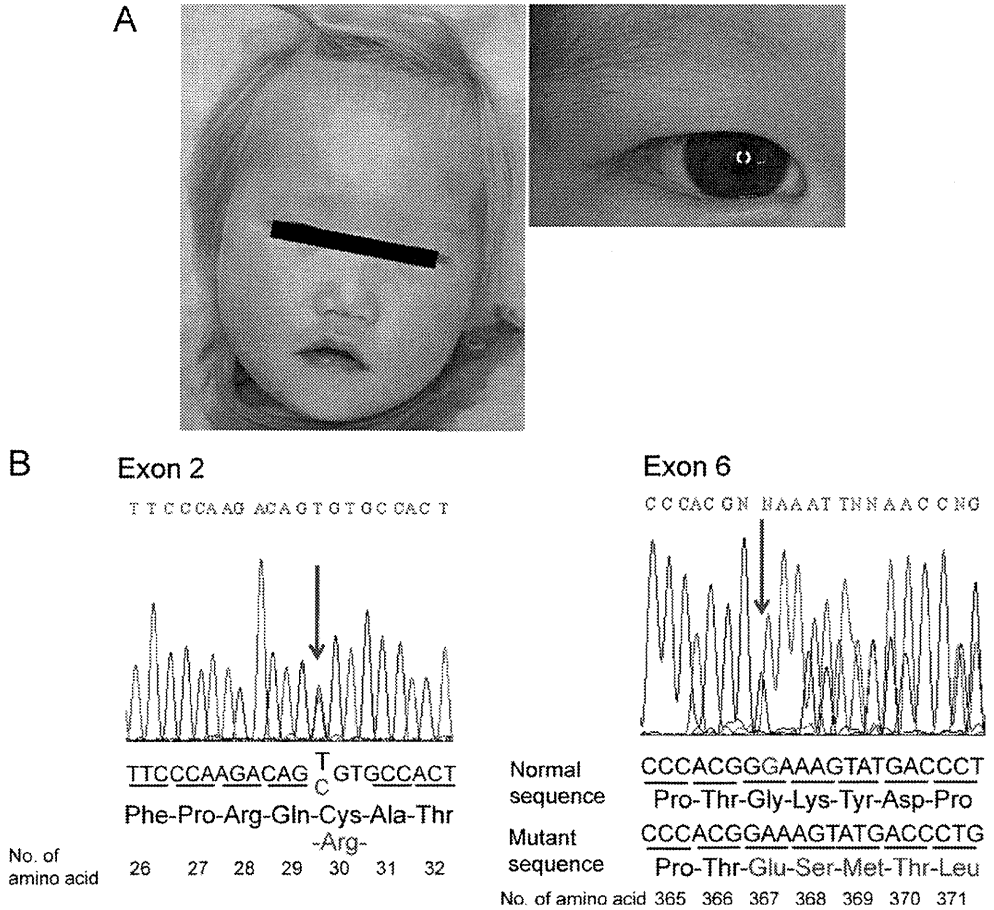

Fig. 1. (A) Clinical features of the patient at 1 year of age. She had blond hair, brown eyebrows, dark brown eyelashes, and irides, and lighter skin. A small Mongolian spot was found on her hip. She also presented possible tanning ability, and no nystagmus. (B) DNA sequence of patient's *TYRP1* gene. In exon 2, T at point marked with an arrow was changed to C in red at position c.88, resulted in a substitution cysteine to arginine at position 30 amino acids. In exon 6, G in red at position c.1100 marked with an arrow was deleted in the mutant sequence of the case. Deletion of the nucleotide occurred at point marked with an arrow, which lead to a frameshift and resulted in a stop at codon 384.

was found on her hip. She also presented possible tanning ability, and no nystagmus. Neither apparent amblyopia nor photophobia was recognized, although the thorough examination was not done because of her infancy. Complementary investigations could exclude a Hermansky-Pudlak syndrome, a form of OCA with bleeding diathesis since her haematological examination and platelet aggregation studies were normal. Family history was unremarkable; she was the only person with clinical signs of albinism in the family. This study was approved by the Ethics Committees of Yamagata University School of Medicine. Informed consent was obtained from the patient's parents.

2.2. Mutation screening and functional analyses

TYRP1 gene was analyzed using PCR-based single strand conformation polymorphism/heteroduplex (SSCP/HD) and direct sequencing as previously described [11]. Briefly, genomic DNA was extracted from peripheral blood and used as a template for PCR. The products showing aberrant patterns on SSCP/HD gels were reamplified and sequenced to identify the mutation.

Functional analysis was performed as previously described [12,13]. Briefly, we constructed wild-type and mutant p.C30R *TYRP1* cDNA. Each of the cDNAs was inserted into the mammalian expression plasmid pIREShyg3 (BD Biosciences, San Jose, CA), creating pIREShyg3-*TYRP1* wild-type and pIREShyg3-*TYRP1* mutant-p.C30R. Following an initial 24 h-culture period, melan-*b* cells [14] were transfected with either 1.6 μg of one of the two constructs or pIREShyg3 alone (mock transfection) per 4 cm^2 flask. As the transfection efficiency of the plasmid to melan-*b* was very low, less than 1%, the experiment with the cells transiently expressing the protein was very difficult. Then, we established stable transformants, which were selected in culture media containing 500 μg/ml hygromycin B. Six independent clones were established from each transformants. For melanin assays, each suspension was pelleted and incubated at 95 °C for 1 h after resuspension in 100 μl of 1 N NaOH. After a 100× dilution, the OD_{475} was measured and converted to melanin content via a standard curve using sepia melanin (Sigma, Poole, UK). The melanin content was normalized to protein content, determined using a Protein Assay Kit from Bio-Rad (Hercules, CA). Melan-*b* was cultured in RPMI1640 medium with 10% fetal calf serum, 200 nM 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), and 100 μM 2-mercaptoethanol, in 10% CO_2 [14].

2.3. Real-time quantitative RT-PCR (RQ-RCR)

RQ-RCR was performed with total RNAs extracted from the transformants cells, TaKaRa RNA PCR kit ver3.0 (TaKaRa, Japan), and the primer set, hTYRP1 F 5′-TCTGGGCTGTATCTTCTTCC-3′ and hTYRP1 R 5′-TCTGTCCCAGGCCCAGACAC-3′. The data was normalized with a beta-2-microglobin (B2 M) fragment. Reactions were performed using the STRATAGENE Mx3000P Real-Time QPCR System under relative quantification with Brilliant II

SYBR Green QPCR Master Mix® (STRATAGENE, La Jolla, CA). Data were analyzed with MxPro™ Software ver 4.0 (STRATAGENE, La Jolla, CA).

2.4. Protein analyses

Cells were washed twice with cold PBS, and lysed with lysis buffer (Tris–HCl pH 7.5, NaCl 150 mM, NP-40 1%) plus protease inhibitor (Roche, Switzerland). Lysates were pelleted, and 5 μg protein was separated by SDS-PAGE, transferred onto Immobilon-P (Millipore, MA). Membranes were blocked in 0.3% fat-free milk in PBS plus 0.05% Tween20 overnight at 4 °C, and were then incubated in blocking solution containing the monoclonal anti-human TYRP1 mouse antibody (LifeSpan BioSciences, WA) for 1 h at room temperature. Membranes were then washed extensively, followed by peroxidase-conjugated IgG for 1 h at room temperature and washed again. Signals were detected using ECL Plus Western Blotting Detection Reagents (Amersham Biosciences, Sweden) and quantified by CS Analyzer ver2.0 for Windows (ATTO, Japan). Rabbit polyclonal antibody raised against a full-length human TYRP1 protein was purchased from Abnova Corporation (Taiwan).

2.5. Immunofluorescence

We cultured transformed cells on glass coverslips. After 72 h, cells were washed with PBS, fixed for 10 min in 99% methanol at −20 °C, and washed with TBS three times. We blocked nonspecific antibody binding for 5 min with Protein Block (DakoCytomation, Denmark), and incubated coverslips sequentially for 1 h at RT with monoclonal anti-human TYRP1 mouse antibody (LifeSpan BioSciences, WA), followed by three washes with TBS. Then, FITC conjugated anti-mouse IgG antibody (DakoCytomation, Denmark) was reacted on coverslips for 1 h at RT, and we washed coverslips for three times and mounted them on glass using the Prolong Antifade Kit (Molecular Probes). Cells were studied with a confocal microscopy, ZEISS-LSM-510Meta (ZEISS, Germany).

3. Results

3.1. Mutation screening

The mutation screening for the genes responsible for OCA1–4 and Hermansky-Pudlak syndrome type 1 revealed compound heterozygous mutations at NM_000550: c.88T > C, p.C30R and c.1100delG, p.G367fsX384 in the *TYRP1* gene for OCA3 (Fig. 1B). And, her father and mother turned out to be heterozygous for the mutation, c.88T > C, p.C30R and c.1100delG, p.G367fsX384, respectively. No pathological mutations were detected in other genes for OCA. The substitution was not detected in any genomic DNA of the 120 individuals of Japanese origin who were used as normal controls.

3.2. Functional analyses

To experimentally assess the function of the p.C30R-mutant TYRP1 protein, we evaluated an ability of the mutant cDNA to produce melanin in melanocytes, melan-*b* [12,13], which were established from the OCA3 model mice, Brown (*Tyrp1*^{b}/*Tyrp1*^{b}) [14], as previously described. Briefly, we first established six independent transformants using melan-*b* melanocytes with pIREShyg3-*TYRP1* wild-type, pIREShyg3-*TYRP1* mutant-p.C30R, or pIREShyg3 alone. Then, expression of *TYRP1* mRNA in each of the six clones was confirmed with RT-PCR (data not shown). Furthermore, we quantified the amount of the mRNA by real-time quantitative RT-PCR (RQ-RCR). As shown in Fig. 2A, all of stable transformants with wild-type and mutant cDNA expressed *TYRP1* mRNA, although the expression level varied in the transformants.

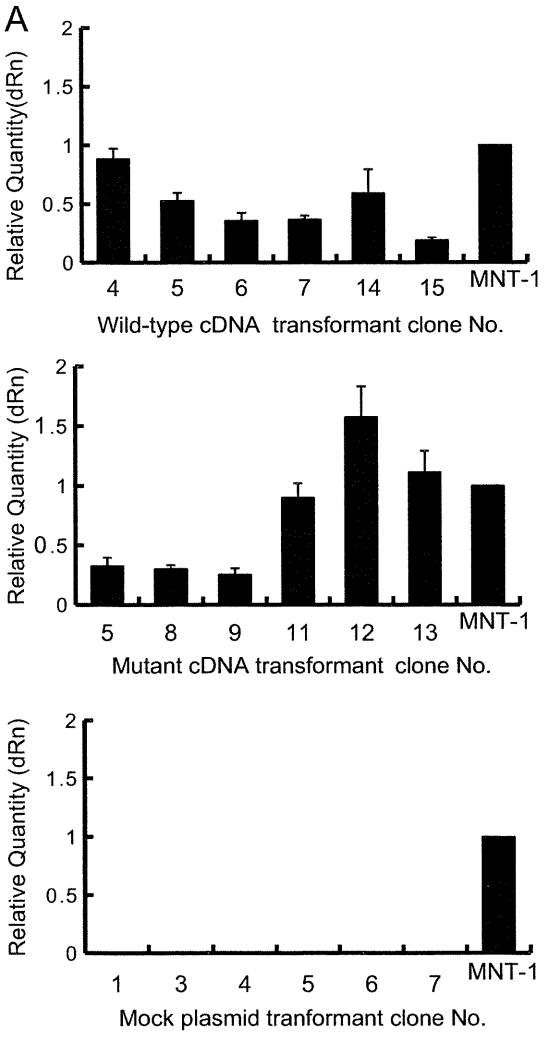

Fig. 2. (A) Expression of *TYRP1* mRNA in each of the clones. Data derived from real-time quantitative RT-PCR is expressed as mean ± S.D. of three independent experiments performed in triplicate. Total RNAs were extracted from the each clones with wild-type cDNA (upper lanes), mutant cDNA(middle lanes), and mock plasmid (bottom lanes). MNT-1 melanoma cells were used as a control, and the expression level of *TYRP1* mRNA in MNT-1 cells was defined as relative quantity 1. (B) Differential complementation of hypopigmentation of melan-*b* cells after transfection with normal and mutant human *TYRP1* cDNAs. Melanin was observed using bright-field microscopy (upper) and phase-contrast (lower) was used to examine cells. (a and b) Mock-transfection without added DNA; (c and d) p.C30R mutant; (e and f) wild-type human *TYRP1* cDNA. The transfected cells with the wild-type cDNA showed some visible pigmentation. In contrast, the cells with the p.C30R mutant cDNA had very little melanin. (C) Melanin content of melan-*b* transfected with different cDNAs. Means ± SEM were calculated using six independent clones from each treatment. Transfected cells were compared to mock-transfected cells. **A significant increase in melanin content was observed only after transfection with the wild-type sequence ($p < 0.001$, Student's *t*-test).

Over-expression of wild-type human TYRP1 protein in melan-*b* cells restored melanin production, while the transformants with the pIREShyg3-*TYRP1* mutant-p.C30R failed (Fig. 2B). These subjective assessments were substantiated by melanin content. Cells transfected with wild-type cDNA contained more than 1.7-fold the melanin found in mock-transfected cells, while the melanin levels in cells transfected with p.C30R-mutant cDNA were similar to those in mock-transfected cells (Fig. 2C).

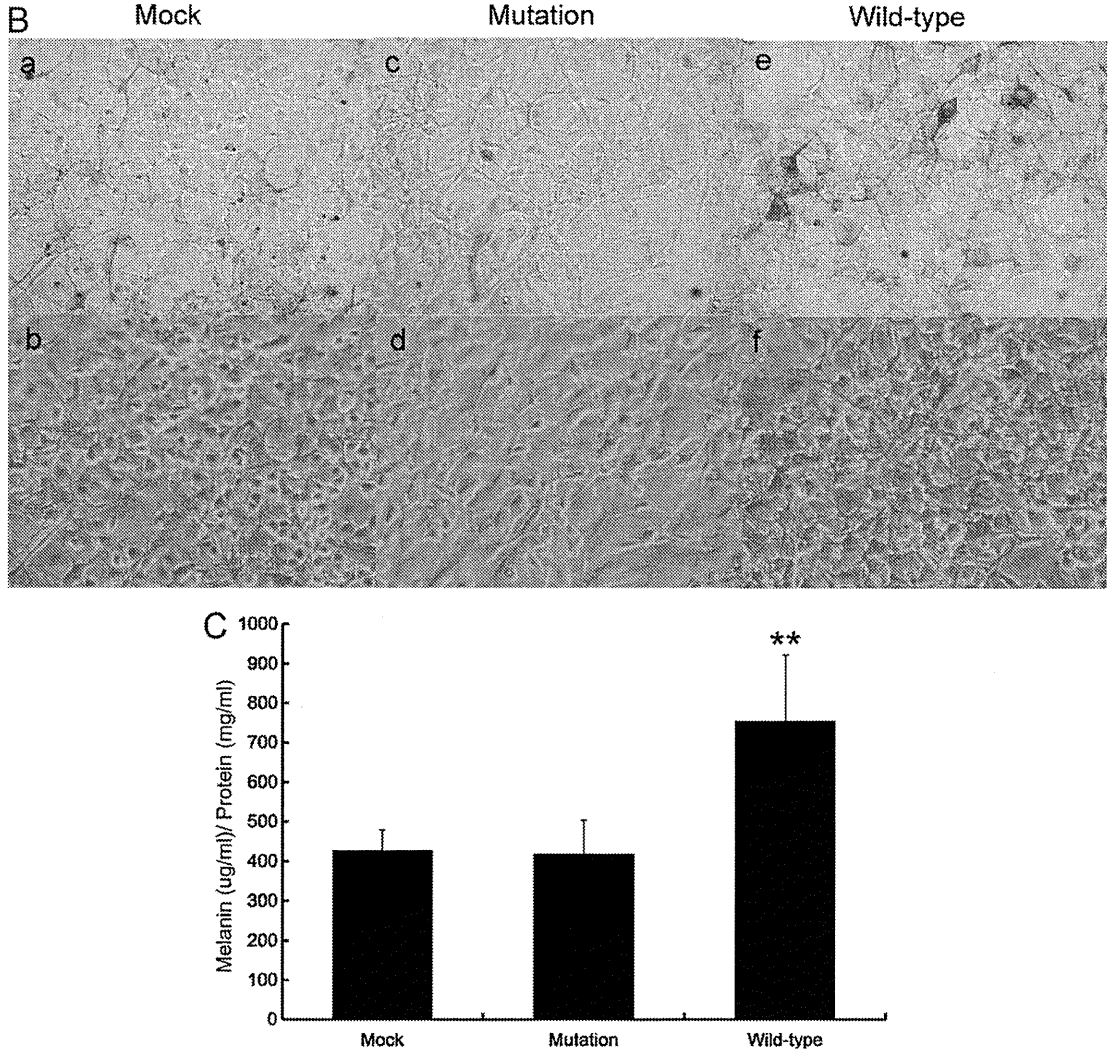

Fig. 2. (*Continued*).

3.3. Protein analyses

The protein expression in the transformants was also investigated with Western blotting. The result revealed that the transformants with wild type certainly expressed the TYRP1 protein. We unexpectedly failed to find the mutant TYRP1 protein in the transformants with mutant cDNA as well as mock transfection cells (Fig. 3), although the expression of mRNA has been confirmed in the transformants with mutant cDNA, especially much expression of mRNA in mutant clone Nos. 11, 12 and 13. We did Western blotting again to confirm the result of the protein expression using another polyclonal antibody raised against a full-length human TYRP1 protein, however, the result was similar to Fig. 3.

Then, we analyzed expression and intracellular localization of the wild-type and the mutant TYRP1 proteins in the individual stable transformant cells with immunofluorescence method, especially in order to detect a few cells expressing the mutant protein and where it would accumulate in the cells. The wild-type protein was easy to find the specimen, because almost 70% of the cells contained the TYRP1 protein, which localized mostly in peripheral area of the cells (Fig. 4A). On the other hand, it was very hard to find the cells expressing the mutant protein in the specimen, because the positive ratio was less than 0.1%. In the positive cells, the mutant protein localized around nucleus and not in peripheral area (Fig. 4B).

Finally, we analyzed a relationship between the expression level of TYRP1 protein and the amount of melanogenesis in the transformants with pIREShyg3-*TYRP1* wild-type. As shown in Fig. 5, no clear correlation between them was found. The wild type clones Nos. 4 and 6, which expressed relatively much TYRP1 (Fig. 3), however, melanogenesis in both of the cells were not promoted (Fig. 5). While the amount of TYRP1 protein was relatively less in clone No. 14, melanin production was relatively much in those cells.

4. Discussion

OCA3 is a rare form worldwide, especially in East Asian area. So far, only two Chinese patients have been recently reported [10]. We had an opportunity to diagnose genetically a Japanese girl with albinism type 3 who showed an apparent clinical tyrosinase-positive OCA. The result revealed that she was a heterozygote with two novel mutations, c.88T > C, p.C30R and c.1100delG, p.G367fsX384 in the *TYRP1* gene. The former missense mutation, p.C30R, involves a conserved amino acid residue since it is known to be present among all species carrying *TYRP1* ortholog, including the chimpanzee, pig, horse, dog, mouse, cow, chicken, zebra fish, platypus, axolotl, and frog. This data indicated that cysteine

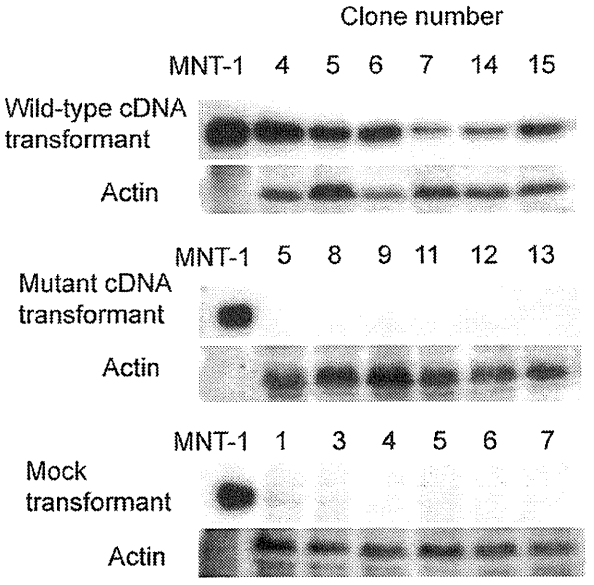

Fig. 3. Western blot analysis of TYRP1 in each of the clones. Lysate was extracted from stable transformants, and 5 μg protein of the each clones with wild-type cDNA (upper lanes), mutant cDNA (middle lanes), and mock plasmid (bottom lanes) was separated by SDS-PAGE. The lysate from MNT-1 melanoma cells was used as a positive control. The reason why the band of actin in the MNT-1cells was so faint was that only 0.5 μg protein was applied on the gel, because MNT-1 cells contained much TYRP1 protein. The protein expression of TYRP1 was confirmed in the transformants with wild-type cDNA, but not detected in those with mutant cDNA or those with mock plasmid.

residue at codon 30 might be functionally important. The latter mutation with one nucleotide deletion should be pathologic, because a termination codon appeared after 16-amino acid sequence resulted in a truncated peptide. Deletion G at codon 367 is just before position of codon 368 in which the first mutation in the *TYRP1* gene was detected by Boissy et al. [2]. These positions might be hot spot for the deletion mutation.

The clinical feature of OCA3 has been considered as rather mild, and in non-African patients, reddish hair color has been reported [9]. Our patient had blond hair, and lighter skin with a small Mongolian spot, and also presented possible tanning ability and no nystagmus. These symptoms indicated mild phenotype of OCA, supporting the previous reports.

We investigated ability of the wild-type versus the mutant polypeptide to produce melanin in melanocytes (*b* cells) obtained from the OCA3 mouse. The melanin levels in cells transfected with p.C30R mutant cDNA were similar to those in mock-transfected cells (Fig. 2C). Therefore, the p.C30R-mutant cDNA was functionally incapable of melanin synthesis and should be pathologic thus causing albinism.

Western blotting showed that the mutant TYRP1 protein in the transformants with p.C30R mutant cDNA was not detected as well as mock transfection cells (Fig. 3) in spite of much expression of mRNA in mutant clones (Fig. 2A). The p.C30 residue is located close to the membrane localization signal peptide region. Due to the charge, size and hydrophilic properties, the amino acids, cysteine at position 30, was predicted to disrupt the topological structure of the protein, which could result in protein misfolding. And also, the result of the intracellular localization of the mutant TYRP1 protein in the stable transformant cells revealed that the mutant protein localized around nucleus and not in peripheral area (Fig. 4B), supporting the disruption of membrane traffic. These results suggested that the mutant protein might be degraded soon just after its synthesis because normal transport of the mutant protein to melanosomes might be disturbed.

There was no clear correlation between amount of mRNA of *TYRP1* and melanogenesis in the transformants, indicating that TYRP1 protein did not regulate the level of melanogenesis as a rate limiting factor. This fact might be one of the reasons why patients with OCA3 reveal mild phenotypes.

In conclusion, we identified novel mutations of the *TYRP1* gene, c.88T > C, p.C30R and c.1100delG, p.G367fsX384, in a Japanese girl. This study confirms that the parents were carrier of the mutations, that it is an autosomal recessive inheritance and that the recurrence rate for this couple to have a child with albinism (OCA3) is 25%. This is the first report of the occurrence of OCA3 in Japanese population.

Funding sources

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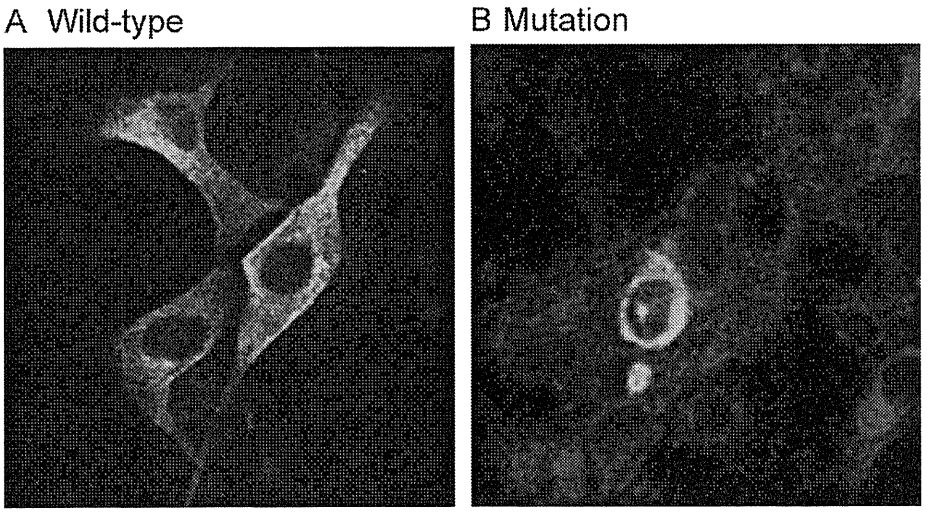

Fig. 4. Immunofluorescence analysis of TYRP1 protein intracellular localization in the stable transformants with the wild-type (wild-type clone No. 4) (A) and the mutant cDNA (mutant clone No. 12) (B). The cells were grown on glass coverslips, fixed and labeled with a mouse monoclonal anti-TYRP1 antibody. The wild-type protein was located mostly in peripheral area of the cells. The mutant protein was expressed only in less than 0.1% cells among the specimen and located around nucleus and not in peripheral area of the cells.

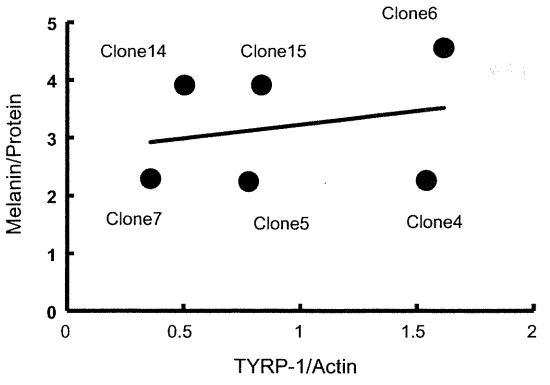

Fig. 5. Assessment of relationship between the amount TYRP1 protein and melanogenesis. The amount of TYRP1 protein shown in Fig. 3 was quantified by CS Analyzer ver2.0 for Windows (ATTO, Japan), and normalized with the amount of actin protein as a inner control. No correlation was found in the expression and the melanogenesis.

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Although most of the malignant tumors in chronic burn scars are SCC, other types of malignancies such as BCC, MM and sarcomas can also be seen rarely. The cause of the low incidence of sarcomas compared to carcinomas in chronic burn scars is speculated as the relatively deep position of mesenchymal cells of dermal or subcutaneous tissue that is less vulnerable to trauma and undergoes less tissue regeneration than the epidermis.[9]

Atypical fibroxanthoma may mimic spindle cell SCC, MM, leiomyosarcoma and malignant fibrous histiocytoma on histological examination.[3] Some of the previously published MFH cases may eventually be examples of AFX. But the lack of a specific positive immunophenotypic marker at that time may have prevented proper diagnosis. CD10 positivity seems to be a valuable adjunct to the current antibody battery for immunophenotyping.[2,10] Tumor cells in our case showed diffuse and strong positivity for CD10, while negative results for antibodies pointing to an epithelial, melanocytic or smooth muscle phenotype permitted us to rule out with certainty the potential mimics mentioned above.

Wide surgical excision or Mohs micrographic surgery were suggested for the treatment of AFX. Follow up for a number of years is recommended for the possibility of local recurrence or metastasis.

In conclusion, the represented case had a very uncommon complication of burn injury and this situation emphasized the importance of appropriate primary treatment of the burn. Obtaining a stable covering of the burn wound either by graft or flap coverage and sun protection of these areas are the best prophylaxis for preventing of tumors originating from burn scars. Careful observation of the chronic ulcers and lesions on the burn scars are also essential for early detection of tumors originating from burn scars.

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Case of subcutaneous lobular capillary hemangioma

Dear Editor,

Lobular capillary hemangioma (LCH), also called granuloma pyogenicum (GP), is a common reactive neoplasm arising on the mucosa and skin. It usually occurs as a polypoid or sessile nodule with rapid growth, and surface erosions are common. Its

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histological feature is lobular arrangement of numerous capillaries in the upper dermis with stromal edema and inflammatory infiltration.[1] Some clinical research has shown that the cutaneous lesions show a slight predilection for males[2] and are prone to occur by even minor trauma or irritation. LCH lesions arising on the mucosa are twice as likely to occur in females as in males;[3] therefore, sex hormones have been suggested as etiological factors in LCH development.[4] LCH can arise in the subcutis, though it is rare.[5] Subcutaneous LCH usually occurs as a well-circumscribed, elastic, hard tumor, sometimes together with slight tenderness. Because clinical findings are unspecific, it is difficult to distinguish clinically from other subcutaneous tumors. Here we show a case of subcutaneous LCH, discuss the necessity of recognition of this entity, and review the Japanese published work on subcutaneous LCH.

A 76-year-old woman presented to our division for a subcutaneous nodule on her fourth finger. The distal portion of her left third and fourth finger was amputated as the result of an accident in her 20s. She noticed the nodule 8 years before presentation and it had enlarged gradually. There was no obvious trauma or irritation before the appearance of the tumor. At first presentation, an elastic, hard, subcutaneous tumor 11 mm in diameter was seen on the dorsal aspect of her left fourth finger (Fig. 1). The tumor was not adhesive with underlying tissue. Neither fluid nor content was obtained by aspiration using a sterile needle. Tumor resection was performed under local anesthesia. During the surgical procedure, the tumor did not adhere to the adjacent connective tissue and was able to be removed relatively easily. There were some fine feeding arteries around the tumor. Histologically, the nodule was well circumscribed, located in the deeper dermis to subcutis, and surrounded by thickened fibrous stroma. Inside the nodule, the deeper portion was markedly edematous and filled with eosinophilic partially hyalinized stroma (Fig. 2a).

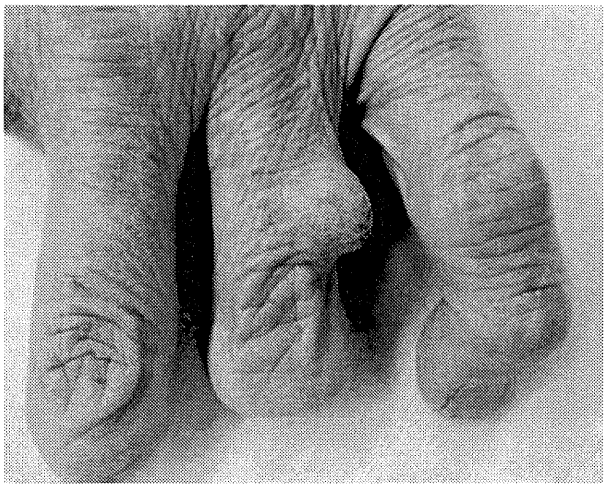

Figure 1. Clinical finding of a subcutaneous tumor on the left fourth finger.

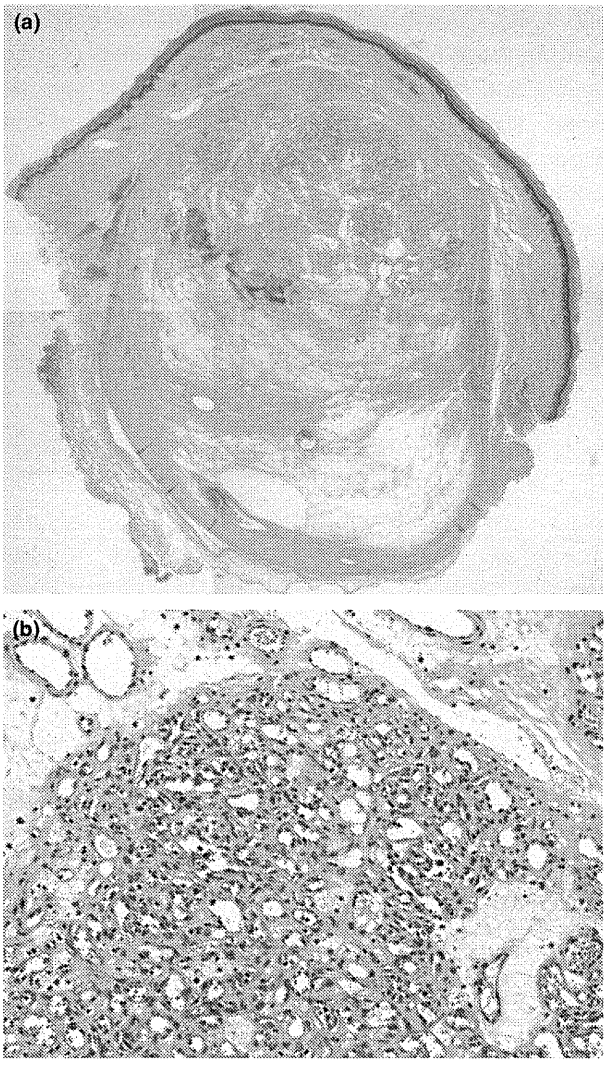

Figure 2. (a) Well-circumscribed tumor surrounded by fibrous thickened connective tissue containing both angiomatous and edematous components is seen in the deeper dermis to subcutis (hematoxylin–eosin [HE], original magnification ×10). (b) Angiomatous component is comprised of numerous congestive and diluted capillaries forming a lobular architecture. No cellular atypia is observed (HE, original magnification ×200).

A lobular angiomatous component was seen in the upper portion, containing numerous capillaries comprised of flattened endothelial cells (Fig. 2b). No cellular atypia was evident in the endothelial cells. Above the nodule, dilated capillaries were seen. Capillary endothelial cells stained positive for CD31 and CD34 (data not shown) by immunohistochemistry. Based on these findings, we diagnosed this case as a subcutaneous LCH. Three years after surgical removal, no local recurrence has been observed.

In 1980, Cooper and Mills[3] reviewed five cases of subcutaneous LCH and described the details of their clinical and histological findings. According to their report, subcutaneous LCH is likely to arise on the upper extremities as an asymptomatic or slightly tender nodule. Histologically, it is characterized by a partially or completely encapsulated subcutaneous tumor. The inside of the tumor consists of angiomatous lobules separated by fibromyxoid stroma. Because this lobular angiomatous architecture is identical with GP with a polypoid or sessile appearance, the investigators suggested LCH would be a more appropriate term than GP. Harris *et al.*[2] reviewed 63 759 dermatopathology reports of a private dermatopathology laboratory in the USA and found 325 cases of LCH. Of these, subcutaneous lesions were observed in only two cases. As this time, we reviewed the Japanese published work on subcutaneous LCH. While some lesions were located in the deeper dermis, we considered them to be essentially the same entity and have shown them together with the depth of the lesion in Table 1.[6–15] Age at the time of consultation ranged 6–84 years (mean age, 36 years), and female cases were more than twice as common as male cases (male : female, 5:11). The extremities and the face were predilection sites, and the tumor size was relatively small, less than 15 mm in diameter. None of the cases noticed antecedent trauma or irritation (not described in cases 2 and 3). Surgical removal was performed in all cases, and there was no recurrence. It is interesting that subcutaneous LCH occurs predominantly in females. The

Table 1. Summary of subcutaneous lobular capillary hemangioma reported in the Japanese published work

Case no.	Age (years)	Sex	Site	Size	Time until first consultation	Depth of location	References
1	47	F	Flexor aspect of right elbow	Rice-sized	1 month	Deeper dermis to subcutis	6
2	21	F	Right upper eyelid	7 mm × 7 mm	6 months	Subcutis	7
3	6	F	Right shoulder	12 × 13 mm	2 years	Deeper dermis to subcutis	7
4	60	F	Dorsal aspect of right third finger	Bean-sized	6 months	Deeper dermis	8
5	40	F	Extensor aspect of right elbow	5 mm × 6 mm	6 months	Deeper dermis to subcutis	9
6	14	M	Right cheek	7 mm × 3 mm	2 months	Deeper dermis	10
7	45	F	Chest	Finger-tip sized	1 month	Subcutis	11
8	25	M	Chest	–[†]	10 months	Deeper dermis	12
9	21	M	Forehead	Bean-sized	2 months	Deeper dermis to subcutis	13
10	8	M	Chest	Bean-sized	8 years[‡]	Deeper dermis	13
11	11	F	Chest	14 mm × 11 mm	3 months	Subcutis	14
12	30	F	Extensor aspect of left second finger	2 mm × 2 mm	2–3 years	Deeper dermis	15
13	52	F	Right palm	7 mm × 10 mm	Several years	Deeper dermis to subcutis	15
14	84	F	Flexor aspect of left second finger	12 mm × 12 mm	1 month	Deeper dermis to subcutis	15
15	41	M	Flexor aspect of left thumb	5 mm × 5 mm	2 months	Deeper dermis to subcutis	15
16	76	F	Dorsal aspect of left fourth finger	11 mm × 11 mm	8 years	Deeper dermis to subcutis	Our case

[†]This lesion was discovered histologically from the biopsy of an asymptomatic region adjacent to a typical superficial lobular capillary hemangioma.
[‡]The tumor was noticed from the time of delivery.

reason for this is uncertain, and additional investigations should be undertaken to clarify it.

Fortna and Junkins-Hopkins[16] have reported a case of locally aggressive subcutaneous LCH that required the excision of underlying skeletal muscle. In our case, the tumor was well demarcated clinically and not adhesive to adjacent tissue. Histological findings revealed that the tumor was encapsulated by fibrous connective tissue. Fortunately, there has been no finding of local recurrence, similar to the other Japanese cases. We should recognize the clinical entity of subcutaneous LCH and the existence of locally aggressive cases, though they may be rare. We should also take subcutaneous LCH into consideration as a differential diagnosis of a subcutaneous nodule, especially one that occurs on the extremities and face.

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Cranial fasciitis resembling infantile fibrosarcoma differentiated by genetic assay

Dear Editor,

Cranial fasciitis is a variant of nodular fasciitis, first described by Lauer and Enzinger in 1980.[1] It forms a solitary tumorous lesion in the head and neck region almost exclusively in children, especially in infants.[2] Cranial fasciitis is a very rare condition. According to

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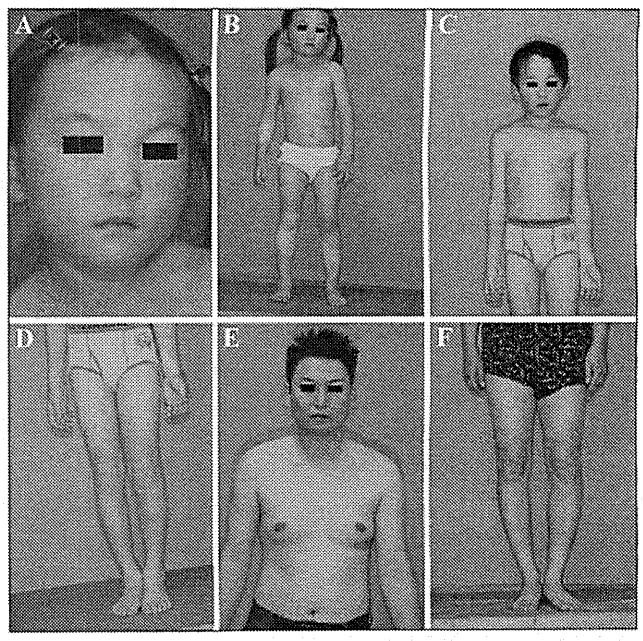

Figure 1. Clinical assessment of the present family: **A, B)** the 4-year-old girl; C, D) the 7-year-old brother of the proband; **E, F)** the 35-year-old father of the proband.

Two children with a mild or moderate piebaldism phenotype and a father without leukoderma in a family with the same recurrent missense mutation in the kinase domain of *KIT*

Piebaldism is a dominantly inherited disorder characterized by a white forelock and leukoderma on the frontal scalp, forehead, ventral trunk and extremities. Some patients have café-au-lait spots, which may be confused with neurofibromatosis type 1. Piebaldism is caused by a mutation of the *KIT* gene encoding the transmembrane receptor tyrosine kinase (TK), c-kit. Leukoderma has been thought to involve complete penetrance of a mutation in *KIT* [1-5].

A 4-year-old Japanese girl presented with aberrant skin color. Physical examination revealed (i) no poliosis on the frontal scalp, (ii) leukoderma on the forehead, right elbow, right knee, right foreleg, and left foreleg, and (iii) café-au-lait spots on the normally pigmented skin *(figures 1A, B)*. The patient had symmetrical dark brownish irises and no hearing loss. The proband's 7-year-old brother had patchy leukoderma on the left wrist and café-au-lait spots on the normally pigmented skin *(figures 1C, D)*. The leukoderma of the proband and the brother was congenital and stable in its relative size and distribution. The proband's 35-year-old father had no poliosis or leukoderma on the body, but had café-au-lait spots on the normally pigmented skin *(figures 1E, F)*. The father had no history of leukoderma on any portion of the body. The mother had no history of pigmentary disorders.

The proband's parents provided written informed consent allowing their family to participate in the study according to a protocol approved by the Ethics Committee of Yamagata University School of Medicine and the Genetic Ethics Committee of Kinki University Faculty of Medicine. The protocol was conducted according to the Declaration of Helsinki Principles. All exons and flanking intron sequences of the *KIT* gene were amplified by polymerase chain reaction (PCR) as described [2]. PCR products of exon 11 in the *KIT* gene from the family members and normal healthy volunteers were studied by single-strand conformation polymorphism (SSCP) as described [6].

Sequence analysis of the *KIT* gene from the proband revealed a missense substitution in exon 11. The T-to-C transition at nucleotide position 1750 resulted in a shift from a phenylalanine residue to a leucine at amino acid position 584 in the tyrosine kinase domain. Direct sequencing revealed the substitution in the brother and the father. SSCP showed the same aberrant bands in the proband, the brother, and the father. However, they were not present in the mother or any of the 103 Japanese controls, suggesting that the substitution is not a polymorphism but a pathologic mutation. The missense mutation p.Phe584Leu (TTT→TTG) has been reported previously [1], but the missense mutation p.Phe584Leu (TTT→CTT) had not been described.

In piebaldism, leukoderma is thought to involve complete penetrance of a mutation in *KIT*, and a genotype-phenotype correlation in *KIT* is commonly present. Reports of the results of mutation analyses of the *KIT* gene in families with piebaldism indicate the complete penetrance of a mutation [1-5]. Piebald patients having a missense mutation in the TK domain of the *KIT* gene usually show a severe phenotype.

We described two children with milder than expected piebaldism and a father with no leukoderma in a family with the same recurrent mutation p.Phe584Leu in the TK domain of the *KIT* gene. Our study indicates that a main feature of leukoderma in piebaldism may be incomplete penetrance of a mutation in *KIT*. ■

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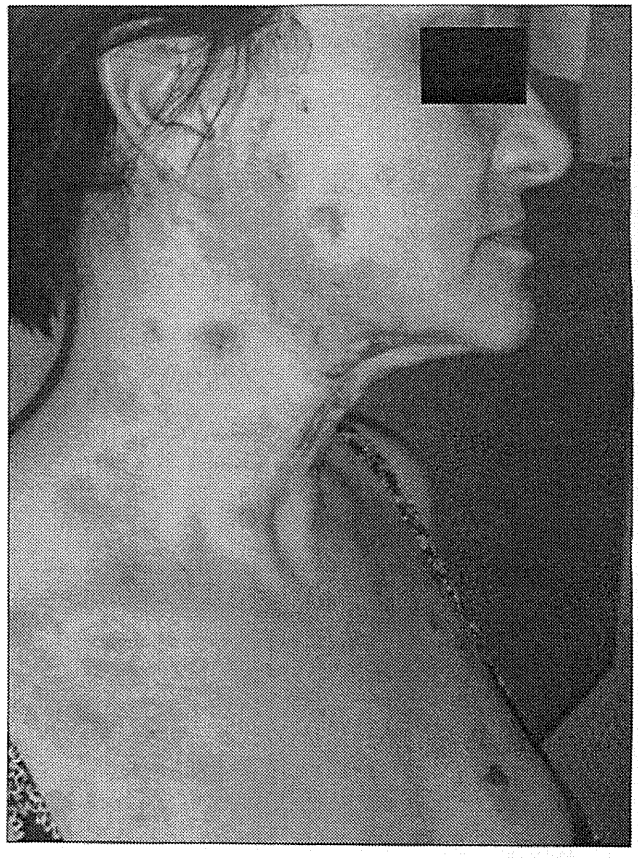

Figure 1. Bullous target-like lesions in the auditory canals, along the sides of the neck and trunk.

Systemic allergic contact dermatitis to black cumin essential oil expressing as generalized erythema multiforme

The seeds of *Nigella sativa*, more commonly known as black cumin, contain 0.4%-2.4% essential oil [1]. In addition to the increasing scientific attention paid to its antioxidant and anticancer activities [2], black cumin essential oil (BCEO) also has immunogenic properties. We report a case of severe systemic allergic contact hypersensitivity induced by both local and oral use of BCEO.

A 56-year-old woman, with a history of allergic contact dermatitis to nickel, presented with a 2-day history of severe bullous target-like lesions, compatible with an erythema multiforme, without Nikolski's sign, nor mucosal involvement *(figure 1)*. Histopathological characteristics of a lesion included a lymphocytic infiltrate at the dermal-epidermal junction, dermal edema, basal vacuolization and keratinocyte necrosis. The eruption began in the auditory canals, spreading to the sides of the neck, trunk and back, and cleared within 1 month with systemic corticosteroid treatment. She had been treated 15 days before the eruption for mental fatigue, with daily ingestion of 2 capsules of BCEO (containing 500 mg of organic Nigella sativa oil and 7.5 mg of vitamin E) and application in the auditory canals of the same oil. No recent herpetic eruption or other drug intake was reported.

Skin tests were done with the form of BCEO used by the patient, i.e. pure BCEO. Patch tests, performed 4 months after resolution, revealed positivity +++ at 72 hours (according to ICDRG criteria), a patch stayed negative with vitamin E. Histological analysis of the positive skin test site revealed abundant spongiosis and lymphocytic exocytosis, resulting in the formation of epidermal vesicles, associated with an infiltrate of lymphocytes and eosinophils in the upper dermis, suggesting a delayed-type hypersensitivity reaction. The ROAT was positive (erythema, infiltration and diffuse papules) after 72 hours of twice daily applications, confirming that the patient was strongly allergic to BCEO. Oral provocation test with BCEO was not performed for ethical reasons and a definite elimination of this product was recommended to the patient.

Herbal medicines are often perceived by the general public as a "soft" alternative to Western Medicine, but the use of these substances can be risky since they can induce both irritant and allergic contact dermatitis [3]. In two previously reported cases, BCEO-induced allergic contact dermatitis was limited to the site of skin contact [4, 5]. We describe here the first case of BCEO-induced systemic allergic contact dermatitis, presenting as generalized erythema multiforme, after the use of both topical and oral BCEO. We hypothesize that the patient was sensitized though the cutaneous route and that the oral intake

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CASE REPORT

Dystrophic epidermolysis bullosa pruriginosa of elderly onset

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ABSTRACT

A 71-year-old man with no family history of skin diseases presented with a 4 month history of recalcitrant pruritic papules and nodules on the lower extremities. He had prurigo-like eruptions with tense bullae on the extensor aspect of his lower extremities with multiple adjacent milia. Toenail dystrophy was observed. Mucous membranes were not affected. Skin biopsy from the shin showed a subepidermal blister with milium. Electron microscopy from lesional and perilesional skin of the leg showed scanty, hypoplastic anchoring fibrils. We detected a heterozygous mutation in the *COL7A1* gene, a G-to-A substitution in exon 87 (c.6859G>A; p.Gly2287Arg). Thus, the clinicopathological and molecular findings supported a diagnosis of dystrophic epidermolysis bullosa pruriginosa. Assessment of other relatives was not feasible. To the best of our knowledge, this is the oldest clinical onset of this unusual variant of dystrophic epidermolysis bullosa reported to date. Why the onset of skin fragility should have occurred so late is not known, but the case serves as a reminder that this particular mechanobullous disease can have a delayed presentation.

Key words: *COL7A1*, dystrophic epidermolysis bullosa pruriginosa, elderly onset, glycine substitution, prednisolone.

INTRODUCTION

Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) (Online Mendelian Inheritance in Man 604129) is a rare variant of DEB characterized by prominent pruritus, trauma-induced blistering, nail dystrophy, and pruritic prurigo-like and/or lichenoid lesions with milia. McGrath *et al.* initially reported eight cases of DEB-Pr and defined the entity.[1] Although autosomal dominant, recessive and sporadic inheritance patterns have been reported, most cases are dominant. As for all forms of DEB, the molecular pathology involves mutations in the type VII collagen gene, *COL7A1* (NM_000094.3), which encodes a 2944 amino acid protein, the main component of anchoring fibrils.[2] In many cases, the clinical manifestations of DEB-Pr will be evident within the first decade or even in infancy; however, in some cases they may be delayed until the third decade or even until patients are in their 50s.[3,4]

Herein, we report a case of sporadic DEB-Pr of elderly onset. To the best of our knowledge, our case is the oldest clinical onset of DEB-Pr reported to date. Although the reason for the variability in the clinical onset of this disease has not been elucidated yet, the emergence of this disease in an elderly subject has important implications for the differential diagnosis of subepidermal blistering diseases in such patients.

CASE REPORT

A 71-year-old man presented with a 4-month history of pruritic erythematous papules on his lower

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extremities. He was treated with a topical steroid and oral antihistamines; however, the number of pruritic papules and nodules gradually increased. He had no family history of skin disease and his parents were not consanguineous. Neither his parents, siblings nor two sons (42 and 49 years old) had skin complaints. He suffered from mild diabetes mellitus and benign prostate hyperplasia and had been taking anti-diabetic and herbal medicine for 1 and 2 years, respectively. Although the topical steroid and oral antihistamines provided some symptom relief, scratching and trauma induced tense bullae and pruritic prurigo-like lesions that gradually appeared mainly on the lower extremities (Fig. 1a). Multiple milia developed following blister re-epithelialization (Fig. 1b,c). He had toenail dystrophy on all toes (Fig. 1d), while his fingernails were intact. Although he had noticed toenail dystrophy in his early teens, it did not cause any inconvenience to him and he had sought no treatment for this. The mucous membranes were not affected. A skin biopsy from the leg revealed dermal–epidermal blister formation with mild lymphocyte infiltration and some eosinophil infiltration in the upper dermis, along with some milia

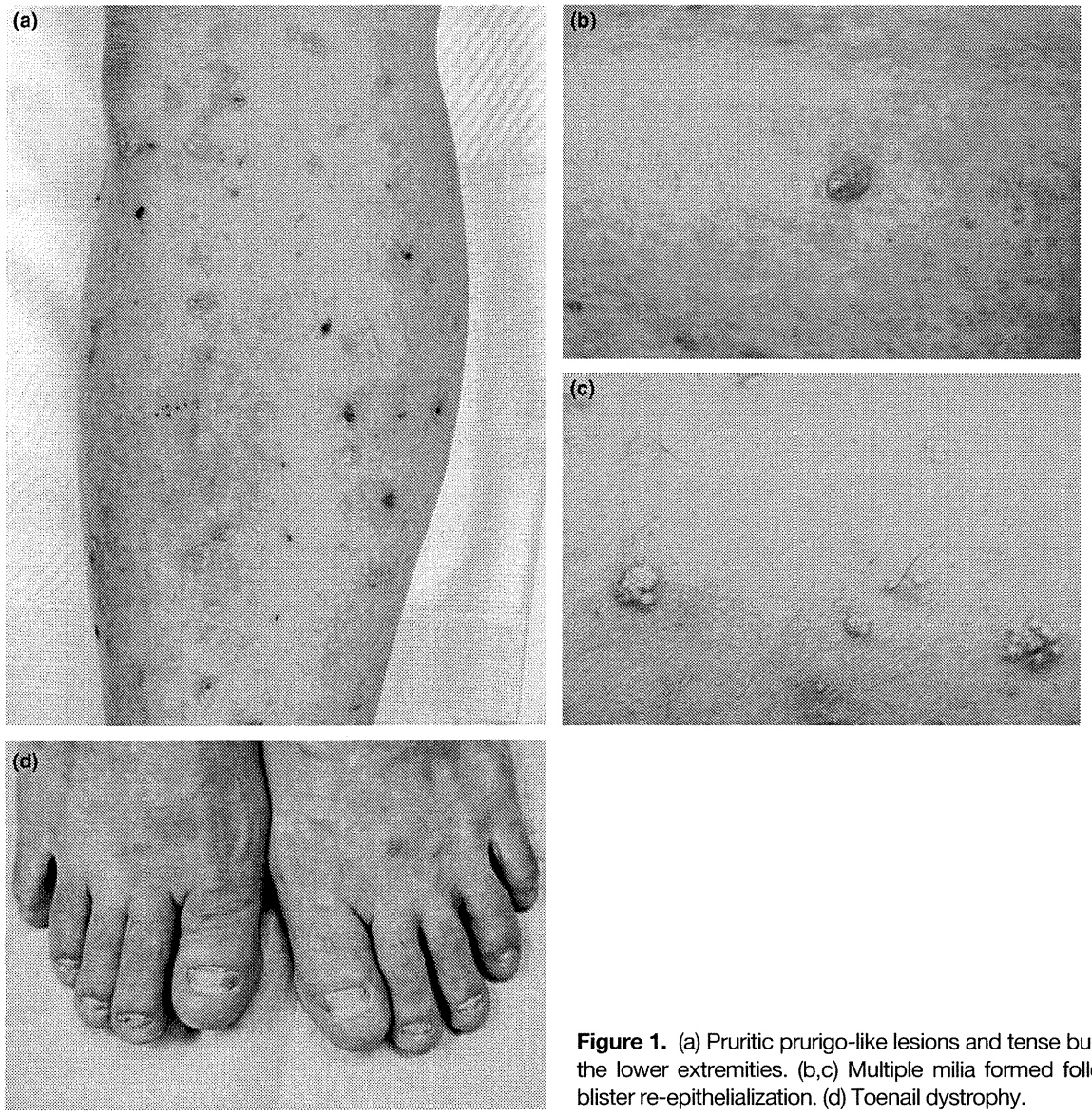

Figure 1. (a) Pruritic prurigo-like lesions and tense bullae of the lower extremities. (b,c) Multiple milia formed following blister re-epithelialization. (d) Toenail dystrophy.

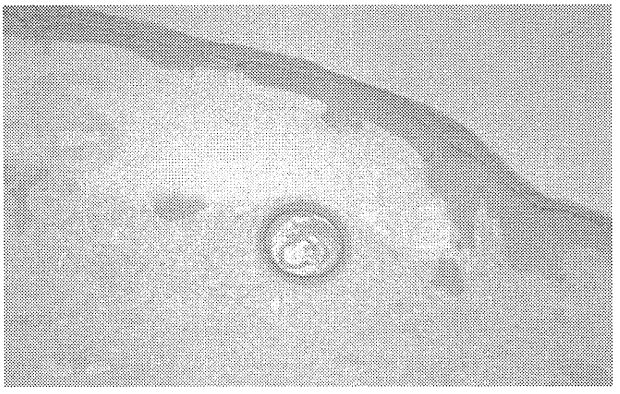

Figure 2. Histopathology of a skin biopsy from the leg. Dermal–epidermal blister formation with mild lymphocytes and partial eosinophil infiltration in the upper dermis and milia (hematoxylin–eosin, original magnification ×100).

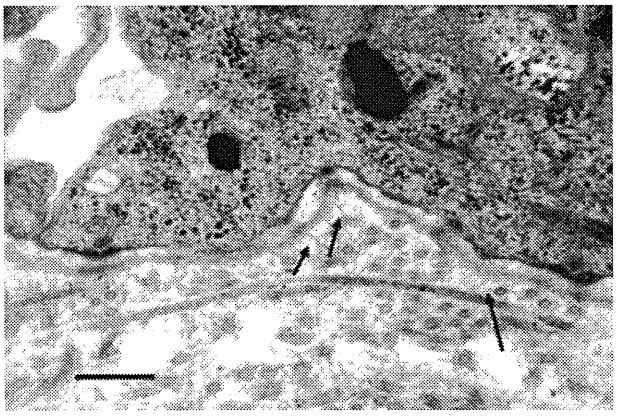

Figure 3. Electron microscopic findings from lesional and perilesional skin of the leg. Anchoring fibrils are scanty and hypoplastic (arrows) (original magnification ×40 000; scale bar, 0.5 μm).

(Fig. 2). Direct immunofluorescence (DIF) showed no immunoglobulin (Ig) or complement deposition at the basement membrane zone. Blood cell count and liver and renal function were all within normal limits. Serum IgE, ferritin and thyroid function were also within normal limits. Immunoblotting studies showed that the patient's serum did not react with any antigen. There were no abnormal findings on chest X-ray, computed tomography, electrocardiogram or upper and lower gastrointestinal endoscopy. An underlying disorder that might cause recalcitrant pruritus was not detected. To rule out drug-induced eruption, all of the drugs he was taking were discontinued and replaced with alternative drugs; however, his symptoms did not improve.

Because the results were not consistent with an autoimmune blistering disease, we examined the skin in more detail. Electron microscopy of lesional and perilesional skin of the leg revealed that anchoring fibrils were scanty and hypoplastic (Fig. 3). After informed consent, genomic DNA was extracted from his peripheral blood samples. Amplified DNA revealed a heterozygous mutation in the *COL7A1* gene, a G-to-A substitution in exon 87 (c.6859G>A; p.Gly2287Arg) (Fig. 4). This mutation has been previously reported by Shimizu *et al.*[5] Considering these findings, we diagnosed the patient with DEB-Pr. Oral prednisolone (PSL) 10 mg/day and 4,4-diamino-diphenyl-sulfone (DDS) 75 mg/day were not effective. Subsequently, PSL 30 mg/day was administrated and the pruritus became markedly

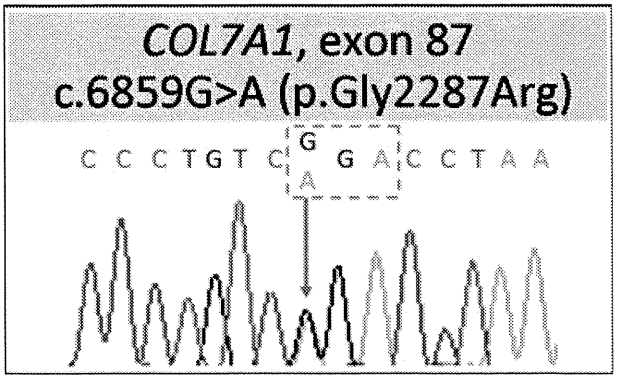

Figure 4. Amplified genomic DNA revealed a heterozygous mutation in the *COL7A1* gene, a G-to-A substitution in exon 87.

less, and no new blisters or prurigo-like lesions were seen. Nevertheless, as the blisters resolved, multiple milia ensued. The dose of PSL was tapered gradually although mild pruritus and a few blisters appeared on the shin when the PSL dose was reduced to 5 mg/day. Topical application of tacrolimus to the prurigo-like lesions also decreased his itching and was considered as effective as oral PSL for symptom control.

DISCUSSION

Dystrophic epidermolysis bullosa pruriginosa was first described by McGrath *et al.*[1] in 1994. It is a rare clinical variant of DEB, characterized by marked

Table 1. Summary of DEB-Pr patients with onset later than 20 years of age

Case No.	Age/Sex	Age at onset of DEB-Pr	Inheritance	Clinical features	Nail dystrophy	Mutation	Complications and past medical history	Treatment	Reference
1	72/M	71	Sporadic	Prurigo-like lesions and blisters with milia of bilateral legs	+	Exon 87 c.6859G>A (p.Gly2287Arg)	Diabetes and prostate hyperplasia	PSL 30 mg/day	Our case
2	34/F	29	Dominant	Pruritic, lichenoid plaques with milia	−	Exon 110 c.8137G>C (p.Gly2713Arg)	Not described	Not described	3
3	27/F	20	Dominant	Prurigo-like papules and milia of pretibial area	+	Exon73 c.6082G>A (p.Gly2028Arg)	Not described	Not described	7
4	44/F	39	Dominant	Pruritus, linear scratching lesions and hyperkeratotic, lichenoid lesions confluent into larger plaques on legs and feet	+	Exon 59 c.5264G>A (p.Gly1755Asp)	Not described	Not described	6
5	39/F	38	Dominant	Pruritus, nodular reddish prurigo-like lesions on left elbow and wrists	+	c.6900 + 4A>G	Not described	Not described	6
6	37/F	38	Dominant	Pruritus, linear scratching lesions and hyperkeratotic lichenoid lesions confluent into large gray-brown plaques on legs and feet	−	c.6900 + 2delTGAT	Not described	Not described	6
7	58/F	53	Recessive	Blistering, excoriated nodules and violaceous scars on lower legs, ankles and elbows	+	Exon 110 c.8206G>A (p.Glu2736Lys)	Diabetes and thyroid cancer	Not described	4
8	52/F	Twenties	Dominant	Intense pruritic blisters on lower legs and extensor surface of both arms	−	Exon 92 c.7097G>T (p.Gly2366Val)	Not described	Not described	8
9	52/F	25	Dominant	Pruritic blisters provoked by scratching on back, nape of the neck, elbows, and both shins	−	Exon 86 c.6752G>A (p.Gly2251Glu)	Subtotal thyroidectomy	Not described	9
10	29/F	27	Sporadic	Multiple lichenified violaceous papules, linear scarring and crusts on extensor sides of feet, lower extremities and elbows	+	Exon 110 c.8137G>C p.Gly2713Arg	Healthy	Topical corticosteroids (not effective)	10

DEB-Pr, Dystrophic epidermolysis bullosa pruriginosa; PSL, prednisolone.

pruritus, trauma-induced blistering, especially on the extensor aspect of the leg, nail dystrophy, prurigo-like lesions and multiple milia. DEB is caused by mutations in the *COL7A1* gene encoding type VII collagen, resulting in a reduced number or disorganization of anchoring fibrils. In DEB-Pr, mainly glycine substitutions have been reported.[6] The onset of clinical symptoms of DEB-Pr is typically during the first decade or even in infancy; however, in some cases clinical onset may be delayed until later in life.[3,4] From these unique clinical features, various differential diagnoses may be considered, such as prurigo nodularis, lichen planus and dermatitis artefacta.

Initially, our patient had pruritic papules and nodules mainly on the leg, which were then followed by tense bullae. Milia formation was seen after the tense bullae re-epithelialized. Our differential diagnoses included pemphigoid nodularis, prurigo nodularis, epidermolysis bullosa acquisita, DEB-Pr and a drug-induced eruption. We ruled out autoimmune blistering disease due to the results of DIF and immunoblotting analysis. Prurigo nodularis usually does not form blisters or milia. Discontinuation of the patient's medication had no clinical impact. However, the combination of the clinical features, the decreased anchoring fibrils on electron microscopy and the detection of the *COL7A1* gene led to a diagnosis of DEB-Pr. Regrettably, we could not acquire informed consent for genetic analysis from his sons; this information would be valuable for knowing whether they might also be at risk for expressing the disorder.

A summary of patients with DEB-Pr with onset later than 20 years of age is shown in Table 1.[3,4,6–10] Of note, there is a female preponderance for all late onset DEB-Pr cases, aside from our patient; the reason for this is unclear. Although the factors responsible for the variability in the time of clinical onset of DEB-Pr have not been elucidated yet, the recognition of this disease having such a late onset adds to the differential diagnosis of subepidermal blistering in elderly subjects.

Dystrophic epidermolysis bullosa pruriginosa has a wide clinical spectrum. In different pedigrees, patients with the same glycine substitution mutation may show clinical heterogeneity.[11] With regard to the pruritus, some DEB-Pr patients have elevated serum IgE and/or atopy,[3,8] but other possible associations such as functional gene promoter polymorphisms in the matrix metalloproteinase-1 (*MMP-1*), which can degrade type VII collagen,[12] and loss-of-function mutations in filaggrin (*FLG*)[4] have not improved clinicopathological understanding of disease mechanisms in DEB-Pr.

Shimizu *et al.*[5] reported a DEB pedigree with the *COL7A1* mutation p.Gly2287Arg, the same mutation as in our case. Their patients showed only nail dystrophy restricted to the great toes and did not show any signs of skin fragility. They determined that this mutation may lead to a very mild phenotype of DEB that might be overlooked. Our patient noticed toenail dystrophy in his early teens, but it was approximately 60 years more before the pruritic eruptions and cutaneous manifestations emerged. Thus, it is likely that our patient has had lifelong dominant DEB, which for most of his life only manifested as nail dystrophy, similar to the cases reported by Shimizu *et al.*[5] However, with the development of pruritus and blistering, the diagnosis evolved to DEB-Pr. Our observations therefore have important implications for the accuracy of genotype-phenotype correlation in DEB and also highlight the potential significance of pruritus in patients with DEB.

The aims of treatment for DEB-Pr are to ease the pruritus and to suppress the scratching activity that leads to the formation of blisters and/or prurigo-like lesions; however, no universally successful treatment has been established. Recent studies have described the efficacy of topical tacrolimus (as we also observed in our patient), systemic cyclosporine and thalidomide.[13–15] McGrath *et al.*[1] reported that treatment with a systemic corticosteroid 10–30 mg/day up to 2 months did not appear to be effective, whereas in our case, oral PSL 30 mg/day was effective and improved the patient's symptoms. We therefore advocate use of topical tacrolimus and a higher dose PSL as potentially useful treatment options for DEB-Pr.

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