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Clinical and immunological profiles of 25 patients with pemphigoid gestationis

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Summary

Background Systematic study of pemphigoid gestationis (PG) has not been performed owing to its rarity.

Objectives To perform clinical and immunological analyses of 25 patients with PG.

Methods In addition to clinical and histopathological assessments, we performed immunofluorescence (IF), immunoblotting (IB) and enzyme-linked immunosorbent assays (ELISAs).

Results PG developed preferentially during the second or third trimester of pregnancy, with a mean age at onset of 30·5 years. Histopathology showed subepidermal blisters less frequently. Direct IF showed C3 deposition in the basement membrane zone (BMZ) in all patients, with rare reactivity with keratinocyte cell surfaces. Ninety-two per cent of patients showed circulating IgG anti-BMZ autoantibodies during indirect IF of either normal or 1 mol L^{-1} NaCl-split skin. Complement IF revealed linear C3 reactivity with the BMZ of normal skin in 68% of patients, and all patients had C3 reactivity on the epidermal side of 1 mol L^{-1} NaCl-split skin. IB and ELISA of the NC16a domain of BP180 recombinant protein was positive in 96% and 92% of patients, respectively, while only four patients had a positive ELISA for BP230. In IB tests, 28% of patients reacted with the C-terminal domain of BP180 and 20% reacted with leucocyte adhesion deficiency-1 protein. Multigravidae developed PG during a significantly ($P < 0·01$) earlier stage (21·1 weeks) of pregnancy than primigravidae (31·3 weeks).

Conclusions IB and ELISA of the NC16a domain of BP180 were shown to be sensitive and diagnostic methods in PG. Patients with PG rarely reacted with BP230, suggesting a different pathogenesis between PG and bullous pemphigoid. Multigravidae developed PG skin lesions significantly earlier in pregnancy than primigravidae.

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Conflicts of interest
None declared.

T.K., an associate professor, Department of Dermatology, St. Marianna University School of Medicine, participated in this study as a part-time associate professor, Department of Dermatology, Kurume University School of Medicine, and performed the statistical analyses.

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What's already known about this topic?

- Pemphigoid gestationis (PG) is a complement-related bullous pemphigoid associated with pregnancy.
- No systematic studies of PG have been performed owing to the rarity of this disease.

What does this study add?

- Immunoblotting and enzyme-linked immunosorbent assay of the NC16a domain of BP180 recombinant protein are sensitive and diagnostic methods in PG.
- Patients with PG rarely react with BP230.
- Multigravidae develop PG lesions significantly earlier in pregnancy than primigravidae.

Pemphigoid gestationis (PG), also called 'herpes gestationis', is a rare pregnancy-associated autoimmune blistering disease.[1] Skin lesions in PG usually start as pruritic urticarial plaques on the trunk and culminate in vesicular lesions.[2] Histopathology shows subepidermal blisters and eosinophilic infiltrates.[3] The major autoantigen is the 180-kDa protein BP180, although the 230-kDa protein BP230 has also been detected occasionally.[4–6] Major epitopes are located in the NC16a domain of BP180, but minor epitopes are present in other domains.[7–9] These features are almost the same as those of bullous pemphigoid (BP), indicating that PG is BP during pregnancy.[10]

It is unknown why PG occurs in young, pregnant women, while BP preferentially affects older people of both sexes; however, there are several clues: cross-reactivity at the basement membrane zone (BMZ) in skin, chorionic villi and amnion; production of BP180 in placenta;[11–13] and specific major histocompatibility complex molecules found in patients and their partners.[14] Thus, PG may develop through the production of autoantibodies to excessive BP180 in placenta under the specific genetic predisposition of couples.

PG commonly occurs during the second or third trimester of pregnancy.[15] While most cases spontaneously resolve after pregnancy, symptoms may continue into the postpartum period.[16] In general, PG symptoms become more serious during a subsequent pregnancy.[17]

Despite many sporadic case reports, there have only been a few systematic studies for PG. In this study, we report on various clinical and immunological features in 25 patients with PG, who were examined and diagnosed at Kurume University Hospital, Kurume, Japan. In addition, we statistically compare primi- and multigravidae.

Patients and methods

This study was a retrospective investigation of patients with PG, over a period of 17 years, and was approved by the ethical committee of Kurume University. We examined 25 patients with PG who visited Kurume University Hospital or were referred for immunoserological tests between 1996 and 2013. Final diagnoses of PG were made either at Kurume University Hospital or other hospitals. Of the included patients, we were able to follow the entire clinical course for 21 patients. Firstly, we clinically analysed the patients in terms of age, time of onset of symptoms, clinical course and treatments. Secondly, we examined the histopathological and direct immunofluorescence (IF) features.

We performed indirect IF of normal and 1 mol L^{-1} NaCl-split skin as described previously.[18] We also performed complement IF of both normal and 1 mol L^{-1} NaCl-split skin as described previously.[19,20] Immunoblotting (IB) analyses of normal human epidermal extracts, the recombinant proteins (RPs) of the NC16a and C-terminal domains of BP180, and concentrated culture supernatant of HaCaT cells were performed as previously described.[8,20,21] Enzyme-linked immunosorbent assays (ELISAs) of the NC16a domain of BP180 RP, and of the N- and C-terminal domains of BP230 RP were performed using commercially available kits (MESACUP; MBL, Nagoya, Japan). The results were expressed as ELISA indices with a cut-off value of 15·0 for the BP180 ELISA and 9·0 for the BP230 ELISA.

Statistical analysis

We investigated differences in the clinical and serological features of primi- and multigravidae. Relationships among mean age at onset and time of gestation at onset in primi- and multigravidae were analysed by the Student's *t*-test. Postpartum remission time and ELISA indices were analysed by the Wilcoxon rank sum test. Other clinical features and serological results were analysed using Fisher's exact test. *P*-values $< 0{\cdot}05$ were considered to be statistically significant. Statistical analyses were performed using Stata® 13 (Stata Corp., College Station, TX, U.S.A.).

To calculate the mean time of gestation at onset, postpartum onset was considered to be at week 40 of pregnancy. Prognosis was assessed by the mean postpartum remission time, which was the time of complete remission after delivery. For cases achieving remission before delivery, the mean postpartum remission time was calculated as a minus value. Thus, patients who had no skin lesions at 8 months of gestation were considered to have achieved remission at −2 months after delivery.

Results

All clinical and immunological findings in all 25 patients with PG are given in Table S1 (see Supporting Information). A summary of these results is given in Table 1.

Case reports

Among the 25 cases of PG, we describe two new cases (cases 9 and 15) in detail below. In addition, we also describe an additional 13 cases of PG (cases 2, 5, 7, 11, 16, 17 and 19–25) in detail in Appendix S1 (see Supporting Information). All the responsible dermatologists for these cases are listed as coauthors in this study. The clinical features of these 13 cases of PG are depicted in Figure S1 (see Supporting Information). Histopathological and direct IF findings for representative cases are shown in Figures S2 and S3, respectively (see Supporting Information).

Case 9 developed severely pruritic erythematous skin lesions on the trunk and extremities in week 36 of her first pregnancy. The lesions gradually worsened, and the patient visited the Division of Dermatology, Yamagata City Hospital Saiseikan. Physical examination revealed oedematous erythema multiforme-like lesions with tense bullae on the face, abdomen, extremities and palms (Fig. 1a,b). Histopathology showed a subepidermal blister with eosinophils, spongiosis, and perivascular and perineural infiltration of eosinophils in the dermis

Table 1 Clinical, histopathological and immunological findings in 25 patients with pemphigoid gestationis (PG)

Case	Age (years)	Obstetric history	Onset[a]	Treatment	Remission of lesions[b]	Histopathology	IF		IF (IgG)		IF (C3)		IB	BP180		HaCaT	ELISA[c]	
							DIF											
							BMZ	CS	IIF (BMZ)	ss-IIF (Epi)	CIF (BMZ)	ss-CIF (Epi)	Epi	NC16a	C-terminal		BP180	BP230
1	30	MG (1)	23 w	U	U	Subepidermal bulla	C3	–	10×	10×	10×	40×	180+	+	–	–	56·0	0·7
2	26	PG (0)	27 w	PSL	2 mo	Spongiosis, papillary dermal oedema	C3	–	40×	40×	10×	40×	180+/–, 230+	+	–	–	55·0	4·5
3	28	PG (0)	25 w	PSL	3 w	Spongiosis, intraepidermal bulla	C3	–	10×	10×	10×	10×	180+/–, 190+/–	+	+	+	62·0	2·2
4	28	PG (0)	3 d	PSL	1 mo	Subepidermal bulla	C3	–	160×	40×	10×	10×	–	+	+	–	133·0	0·1
5	22	PG (0)	24 w	Betamethasone	4 mo	Subepidermal bulla	IgG, C3	–	40×	–	10×	10×	–	+/–	–	–	12·0	17·0
6	30	MG (1)	12 w	Topical steroid only	–2 mo	Subepidermal bulla	C3	–	40×	40×	10×	10×	–	+	–	–	43·0	0·6
7	32	PG (0)	28 w	PSL	6 mo	Spongiosis	IgG, C3	–	160×	40×	10×	40×	180+/–	+	+	+	131·0	1·3
8	30	MG (1)	34 w	PSL	10 mo	Liquefaction degeneration	C3	–	–	10×	10×	10×	–	+/–	–	–	36·0	0·2
9	33	PG (0)	36 w	PSL, DDS	11 mo	Subepidermal bulla, spongiosis	C3	IgG, C3	160×	40×	10×	40×	–	+	–	–	112·0	–0·2
10	30	PG (0)	31 w	PSL, DDS, steroid pulse, CyA	U	Intraepidermal bulla	C3	–	160×	40×	40×	40×	180+/–	++	–	–	1100·0	12·2
11	26	PG (0)	3 d	PSL	6 mo	Subepidermal bulla	C3	–	10×	40×	–	10×	–	+	–	–	81·0	0·8
12	29	PG (0)	35 w	PSL	2 mo	Subepidermal bulla	IgG, C3	–	160×	40×	40×	40×	180+	++	+/–	+	134·0	2·0
13	33	MG (1)	10 w	PSL, plasmapheresis, IFN-γ, mizoribine, IVIG	30 mo	Subepidermal bulla, intraepidermal bulla	C3	C3	160×	40×	160×	40×	180+	++	+	+	185·0	59·2
14	27	PG (0)	36 w	Antihistamine, topical steroid only	7 w	Subepidermal bulla, intraepidermal bulla	IgG, C3	–	40×	40×	–	10×	–	+/–	–	–	79·0	0·9
15	39	MG (2)	23 w	PSL	1 mo	Subepidermal bulla, spongiosis	IgG, C3	–	10×	40×	10×	10×	–	+	–	–	29·0	0·5
16	39	PG (0)	20 w	PSL	2 w	Liquefaction, degeneration, papillary dermal oedema	IgG, C3	–	160×	10×	10×	10×	180+	+	–	–	155·0	1·1
17	25	PG (0)	29 w	Topical steroid only	3 mo	Subepidermal bulla	IgG, IgM, C3	–	40×	40×	10×	10×	–	+	–	–	94·0	15·3
18	26	U	1 d	U	U	Subepidermal bulla	IgG, C3	–	40×	40×	10×	10×	180+/–	+	–	–	123·0	2·4
19	38	MG (6)	27 w	Topical steroid only	2 w	Subepidermal bulla	C3	–	40×	40×	–	10×	–	+/–	–	–	85·0	2·1
20	34	PG (0)	36 w	PSL	3 w	Subepidermal bulla, spongiosis	IgG, IgM, C3	–	10×	40×	–	10×	–	+/–	+/–	–	46·0	1·3
21	31	MG (2)	28 w	PSL	1 mo	Subepidermal bulla		–	10×	40×	–	10×	–	+	–	–	83·0	–0·5
22	30	PG (0)	28 w	PSL	2 mo	Spongiosis	C3	–	40×	40×	–	10×	180+/–	+	–	–	297·0	2·4

(continued)

Table 1 (continued)

Case	Age (years)	Obstetric history	Onset[a]	Treatment	Remission of lesions[b]	Histopathology	IF DIF BMZ	IF (IgG) CS	IF (IgG) IIF (BMZ)	IF (IgG) ss-IIF (Epi)	IF (C3) CIF (BMZ)	IF (C3) ss-CIF (Epi)	IB Epi	BP180 NC16a	BP180 C-terminal	HaCaT ELISA[c] BP180	HaCaT ELISA[c] BP230
23	27	PG (0)	26 w	Betamethasone	2 w	Subepidermal bulla	C3	–	40×	40×	10×	10×	180+/–	+	–	–	1·1
24	40	MG (1)	12 w	PSL	U	Spongiosis, papillary dermal oedema	ND	ND	–	–	–	10×	–	–	+	0·3	0·1
25	30	PG (0)	4 d	Betamethasone, DDS	2 mo	–	C3	–	10×	40×	–	10×	180+/–	+	–	69·0	2·0

BMZ, basement membrane zone; CIF, complement immunofluorescence; CS, cell surface; CyA, ciclosporin; d, day after delivery; DIF, direct immunofluorescence; DDS, diaphenylsulfone; ELISA, enzyme-linked immunosorbent assay; Epi, epidermis; IB, immunoblotting; IF, immunofluorescence; IFN, interferon; IVIG, intravenous immunoglobulin; MG, multigravidae; Mo, months; ND, not done; PG, primigravidae; PSL, prednisolone; ss-CIF, split skin CIF; ss-IIF, split skin indirect immunofluorescence; U, unknown; w, week. [a]Time of onset refers to stage of gestation. [b]After delivery. [c]BP180 ELISA index > 15 positive, BP230 ELISA index > 9 positive.

(Fig. 1c,d). Direct IF revealed cell surface deposition of IgG and C3 to the entire epidermis (Fig. 1e,f) and linear deposition of C3 at the BMZ (Fig. 1f).

Indirect IF of normal human skin revealed circulating IgG anti-BMZ antibodies, which reacted with the epidermal side of 1 mol L^{-1} NaCl-split skin. Complement IF revealed C3 deposition to the BMZ in normal skin and to the epidermal side of 1 mol L^{-1} NaCl-split skin. IB detected IgG antibodies to the NC16a domain of BP180 RP. ELISAs revealed that anti-BP180 antibodies were positive (index 112, positive > 15) and anti-BP230 antibodies were negative (index −0·2, positive > 9).

A diagnosis of PG was made. Prednisolone 20 mg daily started and was subsequently tapered along with improvement of the skin lesions. However, 2 months after delivery, vesicular skin lesions recurred upon receiving prednisolone 5 mg daily. The addition of diaphenylsulfone (DDS) 50 mg daily improved the skin lesions. Prednisolone and DDS were tapered off 7 and 10 months, respectively, after delivery. Skin lesions completely dissolved 11 months after delivery.

Case 15 developed oedematous erythemas and vesicles on the trunk and extremities in week 23 of her third pregnancy (Fig. 2a,b). The patient first visited the Department of Dermatology, Saiseikai Fukuoka General Hospital. Histopathology showed a subepidermal blister with eosinophilic infiltration and spongiosis (Fig. 2c,d). Direct IF revealed linear deposition of IgG and C3 in the BMZ (Fig. 2e,f).

Indirect IF of normal human skin revealed circulating IgG anti-BMZ antibodies (Fig. 2g), which reacted with the epidermal side of 1 mol L^{-1} NaCl-split skin (Fig. 2h). Complement IF revealed C3 deposition at the BMZ in normal skin (Fig. 2i), and to the epidermal side of 1 mol L^{-1} NaCl-split skin (Fig. 2j). IB detected IgG antibodies to the NC16a domain of BP180 RP (Fig. 2k). ELISAs revealed that anti-BP180 antibodies were positive (index 29) and anti-BP230 antibodies were negative (index 0·5).

A diagnosis of PG was made. A combination of topical corticosteroids and oral prednisolone 20 mg daily was started. One month after delivery, all skin lesions had disappeared.

Clinical findings

The age of onset in the 25 patients with PG ranged from 22·0 to 40·0 years (mean 30·5). Sixteen patients were primigravidae and eight were multigravidae. Three patients developed PG in the first trimester, eight in the second trimester and 10 in the third trimester. Four patients developed PG in the postpartum period.

Data for complications were available for 23 patients. Of these, two patients had Graves disease, and one case each had depression, asthma with atopic dermatitis, spinal cord injury, ovarian tumour with subsequent ileus, spontaneous abortion and pyelonephritis.

Simple erythemas were seen in 15 (60%) patients, urticarial papules in four (16%) and erythema multiforme-like lesions in 23 (92%) patients. In addition, all 25 patients showed blisters, ranging in diameter from 0·5 to 5·0 cm. Clinical data

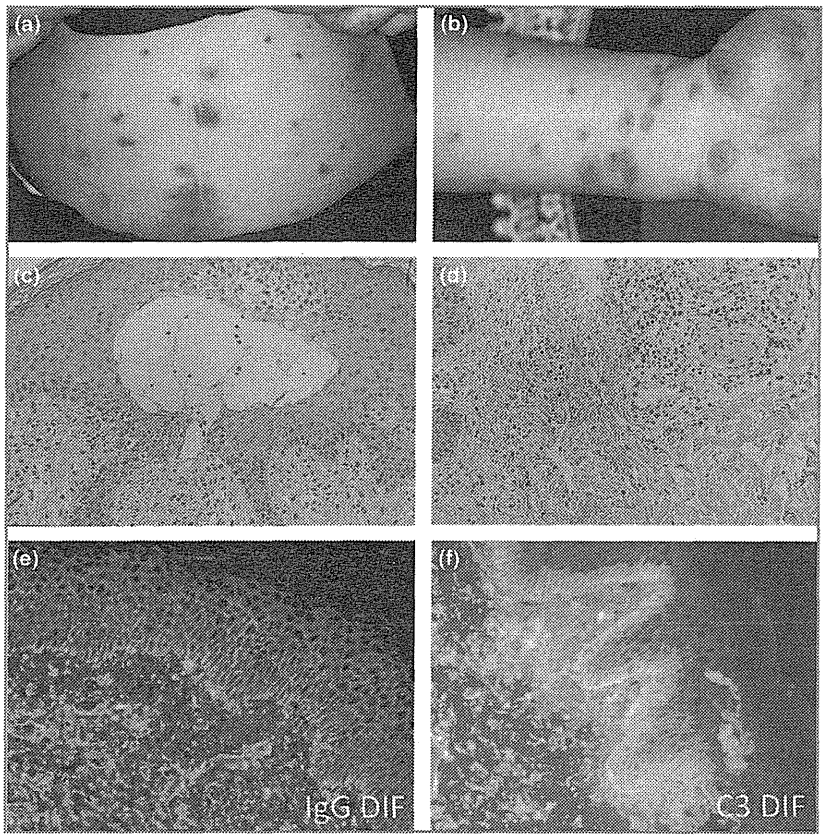

Fig 1. Findings in case 9. (a, b) Clinical features. (c, d) Histopathological features (haematoxylin and eosin staining). (c) Subepidermal bulla. (d) Eosinophilic and lymphocytic infiltrate in the dermis. Direct immunofluorescence (DIF) for (e) IgG and (f) C3.

regarding the types of blister seen were available for 21 patients: tense blisters were seen in all patients and erosions in six. The affected sites were the abdomen (96%), trunk (92%), arms (92%), legs (92%), palms (48%), neck (32%) and face (28%). There was no mucous membrane involvement, with the exception of one patient who had an oral mucosal lesion.

In most cases, systemic prednisolone was started at a dose of 10–30 mg daily; the highest dose prescribed was 50 mg daily. Topical corticosteroids were used in 21 cases. Oral antihistamines were used in 13 patients, although the prescription may have been missed in some cases. DDS, steroid pulse therapy, oral ciclosporin, plasmapheresis, interferon-γ, mizoribine and intravenous immunoglobulin were used in four patients.

Regarding prognosis, skin lesions completely disappeared 4·0 months (mean) after delivery. In the most intractable case (case 13), the skin lesions continued for 30 months after delivery. In contrast, the skin lesions completely disappeared before delivery in one case. No patients were considered to shift to true BP.

Histopathological findings

Skin biopsies were available in 24 patients. Histopathological studies showed subepidermal bulla in 16 (67%) patients, spongiosis in eight (33%), apparent intraepidermal bulla in four (17%), papillary dermal oedema in three (13%) and liquefaction degeneration in two (8%). In all cases, eosinophilic infiltration was observed. In most of the patients, the intraepidermal blisters seemed to be due to severe spongiosis.

Results of the immunological studies

Immunofluorescence studies

Direct IF was performed in 24 patients, and revealed C3 deposition in the BMZ in all patients and IgG deposition in the BMZ in 10 (42%) patients. In addition, one patient showed C3 deposition to keratinocyte cell surfaces, and another patient showed IgG and C3 deposition to cell surfaces.

Indirect IF of normal human skin revealed IgG anti-BMZ antibodies in 23 (92%) of 25 patients. However, we could not find clear IgG reactivity with keratinocyte cell surfaces in indirect IF. Indirect IF of 1 mol L^{-1} NaCl-split skin demonstrated IgG reactivity to the epidermal side in 23 (92%) of 25 patients. Complement IF revealed linear C3 reactivity with the BMZ of normal human skin in 17 (68%) of 25 patients, and C3 reactivity with the epidermal side of 1 mol L^{-1} NaCl-split skin in all patients.

Immunoblotting studies

The results of IB studies of four different antigen sources for all 25 PG sera are depicted in Figure 3. IB of normal human epidermal extract detected IgG antibodies to the 180-kDa BP180 and the 230-kDa BP230 in 12 (48%) and one (4%) patient, respectively. The NC16a domain of BP180 NC16a RP was detected in 24 (96%) patients. The C-terminal domain of BP180 RP was detected in seven (28%) patients. The 120-kDa leucocyte adhesion deficiency-1 (LAD-1) protein in the con-

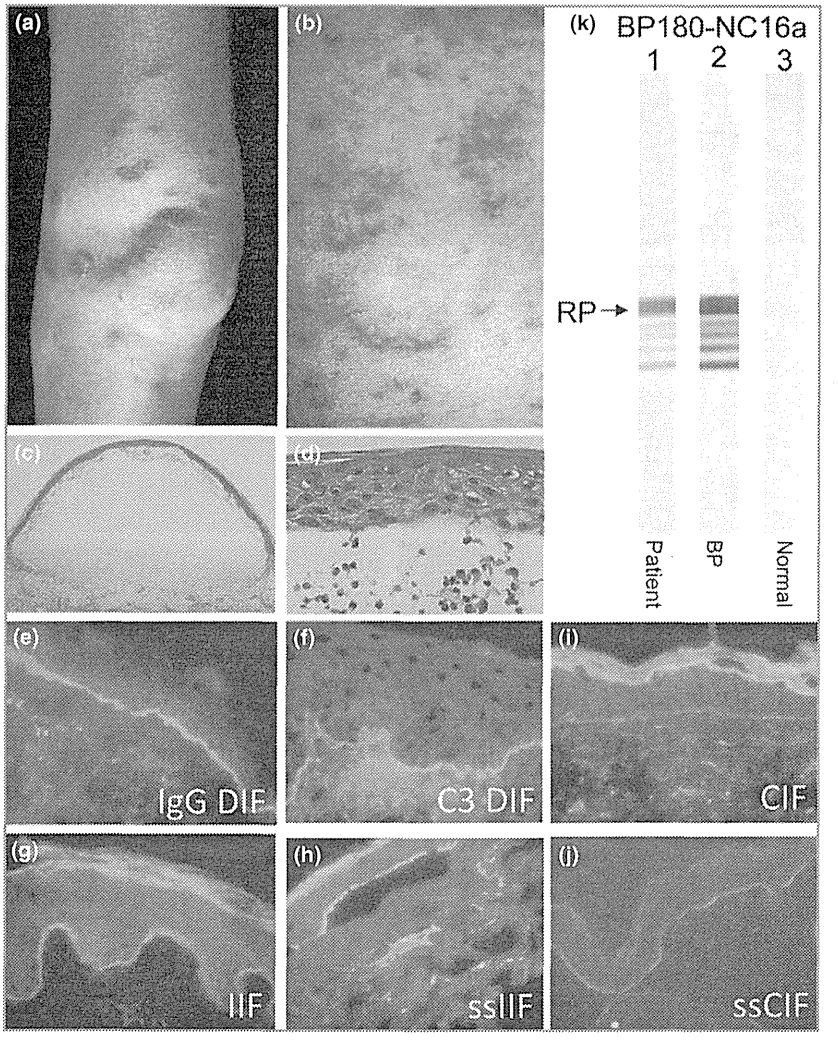

Fig 2. Findings in case 15. (a, b) Clinical features. (c, d) Histopathological features (haematoxylin and eosin staining). Magnification in (c) 100×; magnification in (d) 400×. (e, f) Direct immunofluorescence (DIF) for (e) IgG and (f) C3. (g, h) Indirect immunofluorescence (IIF) of (g) normal skin and (h) 1 mol L^{-1} NaCl-split skin (ss). (i, j) Complement immunofluorescence (CIF) of (i) normal skin and (j) 1 mol L^{-1} NaCl-ss. (k) IgG immunoblotting of the NC16a domain of BP180 recombinant protein (RP). IgG antibodies in patient serum (lane 1) and positive control bullous pemphigoid (BP) serum, but not normal serum (lane 3), showed positive reactivity with the RP.

centrated culture supernatant of HaCaT cells was detected in five (20%) patients.

Enzyme-linked immunosorbent assays

ELISAs for BP180 and BP230 showed positive results in 23 (92%) and four (16%) of 25 patients, respectively. Ranges of indices were 0·3–1100·0 (cut-off < 15) for BP180 and −0·5 to 59·2 (cut-off < 9) for BP230.

Comparison of primi- and multigravidae

All clinical and immunological findings in either multi- or primigravidae, as well as the results of statistical analyses of the difference in all parameters between multi- and all primigravidae, are shown in Table S2 (see Supporting Information).

The mean ± SD week of gestation in which primi- and multigravidae developed PG was 31·3 ± 6·3 and 21·1 ± 8·8, respectively. Skin lesions developed significantly during an earlier stage of pregnancy in multigravidae compared with primigravidae ($P < 0·01$). Among 16 primigravidae, five (31%) and eight (50%) developed skin lesions in the second and third trimesters of pregnancy, respectively, while three (19%) developed PG postpartum. In contrast, among eight multigravidae, three (38%), three (38%) and two (25%) developed skin lesions in the first, second and third trimesters of pregnancy, respectively.

The mean ± SD durations to achieve remission after delivery were 2·9 ± 0·7 months in primigravidae and 6·8 ± 12·1 months in multigravidae. Although there was no statistical difference, the duration until remission of PG after delivery was longer in multigravidae than in primigravidae.

However, because this was a retrospective study, we could not set indices for disease severity. In addition, because this was a multicentre study, we could not compare precisely the severity of various skin lesions. Therefore, we could not compare the severity of skin lesions quantitatively between both groups of patients (i.e. primi- and multigravidae). In addition, there were no statistical differences in the type of skin lesions, sites of skin lesions and types of treatments between primi- and multigravidae. There was no particular histopathological findings specific to either group.

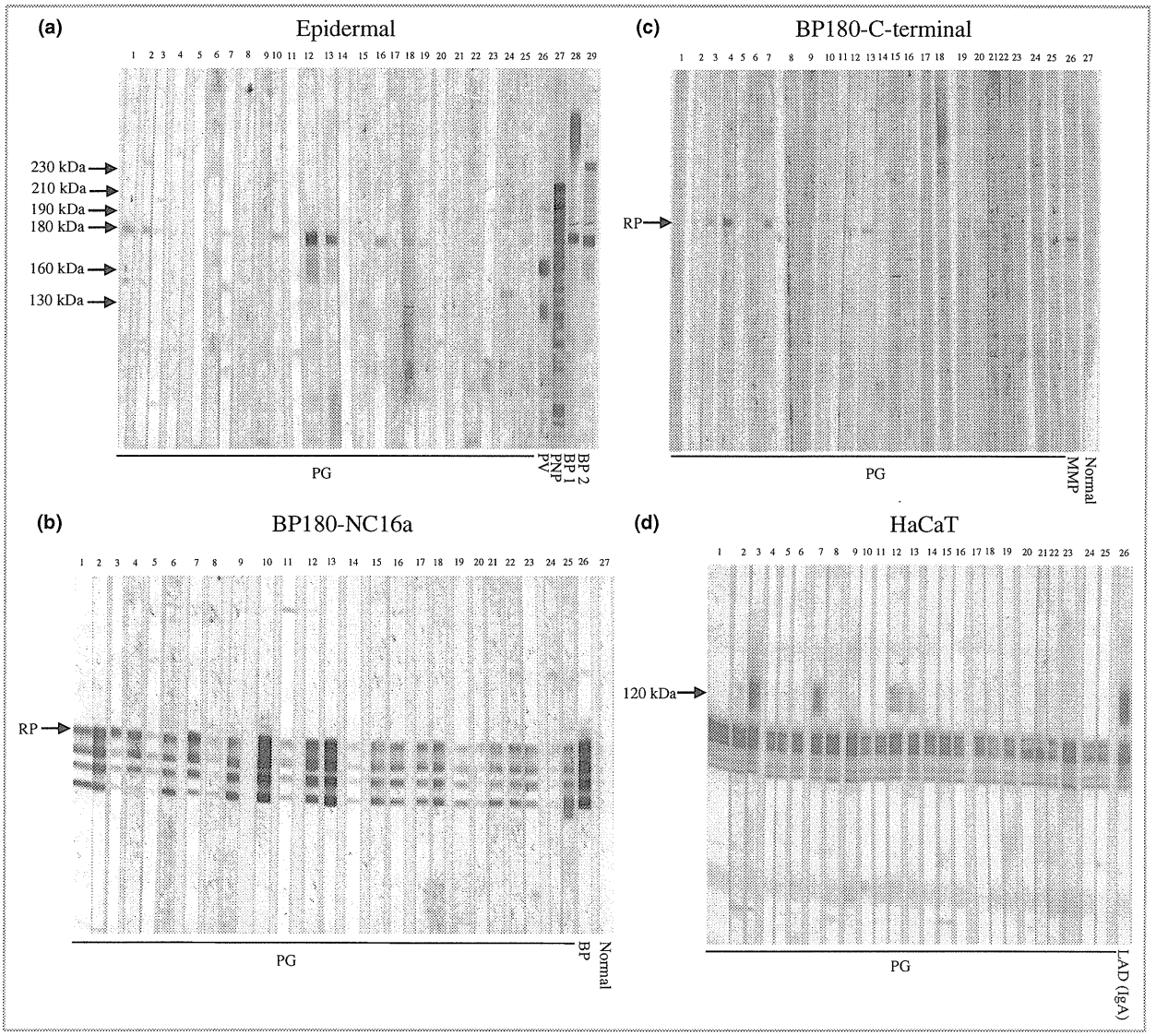

Fig 3. Results of the immunoblotting (IB) studies. The reactivity of the sera from all 25 patients with pemphigoid gestationis is shown in lanes 1–25 in each panel. (a) IB of normal human epidermal extracts. Control pemphigus vulgaris (PV) serum reacted with the 160-kDa desmoglein (Dsg)1 and the 130-kDa Dsg3 (lane 26); control paraneoplastic pemphigus (PNP) serum reacted with the 210-kDa envoplakin and 190-kDa periplakin (lane 27); control bullous pemphigoid (BP) serum 1 reacted with the 180-kDa BP180 (lane 28); and control BP serum 2 reacted with the 230-kDa BP230 and BP180 (lane 29). (b) IB of the NC16a domain of BP180 recombinant protein (RP). Control BP serum (lane 26), but not normal control serum (lane 27), reacted with this RP. (c) IB of the C-terminal domain of BP180 RP. Control anti-BP180-type mucous membrane pemphigoid (MMP) serum (lane 26), but not normal control serum (lane 27), reacted with this RP. (d) IB of concentrated culture supernatant of HaCaT cells. IgA antibodies in control leucocyte adhesion deficiency (LAD)-1 serum (lane 26) reacted with the 120-kDa LAD-1. IgG antibodies in this case did not react with LAD-1 (lane 3).

Regarding immunological studies, there was no clear difference in the results of direct IF and IB of various antigen sources between the two groups. There was no significant difference in titres in either indirect or complement IF of both normal and split skin, nor in the ELISAs for BP180 and BP230 between the two groups.

Discussion

This study describes the clinical, histopathological and immunological features of 25 patients with PG.

Our patients all had typical clinical courses and skin lesions. Two (9%) of 23 patients had Graves disease, which is in agreement with the finding of a previous study that 10% of 75 patients with PG had Graves disease.[22]

Histopathologically, subepidermal bulla with eosinophils was seen in only 16 (67%) of 24 patients. Instead, several patients showed liquefaction degeneration and oedema at the papillary dermis. These results are slightly different from those obtained in BP, and may indicate that anti-BP180 antibodies in PG tend to induce inflammation rather than blisters. Several patients showed marked spongiosis and intraepidermal bullae,

which may indicate that epidermis was preferentially affected in patients with PG.

Among various immunological tests, detection of C3 reactivity to BMZ by direct and complement IF is the gold standard for diagnosis of PG. However, skin biopsy for direct IF may not be available and complement IF is technically difficult. Therefore, we also performed various serological tests to facilitate the diagnosis of PG.

Direct IF showed positive C3 deposition to the BMZ in 24 examined patients with PG, and IgG deposition in 10 patients. Thus, direct IF was a very useful test for PG.

A previous case report of PG showed concurrent BMZ and cell surface reactivity in both direct and indirect IF tests.[23] This curious finding was also shown in 10 of 42 patients with PG in our indirect IF study.[24] In the present study, direct IF showed clear cell surface deposition in two patients. However, the number of patients with PG with reactivity with the cell surface was much less than that of our previous study.[24] In addition, in this study no clear cell surface staining was detected with indirect IF. The frequency and pathomechanisms of cell surface reactivity in PG should be clarified in future studies.

Indirect IF of both normal human skin and 1 mol L^{-1} NaCl-split skin showed positive results in 92% of patients. Thus, indirect IF also detected PG autoantibodies, although complement IF was the original method used to detect IgG anti-BMZ autoantibodies. In contrast, complement IF of normal skin and 1 mol L^{-1} NaCl-split skin showed positive results in 68% and 100% of patients, respectively. In a previous study, 93% of PG sera showed positive reactivity by complement IF.[25] The cause for the relatively lower rate of detection in this study is not clear. However, it may have been due to technical difficulties in the complement IF.

We also examined the sera of all 25 patients with PG using various biochemical studies. IB of normal human epidermal extracts detected BP180 in 48% patients, while only one patient reacted with BP230. In contrast, during IB, the sera of 96% of patients reacted with the NC16a domain of BP180 RP. In addition, ELISA of the NC16a domain of BP180 RP showed positive results in 92% of patients, while it detected anti-BP230 antibodies in 16% of patients. The positive detection rates of 96% (IB) and 92% (ELISA) for the NC16a domain of BP180 RP are significantly higher than those reported previously [79% (IB) and 86% (ELISA)].[8,25]

In contrast, in this study, IB and ELISA detected anti-BP230 antibodies in only a few patients compared with a previous study which showed that 26% of patients with PG reacted with BP230 (IB).[6] In addition, the positive result for BP230 obtained by ELISA in the present study was lower than that found in patients with BP.

It is currently unknown why PG rarely shows reactivity with BP230, although PG is considered as BP developed in pregnant females. A more rapid and more temporary disease course and less severe skin lesions in PG may cause the failure in anti-BP230 antibody production as anti-BP230 antibodies are considered a secondary phenomenon after disturbance of the cell membrane integrity by anti-BP180 antibodies.[26] However, we could not confirm this possibility because two of the three patients with antibodies to both BP230 and BP180 showed a relatively intractable disease course.

Using IB, the sera of 28% of patients with PG reacted with the C-terminal domain of BP180 RP, and the sera of 20% reacted with LAD-1 in concentrated culture supernatant of HaCaT cells. IgG and IgA antibodies to the C-terminal domain of BP180 are diagnostic criteria for anti-BP180-type mucous membrane pemphigoid,[27] while IgA reactivity with LAD-1 is a hallmark for the diagnosis of lamina lucida-type linear IgA bullous dermatosis.[28,29] However, these antibodies can also be detected in BP.[30,31] Therefore, the pathogenic significance of these antibodies to the C-terminal domain of BP180 in PG is currently unknown.

In general, it is believed that more severe skin lesions develop during repeated pregnancies, although some patients have been reported to not develop them during subsequent pregnancies. However, the relationship between clinical severity and multiple pregnancies has not been systematically studied. Therefore, as the second main aim of this study, we performed extensive statistical analyses for various clinical, histopathological and immunological parameters between primi- and multigravidae.

The results of our study indicate that, in general, the clinical features of PG in multigravidae are more severe than those in primigravidae. Thus, compared with primigravidae, multigravidae developed PG in a significantly earlier stage of gestation. The prognosis, which was assessed by the postpartum remission time, seemed longer in multigravidae, although no statistical significance was found. These results may indicate that immunologically, multigravidae are more susceptible to the development of PG than primigravidae. In contrast, there was no statistically significant difference found in indirect IF titer and ELISA indices between the two groups.

In conclusion, the present study clearly shows that IB and ELISA of the NC16a domain of BP180 RP are highly sensitive and diagnostic methods in PG. Reactivity with BP230 was quite low in PG, suggesting different pathomechanisms between PG and BP. PG developed significantly earlier in gestation in multigravidae than in primigravidae, suggesting that multigravidae are immunologically more susceptible to the development of PG.

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We thank Dr D. Tsuruta, Osaka City University Graduate School of Medicine; Dr B. Ohyama, Dr T. Nakama and Dr M. Furumura, Kurume University School of Medicine; Dr C. Tokunaga and Dr T. Hori, Yamato Municipal Hospital; Dr Y. Yamakawa, Yokohama City University Medical Center; Dr R. Okuyama and Dr T. Edamitsu, Shinshu University School of Medicine; Dr T. Tsunoda, Yamagata City Hospital Saiseikan; Dr K. Nakamura, Saitama Medical University; Dr K. Nakajima, Kochi University School of Medicine; Dr S. Takahashi, Ichikawa General Hospital, Tokyo Dental College; Dr Y.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Case reports of 13 patients with pemphigoid gestationis (PG).

Figure S1. Clinical appearance of 13 patients with pemphigoid gestationis.

Figure S2. Representative histopathological features of 11 patients with pemphigoid gestationis.

Figure S3. Representative immunofluorescence features of 10 patients with pemphigoid gestationis.

Table S1. Clinical, histopathological and immunological findings in 25 patients with pemphigoid gestationis.

Table S2. Comparison of primi- and multigravidae.

THE JOURNAL OF DERMATOLOGY

Letters to the Editor

A sporadic elder case of erythrokeratodermia variabilis with a single base-pair transversion in GJB3 gene successfully treated with systemic vitamin A derivative

Dear Editor,

Erythrokeratodermia variabilis (EKV) is considered to be caused by deleterious mutations in two different genes, GJB3 and GJB4, which encode connexin (Cx) 31 and Cx 30.3, respectively.[1-3] We report an EKV patient with a single base-pair transversion (c.625C>T; p.L209F) in GJB3 gene. This is a first Japanese sporadic elder and *de novo* case with the mutation.

A 69-year-old man had presented with hyperkeratotic plaques since his childhood. Transient erythematous areas had appeared since he was 17-years old. He was born to non-consanguineous parents and his family members did not have these skin lesions. Cutaneous examination revealed relatively fixed, symmetrically distributed, well-defined, erythematous hyperpigmented keratotic plaques over his lower extremities and the extensor surface of his upper extremities, and lower back (Fig. 1a). Hyperkeratotic plaques had been noticed on the palms and soles. He also had asymptomatic, irregularly shaped, sharply demarcated, erythematous patches on his back and upper extremities. These lesions were transient, but appeared repeatedly. Histopathological examination of a biopsy taken from a hyperkeratotic fixed plaque on the right leg revealed orthokeratotic basket-weave hyperkeratosis, irregular acanthosis, and mild papillomatosis with occasional church spire configuration. Histopathology of transient erythematous patches on the upper back were revealed to be non-specific and included hyperkeratosis, acanthosis, papillomatosis and capillary dilatation.

To confirm the clinical diagnosis at the genetic level, we obtained informed consent to isolate genomic DNA from samples of his peripheral blood and search for mutations in the GJB3 and GJB4 genes. PCR sequence analysis for GJB3 gene revealed heterozygous C→T transition at position 625 from the ATG translation start site, which results in substitution of leucine 209 with phenylalanine (L209F). The mutation replaces a neutral, nonpolar, hydrophobic residue with a hydrocarbon alkyl side chain (leucine) in the beginning of the cytoplasmic carboxy-terminus of Cx31 with an even more hydrophobic residue carrying an aromatic side chain (phenylalanine). No mutation was found in the GJB4 gene. The patient was treated with etretinate (Tigason^{Rx}; Chugai Pharmaceutical Co., Ltd, Tokyo Japan), an oral vitamin A derivative, 20 mg/day along with topical emollients. Two months later, remarkable improvement in

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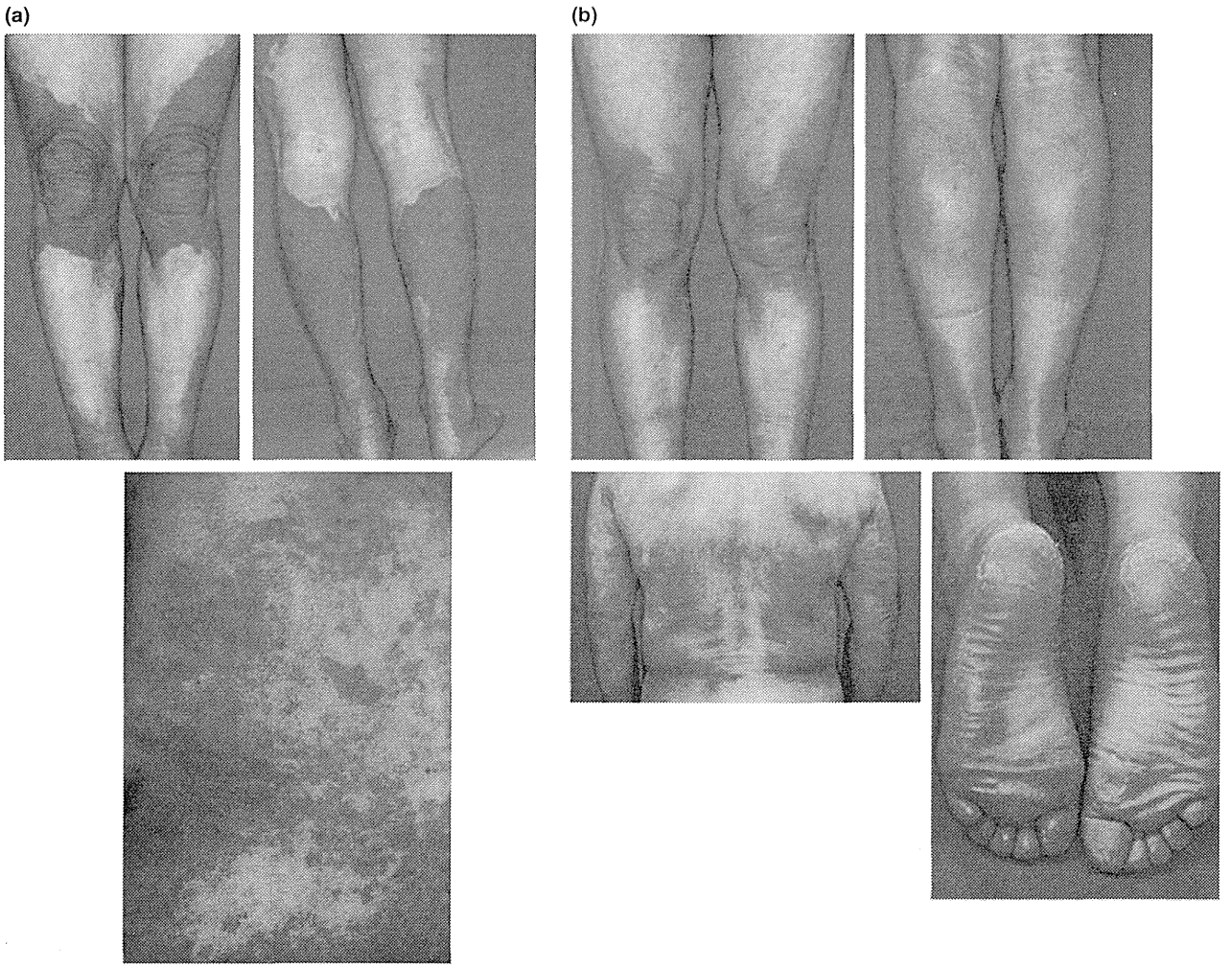

Figure 1. (a) Clinical features on the lower extremities and the back. (b) Complete healing of the lesions on legs, soles, and back, 2 months after systemic vitamin A derivative treatment.

skin lesions, including those on the palms and soles, was observed (Fig. 1b).

The disease-causing mutation (c.625C>T; p.L209F) has been reported previously in two patients (a 14-year-old boy from Australia and a 22-year-old woman from Scotland) with EKV.[4] The boy was a sporadic and *de novo* case. The two patients manifested EKV soon after birth and developed slowly progressive, relatively stable hyperkeratotic plaques in a symmetrical distribution. We suggest that clinical EKV phenotype of the mutation could cause slowly progressive disease course and lead to less severe condition in the early disease stage.

Cx31 contributes to normal keratinocyte growth and differentiation.[5] Retinoids including oral vitamin A derivative enhance the normal differentiation of keratinocytes, partly due to increased production of other Cx thereby compensating for deficiency in Cx31. We proposed the systemic vitamin A derivative agent could be useful to treat elder EKV patients though children and young adults are not treated with systemic retinoids because of the possible long-term side effects.

CONFLICT OF INTEREST: None declared.

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THE JOURNAL OF DERMATOLOGY

Letters to the Editor

Splicing mutation in the *COL7A1* gene mRNA exon 71 in a female patient with pretibial epidermolysis bullosa

Dear Editor,

Pretibial epidermolysis bullosa is an extremely rare form of localized, dystrophic epidermolysis bullosa, characterized by recurrent blisters and hypertrophic scars, which predominantly involve the pretibial skin, with variable nail dystrophy.[1] The disorder is believed to result from mutations in the *COL7A1* gene, which encodes type VII collagen, the major component of anchoring fibrils at the dermoepidermal junction.

A 31-year-old Japanese woman presented with persistent blistering eruptions and complaints of intermittent itchiness,

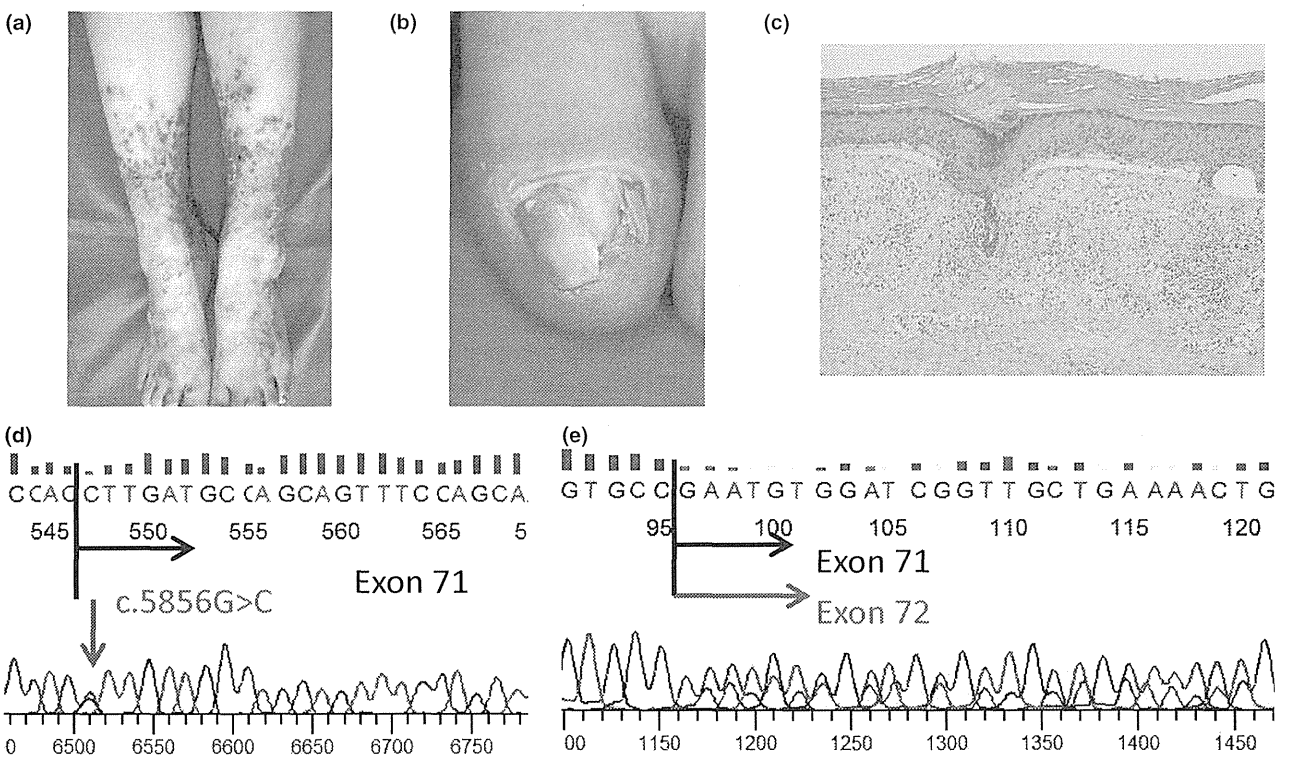

Figure 1. (a) A 31-year-old Japanese woman had persistent blistering eruptions and hypertrophic scarring in the pretibial areas. (b) Nail dystrophy of the big toenail was observed. (c) Skin biopsies taken from vesicles of the pretibial skin revealed subepidermal blisters and a dense inflammatory cell infiltrate in the subepidermal blister and upper dermis (hematoxylin and eosin, original magnification ×200). (d) Direct nucleotide sequencing of genomic antisense DNA from the patient disclosed a heterozygous G>C transition at nucleotide c.5856 in the *COL7A1* gene. (e) Reverse transcription polymerase chain reaction using white blood cells across the site of the c.5856G>C transition in the *COL7A1* gene.

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mainly in the pretibial area of both legs. She had a 20-year history of abnormalities associated with her toenails. The blister formation with pruritus had occurred in her pretibial area since she was 20 years old. She had progressive increased bilateral erythematous, indurated plaques on her shins, which extended to the medial and lateral aspects of the calf. On physical examination, the patient had blistering eruptions, well-defined, slightly indurated, erythematous plaques, and hypertrophic scars occurring predominantly in the lower extremities, including mainly the pretibial area (Fig. 1a). Her toenails were dystrophic with nail-plate crumbling, but her fingernails were normal (Fig. 1b). The sister of the patient experienced similar blistering eruptions on the pretibial areas and toenail dystrophy. Her parents were healthy and their toenails were normal. Her routine laboratory investigations were normal.

On histological examination of a biopsy from the lesional pretibial skin, subepidermal blisters and a moderate inflammatory cell infiltrate comprising lymphocytes in the upper dermis were observed (Fig. 1c). Direct immunofluorescence of the skin biopsy specimen was negative. Based on the clinical and histological findings, the patient was referred for genetic testing to screen for mutations in the *COL7A1* gene. Direct nucleotide sequencing of genomic antisense DNA disclosed a heterozygous splice-site mutation c.5856G>C in the last base of the exon of the *COL7A1* gene (Fig. 1d). Reverse transcription polymerase chain reaction across the site of the c.5856G>C transition in the *COL7A1* gene mRNA had two patterns, exon 71 and exon 72 (Fig. 1e). Splice-site mutation is caused by a mutation in the exon of the exon/intron boundary.[2] These genomic findings showed that the *COL7A1* gene mRNA exon 71 abnormality caused the in-frame deletion on splicing of the genomic DNA. The patient was treated with topical steroids and minimal sedation H_1 antihistamines. Moderate improvement in her skin lesions was observed.

Over 300 pathogenic mutations have been described within the *COL7A1* gene in various clinical forms of dystrophic epidermolysis bullosa.[3] To our knowledge, this is the first case of a patient with pretibial epidermolysis bullosa in which there is a splice site mutation affecting the *COL7A1* gene mRNA exon 71 skipping. Pretibial epidermolysis bullosa shows appreciable clinical overlap with dystrophic epidermolysis bullosa pruriginosa.[4] As Saito *et al.*[5] described, it seems probable that the *COL7A1* gene mRNA exon 87 skipping is related to the phenotype of dystrophic epidermolysis bullosa pruriginosa. We suggested that the transcription and processing of *COL7A1* gene mRNA exon 71 skipping could determine the spectrum of clinical phenotypes of pretibial epidermolysis bullosa.

CONFLICT OF INTEREST: None.

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THE JOURNAL OF DERMATOLOGY

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LETTERS TO THE EDITOR

Japanese case of oculodentodigital dysplasia caused by a mutation in the *GJA1* gene

Dear Editor,

Oculodentodigital Dysplasia (ODDD; Online Mendelian Inheritance in Man no. 164200) is a rare condition characterized by ocular symptoms, enamel hypoplasia, long and narrow nose, and syndactyly type III, which are common clinical features across different populations.[1–3] In some cases, affected individuals can show a variety of cutaneous and neurological manifestations.[1–3] ODDD typically shows an autosomal dominant inheritance trait, while sporadic cases also frequently exist.[1] The causative gene *GJA1* encodes connexin 43 (Cx43), which is a 4-pass transmembrane protein and is a major component of gap junctions.[1] To date, many cases of ODDD have been reported in the published work, but information of the disease in a Japanese population is still limited.[2,3] We herein report a Japanese patient with ODDD resulting from a *GJA1* mutation.

A 4-year-old Japanese boy visited Kobe university hospital due to sparse scalp hair. He had various additional symptoms including narrow nose, low-set ear, strabismus, glaucoma, macular hypoplasia of retina, brittle nails of fingers and toes, brachydactyly, and past surgical history of left fourth–fifth finger syndactyly (Fig. 1a–c). He also showed enamel hypoplasia and hypodontia (data not shown), while neurological abnormalities or palmoplantar keratoderma were not evident. Both parents were unaffected and there were no consanguini-

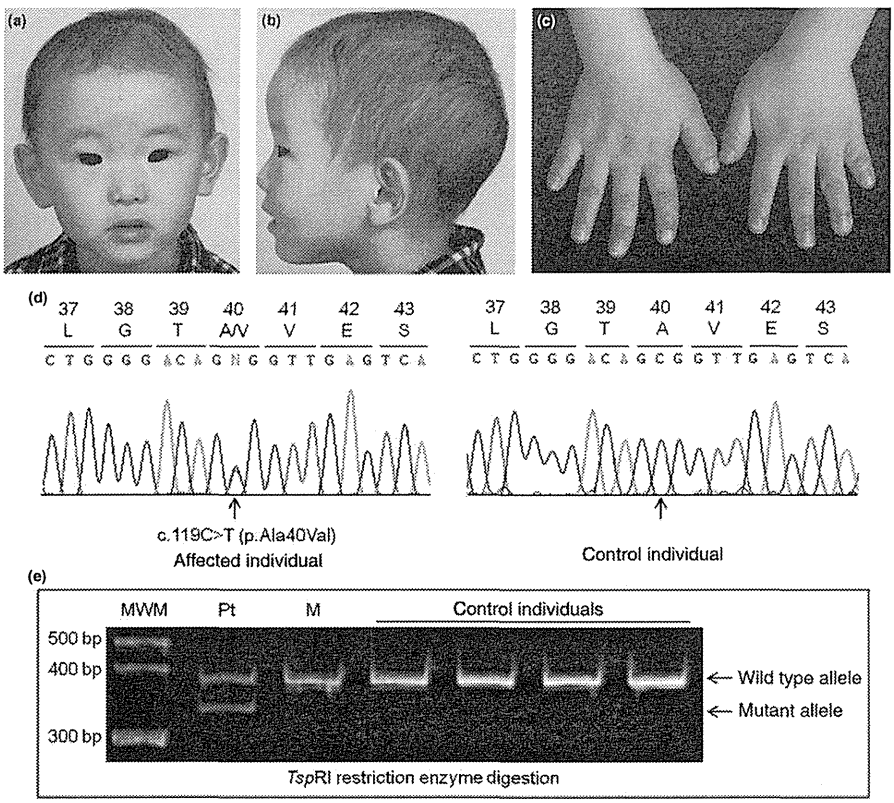

Figure 1. Clinical features of the patient. The patient showed (a,b) hypotrichosis of the scalp, (a) narrow nose and (b) low-set ear. (c) Also, he had brachydactyly of fingers and brittle nails. Note that the syndactyly of the left fourth–fifth fingers was corrected by an operation. (d) Identification of a heterozygous missense mutation c.119 C>T (p.Ala40Val) in the *GJA1* gene of the patient. Position of the mutation is indicated by arrows. (e) Results of screening assay with the restriction enzyme *Tsp*RI. Polymerase chain reaction products from the mutant allele (379 bp) were digested into 320- and 59-bp fragments, while those from the wild-type allele were undigested. The 59-bp fragments were not shown. M, mother; MWM, molecular weight markers; Pt, patient.

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ties between them. Most of these clinical findings were consistent with ODDD.

Following written informed consent, we isolated genomic DNA from blood samples of the patient and his mother (under institutional approval and adhering to the principles of the Declaration of Helsinki). His father's DNA was unavailable. Direct sequencing analysis of the *GJA1* gene was performed as described previously.[1] We identified a heterozygous missense mutation c.119C>T (p.Ala40Val) in the *GJA1* gene of the patient, but not of his mother (Fig. 1d). The results were confirmed by screening assay with the restriction enzyme *Tsp*RI (Fig. 1e). Although we were unable to analyze the patient's father, we postulated that the nucleotide substitution c.119C>T in *GJA1* was most likely a de novo mutation that had occurred in the patient's somatic cells or germ cells of either parent.

The *GJA1* mutation that we detected in our patient was previously identified in a sporadic case of ODDD in another population thus turned out to be recurrent.[1] The c.119C>T change occurred in CpG dinucleotide sequences in which nucleotide substitutions were prone to be introduced, suggesting that this position is a mutational hot spot in the *GJA1* gene.

Cx43 is abundantly expressed in the epidermis and skin appendages.[4] The crucial roles of Cx43 in cutaneous development are supported by the evidence that ODDD can show various skin symptoms, such as hypotrichosis, brittle nails and palmoplantar keratoderma,[1] although it remains unknown how *GJA1* mutations caused these phenotypes. The missense mutation p.Ala40Val is located in the first transmembrane domain of Cx43. Importantly, it has previously been demonstrated that the p.Ala40Val mutant Cx43 protein was able to form gap junction plaques, but lost its channel function *in vitro*.[5] However, because the mutation p.Ala40Val was identified in a heterozygous state, further studies will be required to investigate whether the mutant Cx43 shows a dominant negative effect against wild-type Cx43.

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CONFLICT OF INTEREST: None declared.

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THE JOURNAL OF DERMATOLOGY

Journal of Dermatology 2014; **41**: 937–949

LETTERS TO THE EDITOR

Compound heterozygous mutations in two distinct catalytic residues of the *LIPH* gene underlie autosomal recessive woolly hair in a Japanese family

Dear Editor,

Autosomal recessive woolly hair (ARWH; Online Mendelian Inheritance in Man no. 278150/604379) is a non-syndromic form of hereditary hair disorder characterized by short and tightly curled scalp hair with hypotrichosis at high frequency.[1,2] The disease is caused by mutations in either lipase H (*LIPH*) or

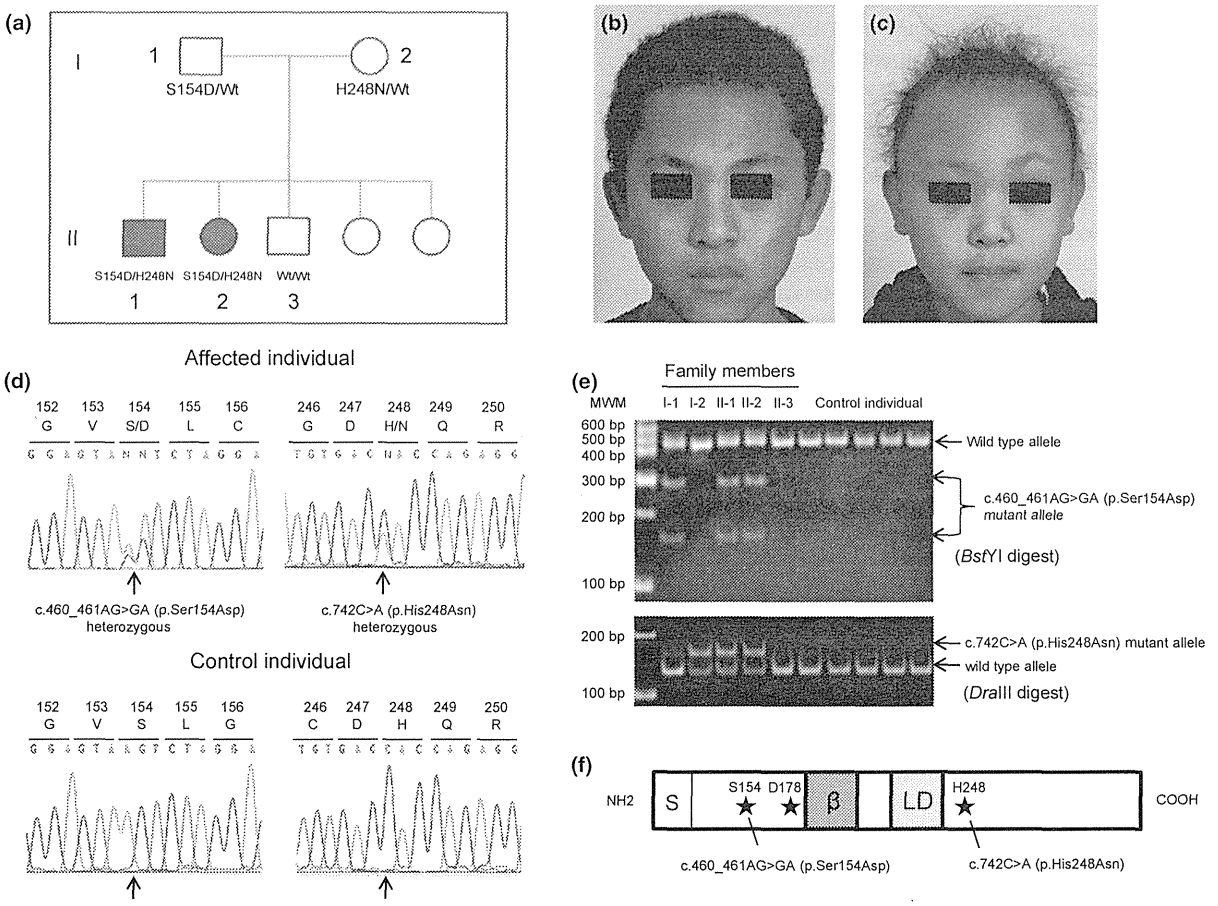

Figure 1. (a) Pedigree of the family. The affected individuals are colored in red. Genotypes of the *LIPH* gene in each individual analyzed are shown. (b,c) Clinical features of the patients. (b) Hair density of the elder patient (II-1) appeared normal, (c) while the younger patient (II-2) showed sparse hair. Keratosis pilaris was not evident in either patient (not shown). (d) Identification of compound heterozygous mutations, c.460_461AG>GA (p.Ser154Asp) and c.742C>A (p.His248Asn), in the *LIPH* gene of the affected individuals. Position of the mutations is indicated by arrows. (e) Results of screening assays for the mutations. Polymerase chain reaction (PCR) products from the c.460_461AG>GA mutant allele (413 bp) were digested into 258-bp and 155-bp fragments, while those from the wild-type allele were undigested with *Bst*YI (top panel). PCR products from the c.742C>A mutant allele (156 bp) were undigested, while those from the wild-type allele were digested into 127-bp and 29-bp fragments with *Dra*III (bottom panel). The 29-bp fragments were not shown. (f) Schematic representation of mPA-PLA$_1\alpha$ protein and position of the mutations identified in this study. The catalytic residues are shown by asterisks. β, β9 loop; LD, lid domain; MWM, molecular weight markers; S, signal peptide.

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LPAR6 genes.[1,2] The *LIPH* gene encodes a phospholipase A_1 named mPA-PLA$_1\alpha$ that produces lysophosphatidic acid (LPA) from phosphatidic acid,[3] and the *LPAR6* gene encodes an LPA receptor known as LPA_6.[4] mPA-PLA$_1\alpha$ is co-expressed with LPA_6 in human hair follicles.[1,2] As *LIPH* is closely related to *LPAR6*, mutations in either gene lead to similar hair abnormalities. In the Japanese population, two founder mutations in the *LIPH* gene, c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn), are detected in most affected individuals with ARWH.[5]

In this study, we identified a Japanese family with ARWH carrying notable mutations in the *LIPH* gene. The family had two affected children. One was a 13-year-old boy (II-1; Fig. 1a) and the other was an 11 year-old girl (II-2; Fig. 1a). Their parents and younger siblings were all unaffected, and there were no consanguinities between the parents (Fig. 1a). Both the affected individuals have had strongly curled scalp hair since their birth, while their eyelashes and eyebrows appear normal (Fig. 1b,c). It is noteworthy that the hair density of the male patient was almost normal (Fig. 1b), whereas that of the female patient was relatively sparse leading to hypotrichosis (Fig. 1c). Both patients had mild atopic dermatitis. Otherwise, neither patient showed any associated findings. Based on these clinical features, we diagnosed the patients as having ARWH.

Following written informed consent, we isolated genomic DNA from blood samples of the family members (under institutional approval and in adherence with the principles of the Declaration of Helsinki). We subsequently performed mutation analysis of the *LIPH* gene (Appendix S1).[1] The results revealed that both the affected individuals carried compound heterozygous missense mutations in the *LIPH* gene: c.460_461AG>GA (p.Ser154Asp) in exon 3 and c.742C>A (p.His248Asn) in exon 6, which were inherited from their father and mother, respectively (Fig. 1a,d,e). The younger brother of the patients (II-3; Fig. 1a) did not have either mutation (Fig. 1a,e). The mutation p.His248Asn was a prevalent founder mutation in the Japanese population.[5] To the best of our knowledge, however, the mutation p.Ser154Asp had not previously been reported. Screening assay with the restriction enzyme *Bst*YI showed that 100 healthy Japanese control individuals did not have the mutation p.Ser154Asp (Fig. 1e; data not shown).

mPA-PLA$_1\alpha$ protein has three catalytic residues, S154, D178 and H248, all of which are critical for its enzymatic activity (Fig. 1f).[3,5] Similar to the mutation His248Asn,[5] the Ser154Asp is a non-conservative amino acid change occurring at a catalytic residue of mPA-PLA$_1\alpha$, suggesting that the Ser154Asp is also a loss-of-function mutation.

Interestingly, there was a significant difference in severity between the two patients within the family. While lacking any evidence, differences in genetic background (e.g. variants in other genes expressed in the hair follicles), sex and/or environmental factors may be involved in determining the severity.

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CONFLICT OF INTEREST: None declared.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary methods.

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ORIGINAL ARTICLE

Expression studies of a novel splice site mutation in the *LIPH* gene identified in a Japanese patient with autosomal recessive woolly hair

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ABSTRACT

Autosomal recessive woolly hair (ARWH) is characterized by short and tightly curled scalp hair without any obvious complications. The disease is known to be caused by either lipase H (*LIPH*) or *LPAR6* genes. Proteins encoded by these two genes are closely related to each other in a lipid-signaling pathway that is believed to play crucial roles in hair follicle development and hair growth. In the Japanese population, most affected individuals with ARWH have been shown to carry two prevalent founder mutations in the *LIPH* gene, c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn), while other *LIPH* mutations have been occasionally identified. In this study, we analyzed a Japanese patient with ARWH, and identified compound heterozygous mutations in the *LIPH* gene, c.736T>A (p.Cys246Ser) and c.982+5G>T. The latter one was a novel splice site mutation in intron 7. Expression studies using blood-derived RNA from the patient detected the *LIPH* transcript from the c.736T>A mutant allele, but not from the c.982+5G>T mutant allele. Furthermore, *in vitro* transcription assay in cultured cells showed that the mutation c.982+5G>T caused an aberrant splicing event, leading to a frame-shift and a premature termination codon (p.Met328Serfs*41). To the best of our knowledge, this is the second splice site mutation in the *LIPH* gene, and our findings further expand the spectrum of the *LIPH* mutations underlying ARWH.

Key words: hypotrichosis, lipase H, *LIPH*, splice site, woolly hair.

INTRODUCTION

Woolly hair (WH) is a hair shaft anomaly characterized by tightly curled hair which typically stops growing at a few inches.[1] In most cases, WH can be associated with hypotrichosis. Hereditary WH is largely classified into syndromic and non-syndromic forms. The non-syndromic form of WH shows either autosomal dominant (Online Mendelian Inheritance in Man [OMIM] no. 194300) or recessive (ARWH; OMIM no. 278150/604379) inheritance trait. Of these, ARWH has been reported to be caused by mutations in either lipase H (*LIPH*) or *LPAR6* genes.[2–5] The *LIPH* gene encodes the membrane-associated phosphatidic acid-selective phospholipase A_1 (mPA-PLA$_1\alpha$) that produces 2-acyl-lysophosphatidic acid (LPA),[6] and *LPAR6* encodes an LPA receptor, known as LPA_6.[7,8] Importantly, both mPA-PLA$_1\alpha$ and LPA_6 are abundantly expressed in the inner root sheath of human hair follicles,[3,5,9] thus it is considered that the mPA-PLA$_1\alpha$/LPA/LPA_6 signaling pathway plays crucial roles in supporting and molding the hair growth.

In regards to the *LIPH* gene, nearly 20 distinct mutations have been identified in several populations including the Japanese population.[2,3,10–22] It is worth noting that most Japanese patients with the disease share common founder mutations in the *LIPH* gene, which are c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn), reflecting the fact that there are heterozygous carriers for both mutations in extremely high frequencies.[16,23] In the present study, we analyzed a Japanese patient with ARWH and identified a novel splice site mutation in the *LIPH* gene. We further investigated how the mutation affected expression of the *LIPH* mRNA.

METHODS

Patient

The patient was an 8-year-old boy of Japanese origin. He visited Shin-Osaka Clinic in Osaka, Japan, due to his hair symptoms. He had had short and tightly-curled scalp hair since his birth, and hair loss had gradually progressed with aging (Fig. 1). His facial and body hair looked normal. In

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