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Gene Corner

A novel *PTPN11* missense mutation in a patient with LEOPARD syndrome

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LEOPARD syndrome (LS) is an autosomal dominant, multiple congenital anomaly syndrome so named because it is characterized by multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormal genitalia, retardation of growth and sensorineural deafness.[1] We report a Japanese boy with LS harbouring the novel PTPN11 (protein-tyrosine-phosphatase nonreceptor type 11) gene mutation c.1381G>T (p.Ala461Ser).

An 8-year-old boy had lentigines at birth which gradually increased in number with age. Physical examination revealed multiple light or dark brown lentigines of various sizes scattered over the whole body, including the face (Fig. 1a–c). He also had pigeon breast (Fig. 1d) and cryptorchidism. There was no sensorineural deafness, ocular hypertelorism, growth retardation or cardiology abnormalities including electrocardiographic conduction defect (Fig. 1e–g). A diagnosis of LS was made based on multiple lentigines, skeletal anomalies and genitourinary abnormalities.

The patient's parents gave their written informed consent as the patient's legal guardians. Direct sequencing of the entire coding regions, exon 2 to exon 15, of PTPN11 (GenBank accession number NT123456) revealed a single nucleotide substitution at codon 461 in exon 12 on one allele of PTPN11 (TGC to TTC; alanine to serine; p.Ala461Ser) (Fig. 2a). The

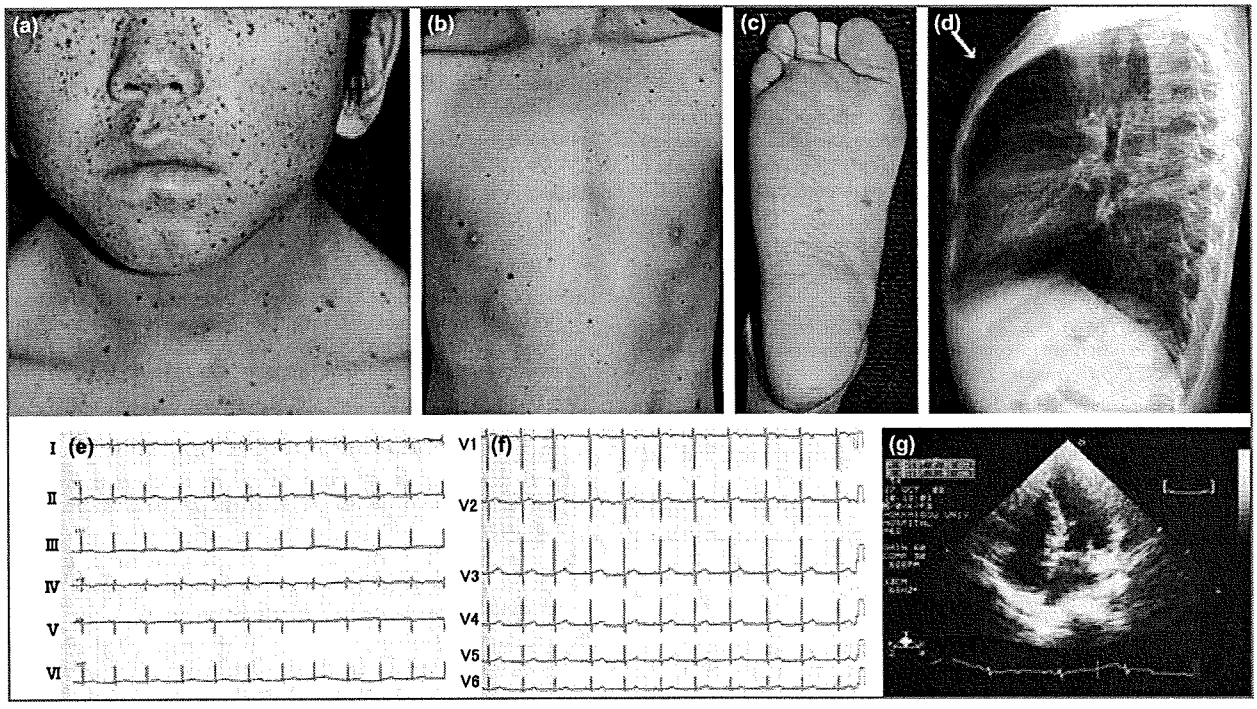

Fig 1. Clinical features of the patient. (a) Multiple light or dark brown lentigines of varied sizes scattered on the face. (b) Pigeon breast and various-sized lentigines are seen on the chest. (c) Lentigines are also observed on the sole. (d) Lateral chest radiograph demonstrates forward protrusion of the breastbone (arrow). (e, f) Electrocardiogram showing a normal sinus rhythm without any conduction defect: PQ duration 0·12 s (normal atrial ventricular conduction delay), QRS axis 90, QRS duration 0·08 s (normal QRS axis, no ventricular conduction disorders), no left ventricular hypertrophy or right ventricular hypertrophy pattern on precordial lead. (g) An apical four chamber view of echocardiogram revealing a normal heart structure and a balanced atrial and ventricular size: normal left ventricular size and wall thickness (end-diastolic left ventricular dimension 37·3 mm, 97% of normal, end-diastolic left ventricular posterior wall thickness 5·8 mm, 99% of normal), normal left ventricular pump function (left ventricular fractional shortening, 0·39), no right ventricular outflow tract stenosis or pulmonary stenosis.

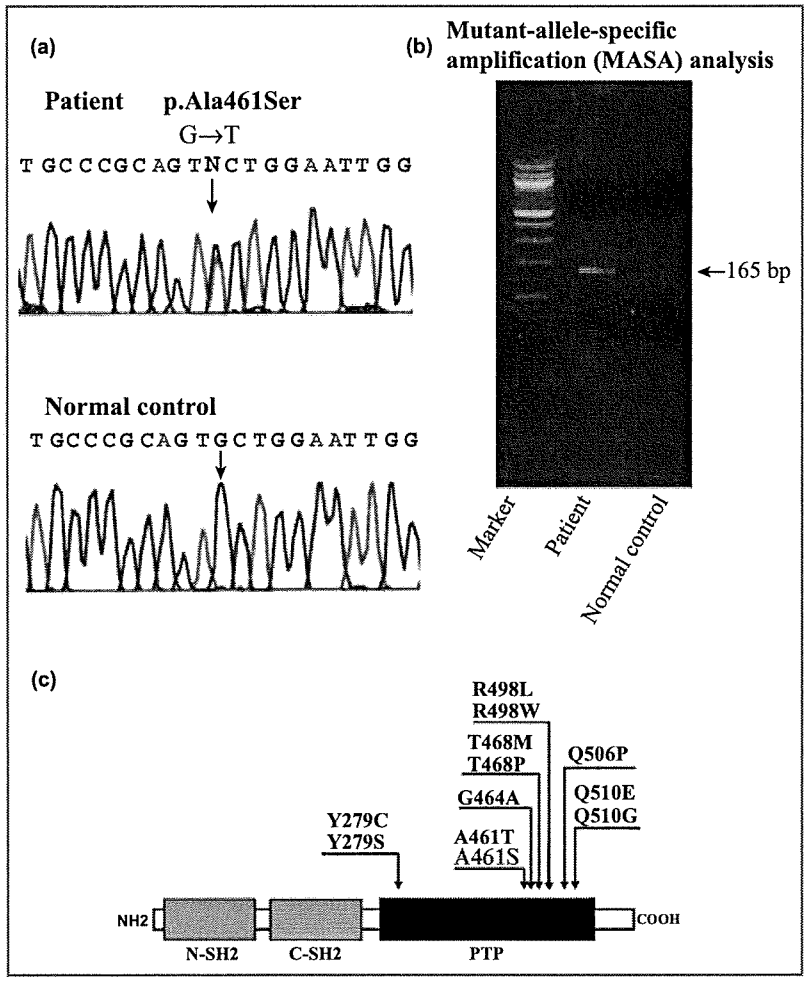

Fig 2. PTPN11 mutation analysis. (a) Direct sequencing of polymerase chain reaction (PCR) products from the patient and a normal control. Heterozygous c.1381G>T (p.Ala461Ser) mutation is found in exon 12 of PTPN11 of the patient, but not of a normal individual. (b) Mutant allele-specific amplification analysis. The amplification band from the mutant alleles is detected as a 165-bp fragment only in the PCR product from the genomic DNA sample of the patient, and not in the PCR product from the control DNA samples, confirming the presence of the mutation p.Ala461Ser in the patient. (c) Summary of PTPN11 mutations reported in LEOPARD syndrome. Note the mutations cluster at the PTP enzyme active site. The mutation found in the present study is p.Ala461Ser (red characters).

oligonucleotide primers and polymerase chain reaction (PCR) conditions were as previously described.[2] No other mutation was found in any part of the coding region or at any of the intron/exon borders of PTPN11. Mutant allele-specific amplification analysis (MASA) was performed with mutant allele-specific primers carrying the substitution of two bases at the 3′-end (mutant allele-specific primers: forward, TTGTCCTTC-TGCCCGCAGCT; reverse, CCCAGACTGTTTTCGTGAGCAC) and a 165-bp fragment derived from the mutant allele was amplified from the patient's genomic DNA (Fig. 2b). MASA showed no PCR product band from the control DNA samples (Fig. 2b). The mutation p.Ala461Ser was not found by sequence analysis in 200 alleles from 100 healthy unrelated Japanese individuals, so it was unlikely to be a polymorphism (data not shown).

The long-term prognosis of patients with LS is usually favourable. General growth, cardiology and hearing assessments were planned annually until adulthood for our patient.

PTPN11 is the major causative gene of LS, and patients with LS with PTPN11 mutations show various LS phenotypes.[3] PTPN11 encodes an SRC homology 2 (SH2) domain-containing protein-tyrosine-phosphatase (SHP-2) protein characterized by two SH2 domains and one protein-tyrosine-phosphatase (PTP) domain. The two SH2 domains interact with the PTP domain, keeping it folded and inactive. Upon binding of an appropriate SH2-binding protein (several growth factor receptors and docking proteins, e.g. GAB family members), this closed structure is opened, allowing a substrate to access the PTP enzyme active site. SHP-2 functions as a cytoplasmic signal transducer downstream of multiple receptors for growth factors, cytokines and hormones.

Mutations in the PTPN11 gene are associated with LS and Noonan syndrome (NS).[3,4] Most NS mutations disrupt key connections between the N-SH2 and PTP domains, resulting in biochemically and biologically 'active mutants' of SHP-2. In contrast, all 11 known LS mutation sites are confined to the seven residues predicted to affect catalytic activity of the PTP domain, in exons 7, 12 and 13 (Fig. 2c),[5] and LS mutations are thought to result in open, catalytically impaired forms of SHP-2.[6,7]

Among the PTPN11 mutations underlying LS, pTyr279Cys in exon 7 and p.Thr468Met in exon 12 are the prevalent mutations. It was reported that 65% of patients with LS harbour pTyr279Cys or p.Thr468Met.[5] As for genotype/phenotype correlations in patients with PTPN11 mutations, an association between exon 7 and exon 12 mutations and hypertrophic

cardiomyopathy, and an association between exon 8 mutations and pulmonary valve stenosis have been established.[5] The present patient with LS harboured p.Ala461Ser in exon 12 and showed multiple lentigines, pigeon breast and cryptorchidism, but he did not have electrocardiographic conduction defect, sensorineural deafness, ocular hypertelorism or growth retardation. Alanine-461 is in a highly conserved catalytic PTP-loop region (454–467) that contacts tyrosine-279.[8] Alanine is a hydrophobic nonpolar amino acid. In contrast, serine is a hydrophilic, polar and neutral amino acid. p.Ala461Ser might contort the catalytic site and interfere with substrate phosphotyrosine binding. It is noteworthy that another mutation at the identical codon of SHP-2, p.Ala461Thr, was reported in two other patients with LS. The two patients showed more severe clinical features than the present case, and the bulkier threonyl substitution at alanine-461 is expected to contort the catalytic site and interfere with substrate phosphotyrosine binding.[8] One of these patients with LS had multiple lentigines, hypertelorism, short stature, cardiovascular lesions, sensorineural deafness and abnormal genitalia.[9] Interestingly, the other had Noonan-like/multiple giant cell lesion syndrome with a complex phenotype that progressed over the years from NS to LS and presented cyst-like lesions of the bones.[10] The present case further confirms that mutations in an identical residue can lead to different phenotypes in LS.

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Key words: LEOPARD syndrome, PTPN11, SHP-2

Conflicts of interest: none declared.

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Disseminated cutaneous *Mycobacterium kansasii* infection in an patient infected with the human immunodeficiency virus

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People infected with the human immunodeficiency virus (HIV) are at a greater risk of mycobacterial infection and more than half of HIV-infected patients in developing countries are co-infected with mycobacteria. Therefore, mycobacterial infection is an important life-threatening complication in patients with HIV. In addition, immunocompromised hosts with both tuberculosis and nontuberculous mycobacterial infections often show atypical clinical features, which can make it difficult for clinicians to make a precise diagnosis. We report a case of *Mycobacterium kansasii* infection in an patient with acquired immunodeficiency syndrome (AIDS) who developed extensive, cutaneous nodules and ulcers without any sign of pulmonary involvement.

A 34-year-old woman was referred to our dermatology clinic with an 8-month history of high fever and disseminated subcutaneous nodules and skin ulcerations.

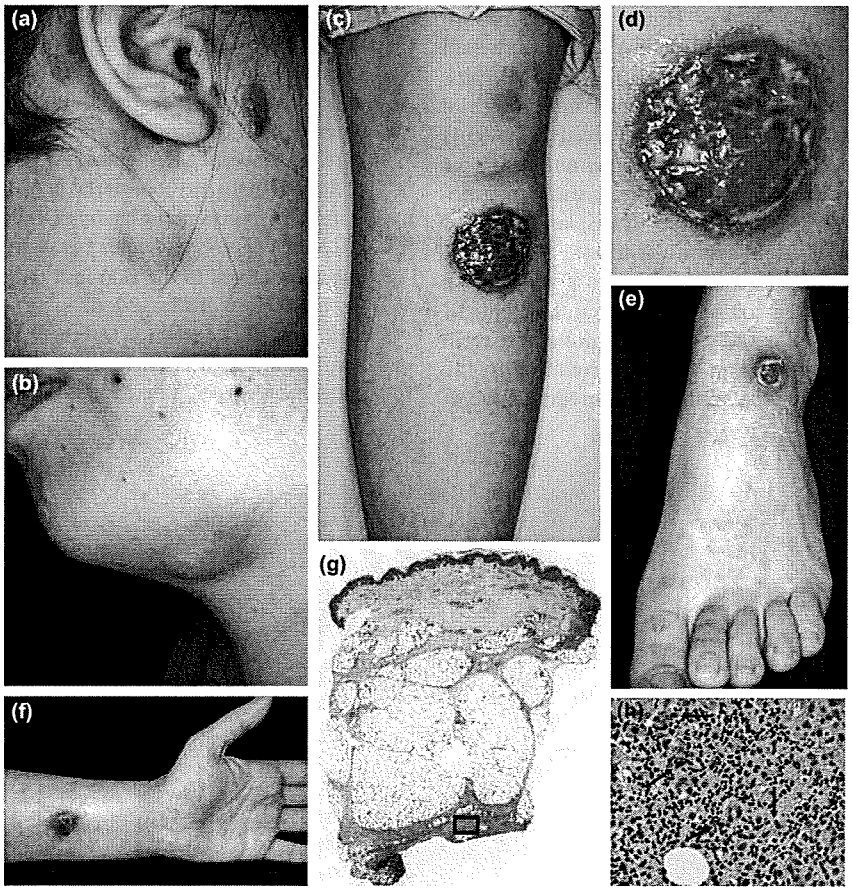

Figure 1 (a, b) Tender erythematous subcutaneous nodules scattered over the patient's neck and face; (c–f) skin ulcers on (c, d) the left lower thigh, (e) ankle and (f) forearm. (g, h) Large infiltrates of neutrophils associated with nontuberculous epithelioid granuloma (arrows). Haematoxylin and eosin; original magnification (g) × 2; (h) × 400.

On physical examination, subcutaneous nodules, up to 60 mm in size, scattered over her face (Fig. 1a) and limbs. On the lower left thigh, ankle and forearm, cutaneous ulcers, 20–40 mm in size were seen (Fig. 1b,c).

Histopathological examination of a biopsy taken from a nodule on the left forearm showed a large infiltrate of neutrophils associated with nontuberculous granuloma (Fig. 1d). Ziehl–Neelsen stain showed numerous acid-fast bacilli, which were confirmed as *M. kansasii* by DNA hybridization studies.

Results of laboratory investigations showed that the patient was positive for HIV-1, and her peripheral CD4 cell count was zero. Systemic examination showed extracutaneous signs of *M. kansasii* infection, including pulmonary nontuberculous mycobacteriosis.

The patient was treated with isoniazid 300 mg/day, ethambutol 750 mg/day, rifampin 450 mg/day and clarithromycin 800 mg/day, which resulted in gradual improvement. One month after beginning antimycobacterial treatment, the patient was started on highly active antiretroviral treatment.

M. kansasii is a slowly growing species that usually inhabits water supplies, swimming pools and sewage, and seldom infects healthy people.[1] However, immunosuppressed patients are often infected with *M. kansasii*, which usually causes pulmonary infection. Cutaneous *M. kansasii* infection is very rare and importantly, most cases of cutaneous *M. kansasii* infection have occurred in patients who are immunocompromised due to chemotherapy or immunosuppressive therapy for conditions such as autoimmune disease, renal or cardiac transplantations.[2,3] Cutaneous *M. kansasii* infection without pulmonary involvement has been reported in only two patients with AIDS, who both a showed solitary skin lesion. One patient had an asymptomatic ulcerative lesion around the right inguinal fold and the other had abscess formation on the thigh associated with regional lymph-node enlargement.[4,5] Our patient differs from these previous reports in that she had severe disseminated skin lesions, probably due to the considerably reduced number of peripheral blood CD4 cells.

The population of people infected with HIV has been increasing annually worldwide. In addition to the commonly observed nontuberculous mycobacterial infections with *Mycobacterium avium* and *Mycobacterium intracellulare*, clinicians should consider other uncommon mycobaceterial species such as *M. kansasii*, in order to ensure prompt and appropriate treatment for patients with HIV.

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Acta Derm Venereol 2009; 89: 445–447

QUIZ SECTION

Progressive Refractory Ulcer of the Nipple: A Quiz

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A 50-year-old woman presented with erosive erythema and effusion on the right nipple one year previously, which gradually became ulcerated and painful. One month after the initial presentation, the ulcer was excised by a breast surgeon. Histopathological examination of the resected tissue revealed non-specific inflammation with no evidence of malignancy. The wound healed completely, but an ulcer reappeared at the same site 3 months later. The same surgical operation was performed again and the wound healed, but the ulcer reappeared 2 months after that operation.

More than 6 months later the patient was finally referred to our hospital with an ulcer on the right breast. Examination revealed an ulcer approximately 3 cm in diameter in the right areola (Fig. 1). Bacterial, fungal and mycobacterial cultures from the ulcer were all negative. Histopathological observations of a skin biopsy from the edge of the ulcer showed necrosis of the epidermis forming the ulcer, and mixed inflammatory cell infiltrate with abscess formation at the base of the ulcer. The patient had been healthy except for hyperlipidaemia and a liver cyst.

What is your diagnosis? See next page for answer.

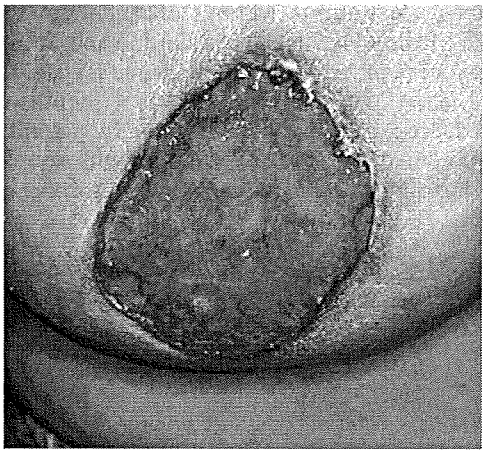

Fig. 1. Painful ulcer of the right nipple.

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Progressive Refractory Ulcer of the Nipple: Comment

Acta Derm Venereol 2009; 89: 445–447 (contd.)

Diagnosis: Pyoderma gangrenosum

At first, severe mastitis and invasive breast cancer were considered as a differential diagnosis for pyoderma gangrenosum (PG) of the breast.

The patient was treated with oral prednisolone, 0.5 mg/day/kg, topical corticosteroid ointment applied to the right side of the ulcer and tacrolimus ointment applied to the left side of the ulcer. Her pain diminished dramatically, and the ulcer epithelialized on both the right and the left sides. The wound healed completely 6 months after the start of the medication, at which time the patient stopped taking prednisolone (Fig. 2). Nine months after stopping the prednisolone, there has been no recurrence.

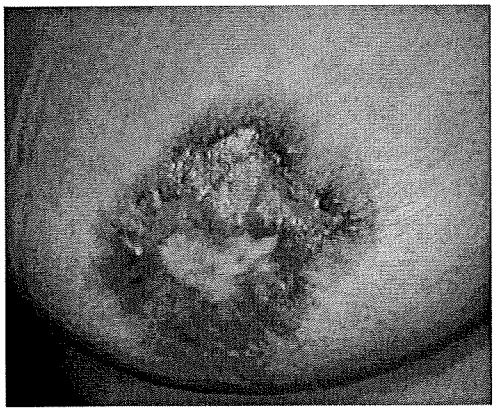

Fig. 2. Complete healing after 6 months of systemic corticosteroid treatment.

PG is a relatively rare non-infectious neutrophilic dermatosis induced by minor skin trauma or underlying systemic disorders (1, 2). For early and mild PG lesions, several kinds of treatment, including topical or intralesional corticosteroids or topical tacrolimus, are reported to be effective (2). Systemic corticosteroids and/or cyclosporine appear to be effective in most cases and should be considered as the first-line therapy (3).

PG can affect any site of the body, but it is most common in the lower limbs. However, its occurrence on the breast is extremely rare. To our knowledge, only 28 cases of PG on the breast have been reported, 22 of which developed after injury, skin biopsy or operation such as mammaplasty (4). To our knowledge, only one case of PG of the breast without any skin trauma or underlying systemic disease has been reported previously (5).

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Delayed contralateral hemiplegia or hemiparesis has been rarely reported as a complication of herpes zoster ophthalmicus.[3] However, there were two suggested causes of this complication. Firstly, the VZV spread directly along the intracranial branches of the trigeminal nerve to the ipsilateral arterial walls via the afferent trigeminal ganglionic fibres.[2,4] Secondly, the inflammatory process spread from the trigeminal ganglion to nearby blood vessels, leading to thrombosis and distal embolization.[3]

PCR analysis of CSF is a specific and sensitive test for VZV detection and is the mainstay for diagnosing the neurological complications of VZV infection in patients.[2] Intravenous aciclovir for 10–14 days is recommended in adults with VZV arteritis.[5] Our patient was an immunocompetent middle-aged man with herpes zoster and zoster ophthalmicus complicated by meningitis and delayed ipsilateral ICH even though he was treated appropriately with intravenous aciclovir. In general, there are two main mechanisms of the ICH related to infection or inflammation; rupture of an intracranial aneurysm or cerebral venous sinus thrombosis.[5] However, our patient had no evidence of an intracranial aneurysm or of venous sinus pathology on the CT angiogram.

To our knowledge, this is the first report of herpes zoster complicated by delayed ICH in the dermatology literature, although there are a few reports of ICH complicating herpes zoster in the medical literature of other fields of study.

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Conflict of interest: none declared.
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Widespread keratosis follicularis squamosa

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Keratosis follicularis squamosa (KFS), first described by Dohi and Momose in 1903,[1] is characterized by small, scattered, scaly lesions up to 10 mm in diameter with tiny pigmented keratotic plugs in the centre. We describe a case of KFS that involved extraordinarily large areas of the body.

A 42-year-old Japanese woman presented with a 2-year history of a symmetrical, scaly eruptions over her body, extending to the legs (Fig. 1a–c). She had been treated with topical corticosteroid ointments for >1 year. She had also

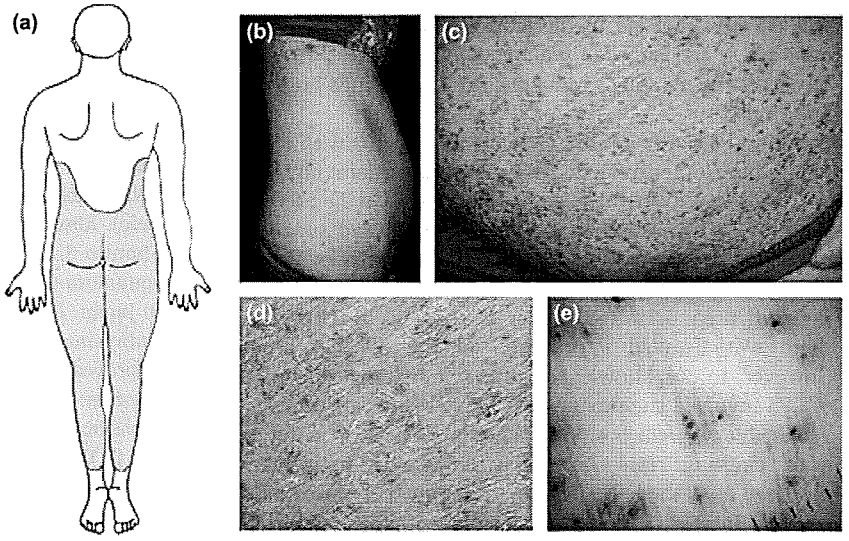

Figure 1 (a) Distribution of lesions involving the axilla, buttocks, lower abdomen, the posterior thighs and lower legs; (b,c) small scattered scaly patches up to 10 mm in diameter with small pigmented keratotic plugging in the centre seen on (b) the lower axilla and (c) buttocks; (d) the margin of each scale was slightly detached from the plaques. (e) Dermatoscopy shows brown dots in the centre associated with hair follicles, but no pigment network was seen.

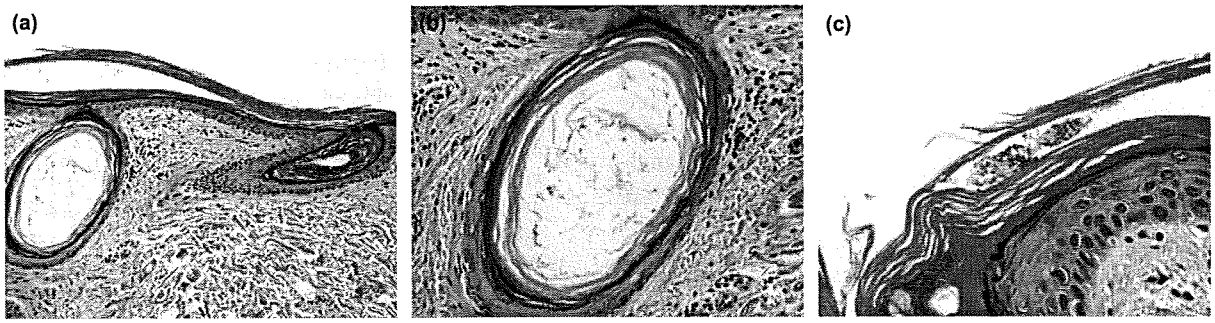

Figure 2 (a–c) Dilated hair follicles with keratotic plugging and separated horny layer from epidermis at the periphery (haematoxylin and eosin; original magnification × 100); bacterial structures were seen in (b) dilated hair follicle and (c) horny layers.

been taking various drugs (chlorpromazine hibenzate, etizolam, flunitrazepam, triazolam and promethazine methylene disalicylate) for 10 years to treat depression. There was no family history of note.

Physical examination revealed small, scattered, scaly lesions up to 10 mm in diameter with tiny pigmented keratotic plugs in the centre (Fig. 1d,e). The margin of each scale was slightly detached from the plaques.

Dermoscopic observation revealed brown dots in the centre of each lesion associated with hair follicles. Laboratory examinations, including measurement of oestrogen, progesterone and prolactin levels, showed no abnormalities.

Histopathological examination of a biopsy taken from a lesion showed hyperkeratosis without parakeratosis, dilated hair follicles with keratotic plugging, and a horny layer separated from the epidermis at the periphery of the lesion (Fig. 2a). Bacterial debris-like material was observed in the dilated hair follicle (Fig. 2b) and horny layers (Fig. 2c). Staining with periodic-acid–Schiff did not reveal any fungal structures. No specific changes were observed in the dermis.

A diagnosis of KFS was made. Topical application of moisturizing cream (0.3% heparinoid cream) to the lesions was effective and led to the clinical disappearance of the majority of the lamellar scaling within 1 year, but some tiny pigmented papules remained on the patient's buttocks and thighs.

Yajima *et al.* reviewed 201 Japanese patients with KFS.[2] According to their review, KFS occurred predominantly in the third and fourth decades of life, with a male : female ratio of 1 : 1.6. The distribution of the affected sites was restricted: abdomen 53.7%, thighs 35.1%, buttocks 34.5%, waist 33.5%, axilla 32.9%, back 12.7%, arms 5.3%, inguinal area 4.7% and lower legs 3.7%, in 188 cases there was detailed information of the affected areas. However, to our knowledge, no previously reported cases of KFS involved such extensive areas as our patient.

Hereditary predisposition, bacterial infection, irritation from clothing, and hormonal imbalance have been proposed as pathogenic factors, although these factors fail to explain the exact pathogenesis of this abnormal keratinization.[3] In some cases, Gram-positive cocci have been observed in the central plugging in the hair follicles.[4] In our patient, continuous long-term topical corticosteroid therapy might have caused the proliferation of bacteria and contributed to the build-up of bacterial material in the dilated hair follicle and horny layers, and led to this peculiar clinical feature.

To date, as many as 245 Japanese cases of KFS have been described, but only three cases have been reported in other countries (China, Korea and Russia).[5] Although KFS is a well-known disease in Japan, it has not been widely reported in the English literature. Additional cases of this skin disorder from other ethnic groups should clarify the prevalence and pathogenesis of KFS.

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CXCL8 in the NOD1 shRNA-expressing cells is mediated by such receptors.

In summary, to our knowledge this is the first report demonstrating that the intracellular receptor NOD1 is functional expressed in human keratinocytes, suggesting that NOD1 may be involved in cutaneous innate immunity. Further studies are needed to understand the contribution of intracellular innate immune receptors to cutaneous host defense.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Prevalent and Rare Mutations in the Gene Encoding Filaggrin in Japanese Patients with Ichthyosis Vulgaris and Atopic Dermatitis

Journal of Investigative Dermatology (2009) **129,** 1302–1305; doi:10.1038/jid.2008.372; published online 27 November 2008

TO THE EDITOR

Mutations in the gene encoding filaggrin (*FLG*) were identified as the underlying cause of ichthyosis vulgaris (IV; OMIM #146700) and also shown to predispose to atopic dermatitis (AD; Palmer *et al.*, 2006; Smith *et al.*, 2006). Although *FLG* is considerably difficult to analyze because of its large size (>12 kb) and highly repetitive nature, PCR strategy that permits routine and comprehensive sequencing of the entire *FLG* has been developed recently (Sandilands *et al.*, 2007).

Abbreviations: AD, atopic dermatitis; FLG, filaggrin; IV, ichthyosis vulgaris

Using this methodology, we have identified four prevalent *FLG* mutations in Japanese patients with IV (Nomura *et al.*, 2008). We also demonstrated that *FLG* mutations were significantly associated with AD and the frequency of these *FLG* mutations observed in our Japanese AD cohort was about 20%. However, the frequency in our cohort

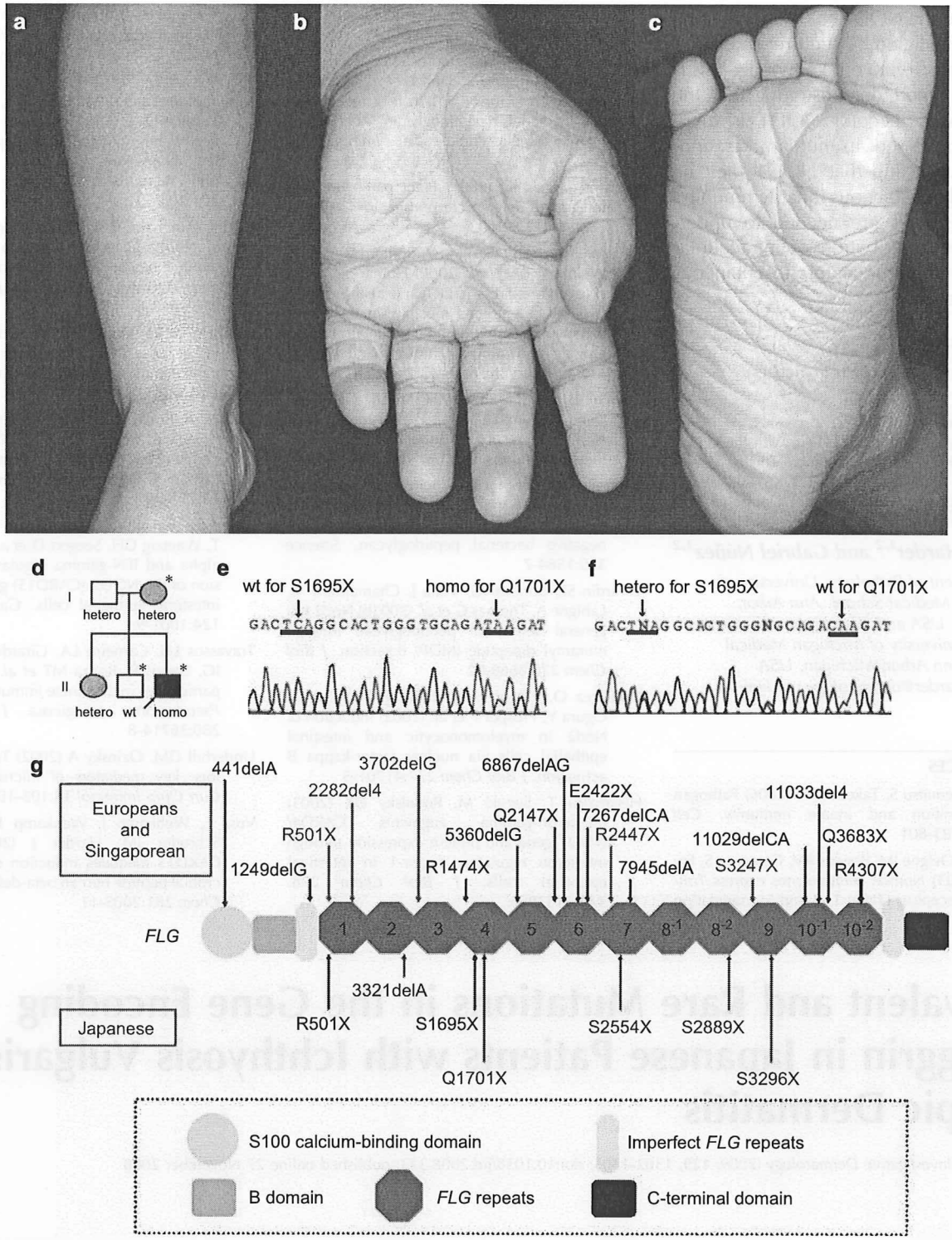

Figure 1. Clinical features and results of mutation analysis. (**a**) Fine scaling clearly visible on the proband's leg. (**b**, **c**) He also showed marked palmoplantar hyperlinearity. (**d**) A family tree of the ichthyosis vulgaris family shows the semidominant inheritance pattern. Solid symbols refer to the marked ichthyosis vulgaris presentation; cross-hatched symbols refer to the milder ichthyosis vulgaris presentation. In addition, the proband, his mother, and sister had concomitant dermatologist-diagnosed atopic dermatitis (*). wt, wild type for Q1701X; hetero, heterozygous; homo, homozygous. (**e**, **f**) A homozygous transition mutation c.5101C>T was identified in the proband, resulting in Q1701X. A heterozygous transition mutation c.5084C>G was identified in one Japanese individual in the control population, resulting in S1695X. Mutation S1695X is located only six amino acids upstream from Q1701X. (**g**) Loss-of-function *FLG* mutations are shown in a schematic of profilaggrin. Mutations shown in red are prevalent; those in black are rare. Some individuals have duplication of the 8th and/or 10th filaggrin repeat(s). Duplicated filaggrin repeats are represented as 8-1, 8-2, 10-1, and 10-2.

was still lower than that seen in analogous European case series, where it is up to 48% (Barker *et al.*, 2007; Sandilands *et al.*, 2007). Furthermore, it was reported that up to 37% of Japanese patients with AD had concomitant IV (Uehara and Hayashi, 1981; Uehara and Miyauchi, 1984). Taken together, there might be further prevalent *FLG* mutations to be discovered in the Japanese population. Here we have studied a further Japanese family with IV and identified two further *FLG* mutations.

A newly recruited Japanese family with IV was studied. The proband, a one-year-old Japanese boy, showed marked scaly dry skin on the extensor limbs and trunk (Figure 1a). Marked palmoplantar hyperlinearity was also evident (Figure 1b and c). A diagnosis of IV was made from these clinical observations. His mother and sister also showed scaly dry skin and palmoplantar hyperlinearity, but the clinical severity was mild compared to the proband (Figure 1d). Therefore, the inheritance pattern seemed semidominant. The proband, his mother, and his brother had concomitant AD.

The medical ethical committee at Hokkaido University Graduate School of Medicine approved all the studies. The study was conducted according to the Declaration of Helsinki Principles. Participants or their legal guardians gave their written informed consent. Following informed consent, genomic DNA from all family members was extracted from peripheral blood according to standard procedures. Initially, all family members were screened for five *FLG* mutations identified in Japanese population so far, R501X, 3321delA, S2554X, S2889X and S3296X, by restriction enzyme digestion, fluorescent PCR, and direct DNA sequencing as described previously (Nomura *et al.*, 2007, 2008; Hamada *et al.*, 2008). However, all individuals were wild type for these variants. Thus, we carried out full sequencing of the *FLG* as described previously (Sandilands *et al.*, 2007), which led to the identification of a previously unreported nonsense mutation Q1701X in repeat 4 in the present family (Figure 1e). The proband turned out to be homozygous for this truncation mutation and his non-consanguineous parents and his sister heterozygous, whereas his brother wild type (Figure 1d). It was also confirmed that they carry no pathogenic mutations in the other *FLG* repeats. Then, we screened 118 unrelated Japanese patients with AD and 134 unrelated Japanese control individuals for Q1701X by direct DNA sequencing. The diagnosis of AD in our case series was made by experienced dermatologists, according to the AD diagnostic criteria by Hannifin and Rajka (1980). Notably, mutation Q1701X was also identified in two Japanese patients with AD (1.7%), which brings the total number of recurrent *FLG* mutations so far identified in Japanese population to five.

During the screening for Q1701X, we identified another previously unreported *FLG* mutation, S1695X, which is located only six amino acids upstream from Q1701X, in the general Japanese control population (Figure 1f). We screened 33 Japanese patients with IV and 118 with AD for S1695X, but all patients were wild type for this mutation. Only one heterozygote was identified in the control population. Therefore, S1695X seems to be an extremely rare *FLG* mutation in Japanese individuals. The control individuals had not been examined in relation to AD or IV status, that is, they were population controls rather than "hypernormal" controls, so no clinical details about the individual carrying S1695X are available. In total, there are at least seven *FLG* variants in the Japanese population, including five that are prevalent and two that are quite rare.

The *FLG* genotype data in the Japanese AD case series and ethnically matched population control series are summarized in Table 1. In this study, case–control association analyses were performed by using Pearson's r^2 statistics, as previously described (Palmer *et al.*, 2006). All alleles were observed to be in normal Hardy–Weinberg equilibrium. Here we demonstrate that about 25% of patients in our Japanese AD case series carry one or more of these seven *FLG* mutations (combined minor allele frequency = 0.127, $n = 236$) and these variants are also carried by 4% of general Japanese control individuals (combined minor allele frequency = 0.019, $n = 268$). There is significant statistical association between the seven *FLG* mutations and AD (r^2 $P = 1.75 \times 10^{-6}$). Moreover, AD was manifested in heterozygous carriers of these *FLG* mutations with a Fisher's exact test odds ratio for AD of 6.8 (95% CI 2.5–18.5, $P = 3.7 \times 10^{-5}$), implying a causal relationship between *FLG* mutations and AD. Taken together, these data strongly suggest that skin barrier impairment because of reduced filaggrin expression is important in the pathogenesis in AD.

To date, 24 *FLG* mutations, including the two identified in this study, have been reported in the European, Japanese, and Singaporean populations (Sandilands *et al.*, 2007; Chen *et al.*, 2008; Nomura *et al.*, 2008). Interestingly,

Table 1. Atopic dermatitis case–control association analysis for *FLG* null variants in Japan

	R501X		3321delA		S1695X		Q1701X		S2554X		S2889X		S3296X		Combined	
Genotypes	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
AA	134	118	133	113	133	118	134	116	133	112	132	105	134	114	129	91
Aa	0	0	1	5	1	0	0	2	1	6	2	13	0	4	5	24
aa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Total	134	118	134	118	134	118	134	118	134	118	134	118	134	118	134	118

For combined genotype, r^2 $P = 1.75 \times 10^{-6}$; Fisher's exact test odds ratio=6.8 (95% CI 2.5–18.5).

mutations found in Japanese are different from those found in Europeans and Singaporean (Figure 1g), except in one case of the common European R501X mutation occurring as a very rare mutation on a different haplotype in the Japanese population (Hamada *et al.*, 2008). These observations imply that every population is highly likely to have a unique set of *FLG* mutations.

In conclusion, we have identified two further *FLG* mutations in the Japanese population. We also showed that at least about 25% of Japanese patients with AD carried one or more of *FLG* mutations. As we have sequenced more than 30 Japanese patients with IV, there is now little possibility that further highly prevalent mutations underlie the Japanese population. Taking the high frequency (up to 37%) of concomitant IV in patients with AD into account, however, it is still possible that there might be further multiple low-frequency *FLG* mutations to be discovered in the Japanese population. Further *FLG* mutation analysis will be necessary to understand the more precise genetic architecture of filaggrin-related AD in Japan.

CONFLICT OF INTEREST

Irwin McLean has filed patents relating to genetic testing and therapy development aimed at the filaggrin gene.

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"White" Nevi and "Red" Melanomas: Association with the RHC Phenotype of the *MC1R* Gene

Journal of Investigative Dermatology (2009) **129,** 1305–1307; doi:10.1038/jid.2008.378; published online 4 December 2008

TO THE EDITOR

In 2002, we reported on three patients presenting with melanocytic nevi lacking pigmentation, which we named "white" dysplastic melanocytic nevi (DMN) due to their peculiar clinical appearance of white to pale red macules with accentuated skin markings and a silvery "shining" when observed with tangential light (Zalaudek *et al.*, 2002). Notably, all three patients had melanoma, and in one patient white DMN were associated with two primary amelanotic melanomas (AMMs).

We present herein a 25-year-old woman (skin type I, red hair, and blue eyes), who sought consultation for a mole check. Clinical examination revealed, besides approximately 30 slightly atypical light brown nevi on

Abbreviations: AMM, amelanotic melanoma; DMN, dysplastic melanocytic nevi; RHC, red hair color

A Novel Humanized Neonatal Autoimmune Blistering Skin Disease Model Induced by Maternally Transferred Antibodies[1]

Wataru Nishie,[2]* Daisuke Sawamura,*† Ken Natsuga,* Satoru Shinkuma,* Maki Goto,* Akihiko Shibaki,* Hideyuki Ujiie,* Edit Olasz,‡ Kim B. Yancey,§ and Hiroshi Shimizu*

All mammal neonates receive maternal Abs for protection against pathogenic organisms in the postnatal environment. However, neonates can experience serious adverse reactions if the Abs transferred from the mother recognize self-molecules as autoAgs. In this study, we describe a novel model for autoimmune disease induced by transferred maternal Abs in genetically transformed Ag-humanized mice progeny. Bullous pemphigoid is the most common life-threatening autoimmune blistering skin disease that affects the elderly, in which circulating IgG autoAbs are directed against epidermal type XVII collagen (COL17). We have established a genetically manipulated experimental mouse model in which maternal Abs against human COL17 are transferred to pups whose skin expresses only human and not mouse COL17, resulting in blistering similar to that seen in patients with bullous pemphigoid. Maternal transfer of pathogenic Abs to humanized neonatal mice is a unique and potential experimental system to establish a novel autoimmune disease model. ***The Journal of Immunology,*** **2009, 183: 4088–4093.**

During pregnancy and after birth, all mammal neonates receive various factors from their mothers to adapt to the new environment, including Abs for protection against pathogenic organisms (1, 2). However, this can result in serious adverse reactions in neonates if the transferred Abs recognize self-molecules as autoAgs. For example, neonatal lupus, which is clinically characterized by skin eruptions and fatal congenital heart block, is induced by autoAbs against Ro/SSA, Ro/SSB, or U1 ribonuclear protein transferred from mothers affected with Sjögren syndrome or systemic lupus erythematosus (3, 4). In addition, maternally transferred autoAbs against acetylcholine receptors can induce the characteristic features of myasthenia gravis in human neonates (5). This suggests that mothers, in experimental animal models, might be able to induce autoimmunity in their offspring.

One possible approach to using maternal Abs to produce disease models for autoimmune diseases is the use of gene-targeted mice (6). Immunizing Ag-knockout female mice with a targeted Ag can induce Abs against the antigenic molecule. Mating these immunized females with wild-type males could mimic autoimmune diseases in the neonates expressing antigenic peptides transcribed by paternal genes in the presence of circulating maternally transferred Ag-specific IgG (6). However, this approach has not achieved practical application, probably because gene-targeted mice often die soon after birth, especially when the targeted genes encode functionally important proteins (7–11). Consequently, another method that does not use lethal gene-deleted maternal mice is desirable. The difference in immune systems between humans and mice is another important problem underlying most of the current experimental autoimmune disease models. In fact, the autoAgs in existing autoimmune disease models have been the mouse's own proteins, which are expected to differ from those in the human autoimmune disease condition (12–14). Therefore, autoimmune disease models with human autoAg expression would be ideal.

In this study, we tried to produce a novel neonatal autoimmune disease model induced by passage of maternal IgG. We aimed at the most common and life-threatening autoimmune blistering skin disease, bullous pemphigoid (BP).[3] In BP, circulating IgG autoAbs are directed against type XVII collagen (COL17, formerly known as BP180 or BPAG2) in the skin (15, 16). COL17 is a type-II-oriented, 180kD hemidesmosomal transmembrane protein that anchors basal keratinocytes to the underlying epidermal basement membrane. The pathogenic epitope in COL17 is tightly clustered within the noncollagenous (NC) 16A stretch of its ectodomain (17, 18). Interestingly, due to significant differences between humans and rodents in the amino acids sequence in the NC16A region, mice that have received human IgG from BP patients fail to show any clinical, histological, or immunological findings consistent with BP (13, 14). We recently generated *Col17a1* gene-targeted (*mCol17*$^{-/-}$) mice as well as COL17-humanized mice by introducing human *COL17A1*cDNA (*hCOL17*$^{+/+}$) transgene driven under keratin 14 promoter into *mCol17*$^{-/-}$ mice (12, 19). Importantly, the *mCol17*$^{-/-}$ mice were too fragile to mate with male mice, but reproductive ability was restored in COL17-humanized (*mCol17*$^{-/-}$, *hCOL17*$^{+/+}$) mice (12). In this study, we used these genetically manipulated COL17-humanized mice to produce a novel neonatal autoimmune disease model induced by passage of maternal IgG.

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[3] Abbreviations used in this paper: BP; boullous pemphigoid; COL17; type XVII collagen; NC; noncollagenous; Tg, transgenic; IIF, indirect immunofluorescence; DIF; direct immunofluorescence, GST; gulutathione S-transferase.

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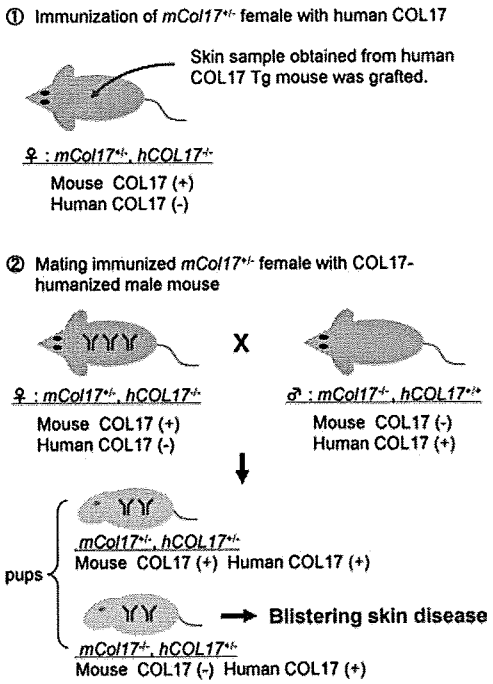

FIGURE 1. Schematic of the method for generating the neonatal BP model. Four-to 6-wk-old heterozygous $mCol17^{+/-}$ female mice (C57BL/6 background) were immunized against human COL17 by grafting skin obtained from gender-matched, syngeneic human COL17 cDNA Tg mouse driven under keratin 14 promoter (1). Two weeks after grafting, the immunized female mice were crossed with 6- to 8-wk-old COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) males. Theoretically, half of the newborns should express only human COL17 in the skin ($mCol17^{-/-}$, $hCOL17^{+/-}$), and these are expected to be a neonatal BP model (2).

Materials and Methods

Gross summary of strategy

We selected a breeding pair consisting of heterozygote *Col17a1*-deficient ($mCol17^{+/-}$) female mice and COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) male mice (Fig. 1). Theoretically, half of the pups from this pair should express only human COL17 in the skin while the other half should express both mouse and human COL17 (Fig. 1). Wild-type mice can develop quite high titers of circulating anti-human COL17 IgG when grafted with human COL17 transgenic (Tg) mouse skin (19). We first immunized $mCol17^{+/-}$ mother mice with skin grafts obtained from human COL17 Tg mouse, and then we mated the immunized $mCol17^{+/-}$ mother mice with COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) male mice. Neonatal COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) mice retained skin stability against mechanical friction (12); similarly, neonatal COL17-humanized mice heterozygously carrying human COL17 cDNA transgene ($mCol17^{-/-}$, $hCOL17^{+/-}$) showed none of the skin abnormalities seen in $mCol17^{-/-}$ mice, although it was possible to detach the epidermis by moderate mechanical friction (our unpublished data). We hypothesized that immunized $mCol17^{+/-}$ mother mice would produce circulating anti-human COL17 IgG that would be transferred into their neonates including those whose skin expressed only human and not mouse COL17 ($mCol17^{-/-}$, $hCOL17^{+/-}$), resulting in natural blistering that replicates human BP disease (Fig. 1).

Immunization of the heterozygote $mCol17^{+/-}$ female mice

Four- to 6-wk-old heterozygote-null $mCol17^{+/-}$ female mice (F_1 mouse was 129/SvEv × C57BL/6 background, back-crossed with C57BL/6 over 10 generations) were immunized against human COL17 as previously described (19), with minor modifications. In brief, 1 × 1 cm of back skin obtained from gender-matched, syngeneic human COL17 cDNA Tg mice was grafted onto the back of the recipient $mCol17^{+/-}$ female mice. As a control, back skin obtained from wild-type C57BL/6 was grafted onto recipient $mCol17^{+/-}$ female mice ($n = 5$). The grafted skin was sutured, and bandages were removed 7 days after skin grafting.

Generation of neonatal BP mice

Two weeks after skin grafting, the immunized and the control $mCol17^{+/-}$ female mice were crossed with 6- to 8-wk-old COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) male mice (12). Half of their newborns ($mCol17^{-/-}$, $hCOL17^{+/-}$) were predicted to express only human COL17 and not mouse COL17 in the skin and the other half of the newborns ($mCol17^{+/-}$, $hCOL17^{+/-}$) to express both mouse and human COL17 in the skin (Fig. 1).

Evaluation of serum anti-human COL17 IgG in the immunized mother mice and their neonates

Sera from immunized $mCol17^{+/-}$ females (before immunization and 1 to 4 wk after immunization) and their neonates (at birth and 1 to 4 wk after birth, respectively) were sampled, followed by ELISA and indirect immunofluorescence (IIF) to evaluate the circulating mouse IgG Abs directed against human COL17 (12, 19). The ELISA index value against the human COL17 NC16A domain peptide was measured using BP180 ELISA kit (MBL) with minor modifications. In brief, this kit is designed to detect human IgG against human COL17; therefore, HRP-conjugated goat polyclonal anti-mouse IgG (1/20,000 dilution, Jackson ImmunoResearch Laboratories) was used as a secondary Ab substitute for prepared HRP-conjugated anti-human IgG. The absorbance was measured at 450 nm by microtiter plate readers (Bio-Rad). For IIF studies, serum from the mice was serially diluted in PBS. Normal or 1M NaCl split human skin samples were obtained from a healthy volunteer and incubated with the sera for 30 min at 37°C, followed by staining with FITC-conjugated polyclonal goat anti-mouse IgG (1/100 dilution, Jackson ImmunoResearch Laboratories) as described previously (12, 19).

Immunopathological analysis of neonatal BP

For histological investigations, back skin of the mice was obtained at birth and 1 to 4 wk after birth, and processed for H&E staining and direct immunofluorescence (DIF) microscopy. For DIF study, FITC-conjugated goat polyclonal anti-mouse IgG (1/100 dilation, Jackson ImmunoResearch Laboratories), rat monoclonal anti-mouse IgG1, IgG2a, IgG2b (1/100 dilution, BD Pharmingen), goat polyclonal anti-mouse IgG2c (1/400 dilation, Bethyl Laboratories), and FITC-conjugated goat anti-mouse C3 (1/200 dilation, Cappel) were used (12, 14, 20).

Passive transfer of maternal IgG with or without immunoadsorption against human COL17 NC16A protein into neonatal COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) mice

Total IgG was purified from pooled sera obtained from 5 immunized $mCol17^{+/-}$ females (10 wks after skin grafting) using HiTrap Protein G HP (GE Healthcare) according to the manufactur's instructions. Recombinant human COL17 NC16A (amino acid: 490–566) protein was generated as a gulutathione S-transferase (GST) fusion protein as previously described (12), and 6 mg of the purified protein was coupled with 1 ml of GSTrap FF (GE Healthcare). Half of the purified total IgG was coupled with the human COL17 NC16A-GST protein in the column to eliminate Abs directing to human COL17 NC16A protein, and flow-through samples were collected. Total IgG with or without immunoadsorption using human COL17 NC16A protein were concentrated by Amicon Ultra-50 ultracentrifuge (Millipore), and each was adjusted to be 2.1 μg/μl. Fifty μl of Abs was i.p. injected into neonatal COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) mice as previously described (12).

All mouse procedures were approved by the Institutional Animal Care and Use Committee of Hokkaido University, and fully informed consent from all patients was obtained for the use of their materials.

Results

High titers of IgG Abs against human COL17 were induced in recipient mother mice and they were efficiently transferred to their neonates

Consistent with the previous report in which high titer of IgG against human COL17 were successfully induced when human COL17 Tg mouse skin was grafted onto the wild-type mice (19), the heterozygote $mCol17^{+/-}$ female mice also developed high titers of circulating anti-human COL17 IgG after skin grafting of human COL17 cDNA Tg mice skin (Fig. 2, *a–c*). ELISA studies clearly showed the presence of circulating anti-human COL17 IgG at 3 wk after skin grafting, and a maximum titer was reached at

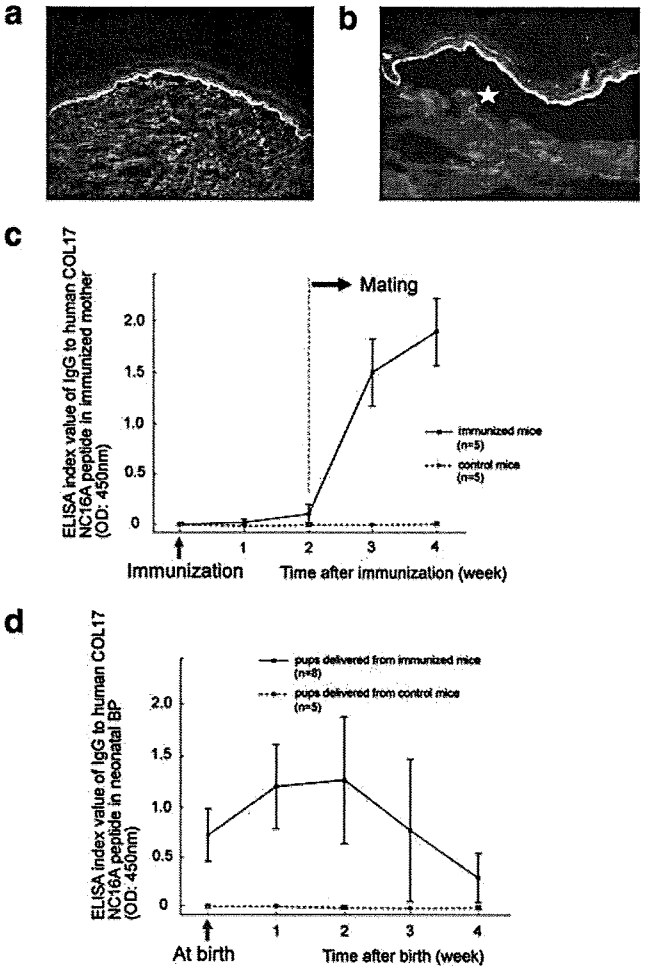

FIGURE 2. Profile of IgG Abs against human COL17 in immunized heterozygote *mCol17*$^{+/-}$ female and neonatal mice. *a*, IIF study using normal human skin as a substrate demonstrated the presence of IgG Abs against the dermal-epidermal junction in the sera of immunized mothers. *b*, These Abs reacted with the epidermal side of the basement membrane zone in skin incubated with 1M-NaCl (star shows cleft between epidermis and dermis). *c*, Heterozygous recipient *mCol17*$^{+/-}$ female mice developed high titers of circulating anti-human COL17 IgG around 3 wk after immunization. *d*, Maternal IgG was efficiently transferred into neonates, rapidly decreasing 2 to 3 wk after birth. The majority of maternal IgG had disappeared by 4 wk after birth.

4 wk (Fig. 2*c*). IIF study using 1M NaCl split normal human skin as a substrate demonstrated that this anti-human COL17 IgG reacted with the epidermal side of the basement membrane (Fig. 2*b*), consistent with the reactivity of human BP autoAbs (15, 16). We then crossed immunized heterozygous *Col17*-deficient (*mCol17*$^{+/-}$) female mice and COL17-humanized (*mCol17*$^{-/-}$, *hCOL17*$^{+/+}$) male mice to give birth to their neonates (Fig. 1). In the neonates delivered from these immunized female mice, maternally transferred IgG against human COL17 was retained at high titers for at least 2 wk after birth, after which it decreased, disappearing by 4 wk after birth (Fig. 2*d*). In the control, *mCol17*$^{+/-}$ female mice, which had been grafted with wild-type mice skin, no anti-human COL17 IgG Abs could be observed nor in their pus delivered after mating them with COL17-humanized (*mCol17*$^{-/-}$, *hCOL17*$^{+/+}$) male (Fig. 2, *c* and *d*).

Neonatal BP mice developed severe blistering

All (n = 12) of the neonatal BP mice that expressed only human COL17 and not the mouse ortholog in the skin with maternally transferred IgG against human COL17 showed severe skin fragility and the epidermis easily detached with minor mechanical friction (Nikolsky phenomenon, Fig. 3*a*). Notably some mice developed spontaneous small blisters and pustules (Fig. 3, *a* and *c*). These skin lesions gradually disappeared in the first week after birth, leaving small, round, crusted lesions similar to those seen in BP patients (Fig. 3*b*). Although epidermal detachment could be induced by moderate (but not minor) friction in humanized mice heterozygously carrying the human COL17 cDNA transgene (*mCol17*$^{-/-}$, *hCOL17*$^{+/-}$), the skin fragility observed in neonatal BP mice was obviously more severe, and minor friction easily produced extensive epidermal detachment. In contrast, none of the other neonates (n = 13) that expressed both human and mouse COL17 in skin (*mCol17*$^{+/-}$, *hCOL17*$^{+/-}$) demonstrated any distinct skin abnormalities following exposure to maternal IgG, including spontaneous blister formation or Nikolsky phenomenon (data not shown).

Neonatal BP mice showed histological and immunological features identical with those seen in patients with BP

This system is characterized by complete humanization of the Ag in neonatal mice with ensuing inflammatory cascades that are completely mouse-derived. Therefore, the system is able to induce specific IgG-Ag reactions and lead to skin inflammation consistent with BP in humans. Notably, histological examinations demonstrated distinctive subepidermal blister formation with numerous inflammatory cell infiltrates predominately consisting of neutrophils (Fig. 3*c*). DIF studies of BP model mice skin revealed deposition of mouse IgG and of mouse complement (C3) in epidermal basement membrane until the third and the first to second weeks after birth, respectively (Fig. 3*d*). Subclass analysis of in vivo deposition of IgG showed that IgG1 and IgG2c predominated at the dermal-epidermal junction (Fig. 3*e*). This characteristic of IgG subclass deposition was the same for immunized *mCol17*$^{+/-}$ females as for their neonates, as shown by IIF on the normal human skin as a substrate (data not shown).

IgG Abs to the NC16A domain of human COL17 play a major role in inducing blistering skin disease

We previously demonstrated that IgG Abs to the NC16A domain of human COL17 play a major role in induce blistering disease; this was demonstrated by passive-transfer experiments using IgG autoAbs from BP patients in neonatal COL17-humanized (*mCol17*$^{-/-}$, *hCOL17*$^{+/+}$) mice (12). To assess and characterize the role of IgG Abs in immunized *mCol17*$^{+/-}$ female mice in the current model, we performed passive-transfer experiment using IgG Abs obtained from immunized female mice with or without immunoadsorption against human COL17 NC16A protein (n = 2, respectively). By IIF study using normal human skin as a substrate, both immune-adsorbed and without immunoadsorption purified IgG reacted to the dermal-epidermal junction until 5120 and 20480 times dilution respectively (data not shown). The passive-transfer experiment showed that purified total IgG without immunoadsorption with human COL17 NC16A protein resulted in skin fragility associated with IgG deposition along the dermal-epidermal junction (Fig. 4, *a* and *b*). In contrast, treatment of IgG with COL17 NC16A protein resulted in no blistering phenotype, although slight deposition of IgG could be observed along the dermal-epidermal junction (Fig. 4, *a* and *b*). These results clearly suggest that IgG Abs to NC16A domain of human COL17 played the major role to induce blistering skin disease in vivo.

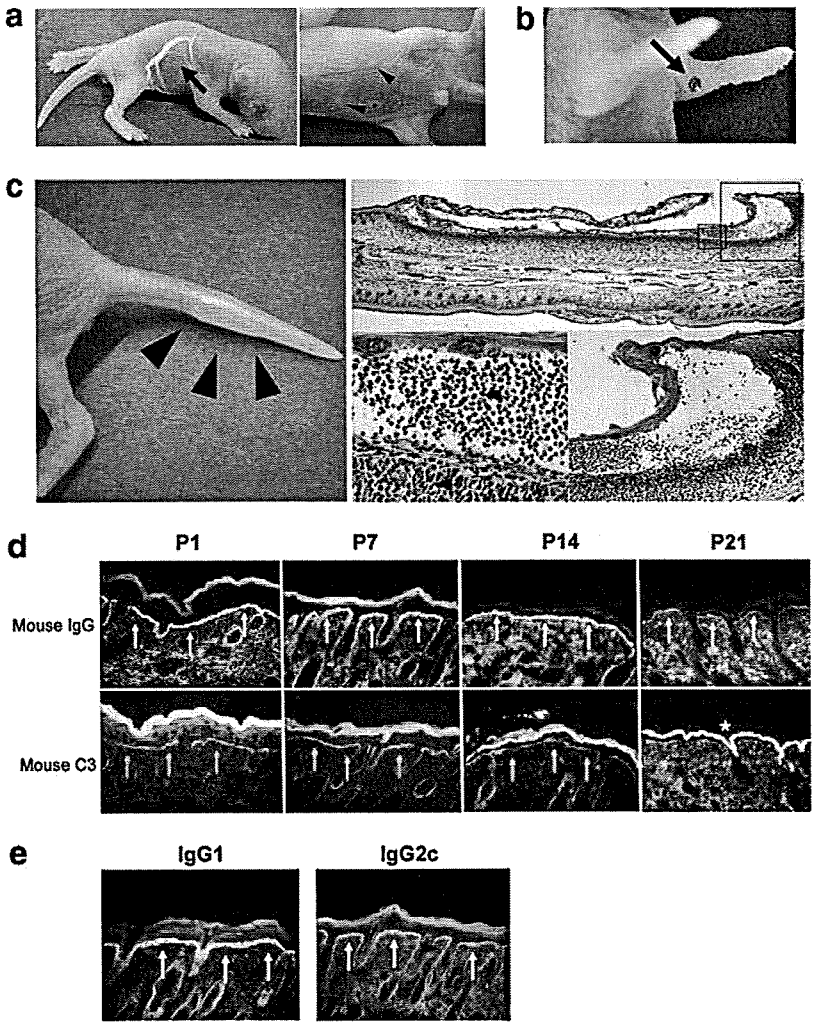

FIGURE 3. *a*, Neonatal BP mice showed severe skin fragility, with the epidermis easily detaching from mechanical friction (Nikolsky phenomenon, arrow). Spontaneous small blisters and pustules were scattered over the entire body (arrowheads). *b*, Small, round, crusted lesion developed around the arm in 4-day-old neonatal BP mice. *c*, Histological finding of blistering lesion on the tail. Subepidermal blister formation associated with numerous infiltrations of neutrophils (arrowheads) was observed. *d*, DIF study revealed in vivo skin deposition of mouse IgG (yellow arrows) until 3 wk after birth, and activated mouse C3 (red arrows) was detected within 1 to 2 wk after birth. Note the Abs to mouse C3 strongly cross-reacted to the corneal layer of the epidemis (star). *e*, In vivo deposition of IgG1 and IgG2c was detected at the dermal-epidermal junction of a neonatal BP mouse soon after birth (arrows).

Maternal IgG to human COL17 was transmissible into neonatal circulation via milk even after birth

Interestingly, some of the mice showed elevated IgG Ab titers to human COL17 by ELISA around 1 to 2 wk after birth (Fig. 2*d*). It has been reported that mouse IgG can be transferred from milk via neonatal FcR expressed in gut, which is different from humans (2, 21, 22). To investigate this possibility, COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) neonatal mice delivered from unrelated pairs were moved soon after birth to a lactating preimmunized $mCol17^{+/-}$ female mouse (ELISA index value to human COL17 of 1.33). As a result, it was found that, at 1 wk of breast-feeding from the immunized female mouse, serum IgG in these pups to human COL17 was markedly elevated (ELISA index titer: 0.73 ± 0.20, $n = 4$), and mouse IgG reacted positively to the dermal-epidermal junction in the skin until 1/1280 dilution (Fig. 5*a*). In contrast, IgG Abs to human COL17 of the pups breast-fed from the nonimmunized female mouse were not increased (ELISA index titer: 0.02 ± 0.03, $n = 4$), and mouse IgG did not react to the normal human skin (Fig. 5*a*). These results clearly indicate that maternally anti-human COL17 IgG Abs were transmitted from milk. However, these COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) neonatal mice with maternal milk-derived Abs to human COL17 in their circulation showed no skin fragility (data not shown). In Neonatal BP mice, active blistering skin disease could be observed for several days after birth, therefore, we assessed in vivo deposition of mouse IgG in the neonatal COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) mice skin at 2 days after being breast-fed by the lactating preimmunized $mCol17^{+/-}$ female to find how maternal IgG from milk could contribute to the blistering skin disease soon after birth. As a result, very low amount of mouse IgG could be detected at the dermal-epidermal junction

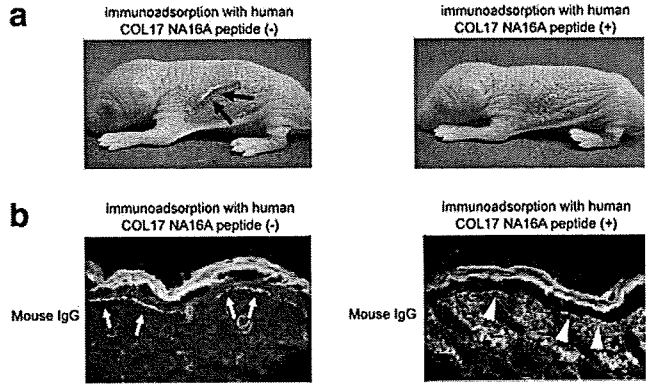

FIGURE 4. IgG Abs to the NC16A domain of human COL17 play a major role in inducing blistering skin disease. *a*, Neonatal COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) mice that received IgG Abs without immunoadsorption with human COL17 NC16A protein from immunized $mCol17^{+/-}$ females resulted in skin fragility (positive Nikolsky sign, arrows), whereas no epidermal detachment could be observed in mice that received immune-adsorbed IgG (50 μl of 2.1 μg/μl IgG Abs, respectively). *b*, In vivo deposition of mouse IgG was more intense in the skin obtained from mice that received IgG Abs without adsorption with human COL17 NC16A protein (arrows) compared with that being adsorbed with the protein (arrowheads).

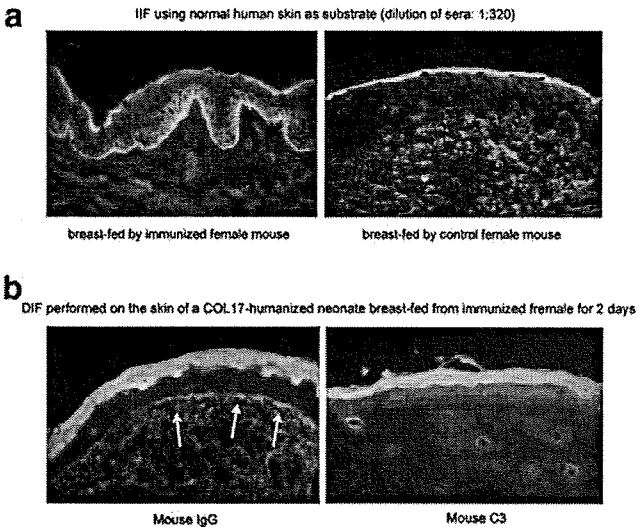

FIGURE 5. Maternal anti-human COL17 Abs transferred from milk. *a*, Serum IgG in these pups to human COL17 reacted to the dermal-epidermal junction at 1 wk of breast-feeding from an immunized female mouse (1/320 dilution). *b*, COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) neonatal mice skin at 2 days of breast-feeding from an immunized mother mouse showed mild in vivo deposition of IgG Abs along the dermal-epidermal junction.

(Fig. 5*b*), suggesting that IgG from milk would play a minor role in the pathogenesis of this Neonatal BP mice model.

Discussion

This study showed successful production of a novel autoimmune disease model in which efficiently transferred maternal IgG induced distinctive Ab-mediated blistering skin disease in COL17-humanized neonates. The COL17 is crucial for maintaining the structural stability of skin. Indeed, loss of COL17 in skin as a result of null mutations results in a novel form of epidermolysis bullosa (OMIM: 226650) (23, 24). Similarly, $mCol17^{-/-}$ mice skin is so fragile that these mice could not be intercrossed (12). Therefore, we used heterozygote-null, but phenotypically normal $mCol17^{+/-}$ female mice to develop Abs against human COL17. Furthermore, by mating immunized female mice with COL17-humanized male mice, human COL17 could be introduced into neonates as an Ag. This simple method enabled us to develop distinctive neonatal BP mice that demonstrate a more severe blistering phenotype characterized by spontaneous blister formation with numerous inflammatory cell infiltrates, a phenotype that is very similar to that seen in humans with BP (15, 16).

Our neonatal BP mice has several advantages over previous mouse models of BP (12, 14). First, unlike other BP models that have relied on the injection of pathogenic IgG Abs into neonatal mice, our model does not require the technically difficult injection procedure. Second, the pathogenic IgG remains in circulation longer in the new model than in conventional models that use injected IgG. Third, the immune reaction is totally dependent on the mouse immune system, while the Ag remains human COL17. The mouse complement system does not work as efficiently during the neonatal period as during adulthood (25). Accordingly, the present system using Abs from the same species is suitable for promoting the subsequent inflammation cascade, including activation of the mouse complement. Finally, immunized heterozygous $mCol17^{+/-}$ female mice induced both IgG1 and IgG2c autoAbs which were transferred to neonates. Mouse IgG1 Abs do not fix complement (20), whereas IgG2c does fix mouse complement (26); therefore, activation of complement in the present system would be induced predominately by IgG2c. Activation of complement has been reported to play a pivotal role in BP blistering (27, 28). In light of this, the current model can be regarded as accurately reproducing human BP disease.

Maternal IgG Abs to human COL17, especially to the NC16A domain, via placenta plays a major role in the current neonatal BP mice model to induce blistering skin disease, in which the most severe disease could be observed soon after birth. However, although it is a rare possibility, maternal transferred lymphocytes as well as pathogenic IgG from milk might contribute to the blistering skin disease. In particular, we were able to clearly demonstrate that maternal IgG Abs transferred into pups via milk 1 wk after birth, although no active blistering skin disease could be observed in the recipient unrelated COL17-humanized ($mCol17^{-/-}$, $hCOL17^{+/+}$) neonatal mice. The reason we could not observe blistering skin disease is probably due to the lower amount of IgG to human COL17 than in neonatal BP mice, in which maternal IgG could be transferred from not only milk but also via placenta, especially soon after birth. IgG transferred from milk might work in part, but it will not be a major player in inducing blistering skin disease, because blistering skin disease was the most severe soon after birth and no active blistering skin disease could be observed at 1 wk, when a lot of fur had grown.

Using maternally transferred pathogenic Abs and introducing human Ags in neonates, we succeeded in inducing autoimmune disease model in neonates whose Ags are functionally important. However, this system does not truly represent autoimmunity in human patients, because Abs to human COL17 in diseased neonates are transferred Abs. In addition, for immunized heterozygote *Col17*-deficient ($mCol17^{+/-}$) female mice, human COL17 is not an autoAg but alloantigen therefore, pathogenic Abs to human COL17 in this system is not strictly an autoAbs. Nevertheless, maternally transferred Abs in genetically transformed Ag-humanized neonates will be useful in the study of autoimmune diseases as a novel method for generating diseases in neonates.

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Disclosures

The authors have no financial conflict of interest.

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