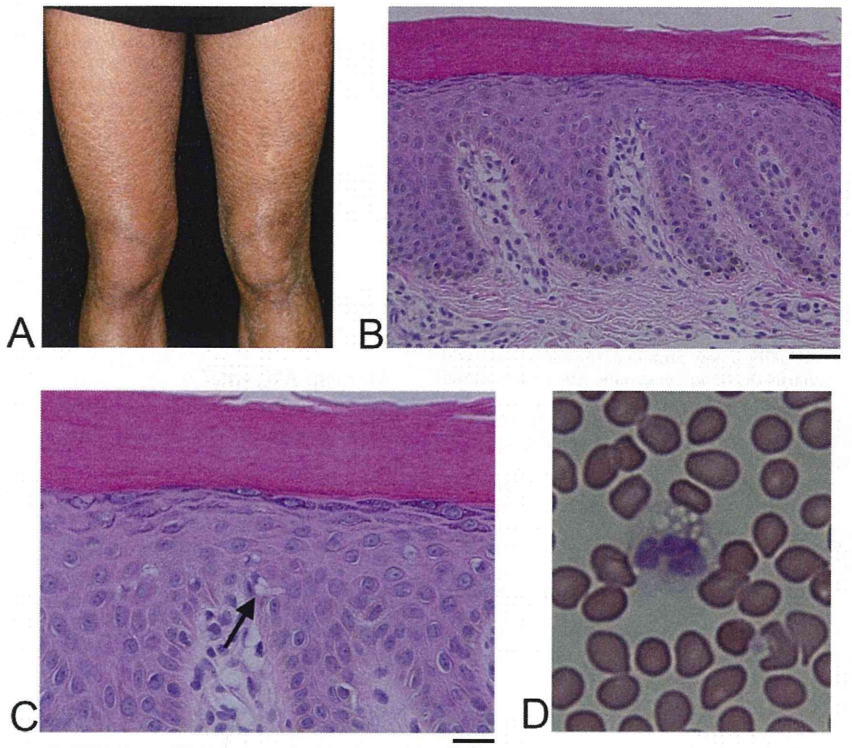

Fig. 1. Clinical presentation, pathological findings, and blood smear of the patient. (A) Clinical presentation of the feet of the patient included fine, gray to brown scales. (B, C) Hematoxylin and eosin staining of the ichthyosis. Marked hyperkeratosis with only a small number of parakeratotic cells was seen. Intra-cytoplasmic lipid droplets within the epidermal keratinocytes were observed (arrow); scale bar: 50 μm (B), 20 μm (C). (D) Jordan's anomaly: lipid droplets were observed in a neutrophil.

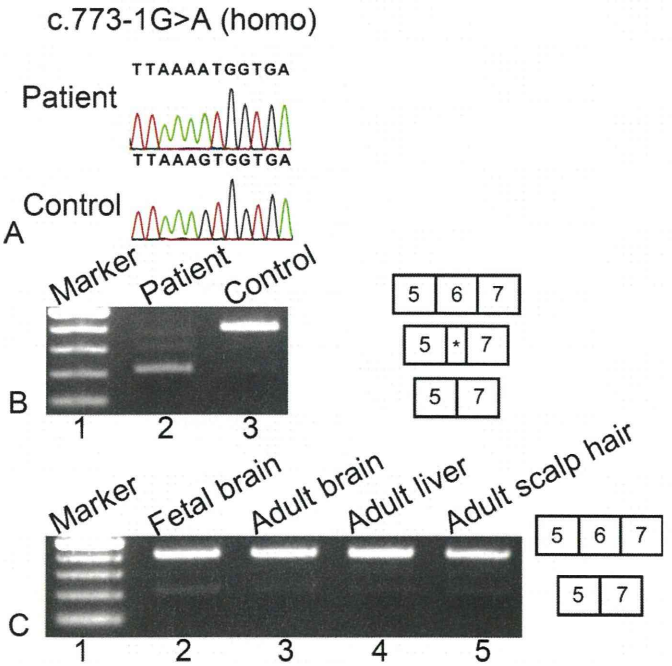

Fig. 2. *ABHD5* sequence data of the patient, and mRNA analysis of the patient's scalp hair and normal human brain and liver. (A) *ABHD5* sequence data for the patient is shown. Arrows indicate c.773 – 1 G > A (homozygous). (B) *ABHD5* mRNA analysis of total RNA derived from plucked hair samples is shown. The sequences of PCR primers, which cover exons 5–7, are provided in Supplementary Table S1. 1, marker; 2, the patient; 3, a healthy human control. The sequence of the uppermost band of the patient's sample was unread. The middle band corresponds to skipping of the 5′–95 bp of exon 6 (*). The upper band of the control is the wild-type *ABHD5* mRNA. The lowest bands of the patient and the control samples represent the completely skipped exon 6 mutation product and an alternatively spliced variant respectively. (C) *ABHD5* mRNA analysis of human tissues. 1, markers; 2, fetal brain; 3, adult brain; 4, adult liver; 5, adult scalp hair. This is the representative data of three independent experiments. The upper bands correspond to full length *ABHD5* mRNA containing exons 5–7. The lower bands represent splice variants, in which exon 6 was skipped. This truncated form is most apparent in the fetal brain.

the *ABHD5* mRNA analysis, we think that the alternatively spliced *ABHD5* mRNA, in which the entire exon 6 is skipped, produces a truncated form of *ABHD5* in normal human tissues, especially in the fetal brain. This suggests that the shorter protein might function or compensate for the full length *ABHD5* in the fetal brain. If this is true, the splice site mutations that form the truncated form of ABHD5 without exon 6 products, might prevent the patients from developing mental retardation.

Supplementry material related to this article found, in the online version, at 10.1016/j.jdermsci.2014.05.009.

In conclusion, to our knowledge, this is the first proposed genotype–phenotype correlation between an *ABHD5* mutation and a DCS phenotype. We also determined the consequence of a splice site mutation of c.773 – 1 G > A. In addition, we found an alternatively spliced *ABHD5* mRNA, in which the entire exon 6 was skipped, more frequently in the fetal human brain than in the adult brain, liver, or skin.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Letter to the Editor

Clinical pharmacology of the anti-IL-17 receptor antibody brodalumab (KHK4827) in Japanese normal healthy volunteers and Japanese subjects with moderate to severe psoriasis: A randomized, dose-escalation, placebo-controlled study

Psoriasis is a chronic, immune-mediated skin disease, with estimated disease rate of 0.6–6.5% in the European Union, 2–3% in the United States, and 0.29–1.18% in Japan [1–5]. Typical treatments for psoriasis include topical therapies, systemic therapies, and phototherapy. More recently, development of biopharmaceuticals has substantially contributed to improve quality of life in patients, particularly with moderate to severe psoriasis.

During the past decade, it has become clear that IL-17 family cytokines are involved in the etiology of inflammatory autoimmune diseases including psoriasis, psoriatic arthritis, and asthma. In psoriasis, over-production of IL-17A, IL-17F, IL-17C, and the IL-17A/F heterodimer stimulate keratinocyte proliferation [6,7].

Brodalumab (KHK4827, also known as AMG 827) is a human IgG2 monoclonal antibody that specifically binds to human IL-17RA and blocks the biological activity of IL-17 family members. In a clinical study conducted in predominantly Caucasian patients, it was reported that brodalumab led to rapid and significant improvement in patients with moderate to severe psoriasis, measured using psoriasis area and severity index (PASI) and static physician global assessment (SPGA) scoring [8,9]. This phase I study was the first clinical trial of brodalumab in the Japanese population. The main purpose of the study was to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of brodalumab in healthy Japanese volunteers and in patients with moderate to severe psoriasis. In addition to that, we evaluated the clinical response of brodalumab in Japanese patients with moderate to severe psoriasis. The protocol was approved by the Institutional Review Board (IRB) at each study site (ClinicalTrials.gov identifier NCT01488201). The study consisted of 2 parts. In the healthy volunteer group, 8 subjects were randomized in a 2:6 ratio to receive a single administration of placebo or brodalumab at 70, 140, 210, or 420 mg subcutaneously (SC), or 210 mg intravenously (IV) (Part A). In patients with moderate to severe psoriasis, 6 patients received a single dose of 140 mg and 7 patients received a single dose of 350 mg brodalumab SC (Part B). Patients (both genders) included in the psoriasis group had active but clinically stable plaque psoriasis involving ≥10% of their body surface area; a minimum PASI score of ≥10 during the screening period; had either received at least 1 previous phototherapy or systemic psoriasis therapy, or were suitable for phototherapy or systemic psoriasis therapy in the opinion of the investigator; and were aged more than 20 and less than 70 years.

A total of 40 healthy male volunteers and 13 (male: 9, female: 4) psoriasis patients were enrolled into this study (Supplementary Table S1 online). A single dose of brodalumab at 140 mg or 350 mg SC resulted in rapid, dose-related improvement in psoriasis symptoms (Fig. 1a and b). A total of 3 out of 6 (50%) and 6 out of 7 (85.7%) patients who received 140 and 350 mg brodalumab SC, respectively, achieved a PASI 75 score or greater (Fig. 1c). Fig. 1d presents a typical photography image indicating the rapid improvement of psoriasis symptoms in a patient who received 350 mg SC brodalumab.

The mean serum brodalumab concentration time profile and PK parameters are comparable between healthy volunteers and psoriasis patients, which exhibited nonlinear PK (Fig. 2a and Supplementary Table S2 online). The mean serum concentration reached T_{max} (Day 1–7) and decreased slowly in a concentration range more than approximately 1 μg/mL, with accelerated elimination when serum concentrations fell below the level (Fig. 2a). The mean IL-17RA occupancy had increased to near maximum level at the first blood collection point after dosing (IV, 0.5 h; SC, Day 3) at all doses. The duration of the maximum level of mean IL-17RA occupancy increased in a dose-

project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945–56.
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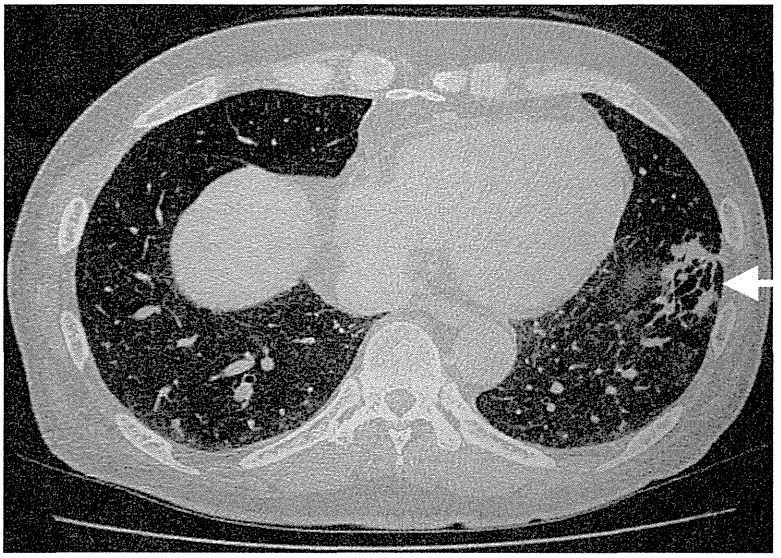
DOI 10.1002/art.38670

Clinical Image: Solitary organizing pneumonia mimicking lung adenocarcinoma in systemic sclerosis



The patient, a 57-year-old man, presented to our hospital with Raynaud's phenomenon. On examination, prominent bilateral skin sclerosis of the fingers, hands, and forearms was observed. Skin biopsy revealed swollen collagen bundles in the dermis, and autoantibody profiling revealed U1 RNP antibody positivity. He was diagnosed as having limited cutaneous systemic sclerosis (SSc). Radiography showed a nodule in the left lower lung. Computed tomography revealed a 39 × 27–mm nodule with multiple cystic structures (**arrow**) in the left lower lung and ground-glass opacities in both lower lungs. Percutaneous lung biopsy showed no evidence of carcinoma. However, open-chest partial excision was performed because lung adenocarcinoma with cystic structures was suspected clinically. The pathologic finding was fibrosis with inflammatory cell infiltration containing lymphocytes and plasma cells. Ziehl-Neelsen staining and Grocott staining were negative. Thus, solitary organizing pneumonia was diagnosed. Organizing pneumonia is a pathologic and clinical entity that is often cryptogenic or secondary to various diseases, including rheumatic conditions. Solitary organizing pneumonia frequently presents as an isolated focal lesion. Organizing pneumonia is considered a rare disease with an incidence of 1.96 cases/100,000, of which ~10–15% are solitary organizing pneumonia. Solitary organizing pneumonia is often misdiagnosed and removed surgically as it is rare and difficult to distinguish from lung carcinoma. The risk of cancer, particularly of the lung, liver, hematologic system, and bladder, is increased among patients with SSc, especially male patients. The present case suggests that solitary organizing pneumonia should be considered when an isolated nodular lung lesion is noted in a patient with SSc.

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IL36RN Mutations Underlie Impetigo Herpetiformis

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TO THE EDITOR

Impetigo herpetiformis (IH) is a rare pustular dermatosis that typically occurs in pregnant women sporadically with unknown etiology (Sauer and Geha, 1961). Early diagnosis is essential, as IH is life-threatening and is associated with placental insufficiency and electrolyte abnormalities. IH appears to have the same clinical and histologic appearance as generalized pustular psoriasis (GPP), which is also a rare severe episodic pustular dermatosis that occurs repeatedly in both sexes at any age. However, some researchers have regarded IH as an entity distinct from GPP, because some patients are affected by IH only in the gestational period (Lotem *et al.*, 1989). Recently, we reported that the majority of GPP that is not accompanied by psoriasis vulgaris (PV; GPP alone) is caused by homozygous or compound heterozygous mutations of *IL36RN*, which encodes IL-36 receptor antagonist (IL-36RN), although only a small number of cases with GPP preceding or accompanied by PV (GPP with PV) were found to have *IL36RN* mutations (Sugiura *et al.*, 2013). Very recently, we reported that *CARD14* c.526G>C is a significant risk factor for GPP with PV, but not for GPP alone in the Japanese cohort, which further supports the idea that GPP with PV differs genetically from GPP alone (Sugiura *et al.*, 2014a). However, to our knowledge, there have been no reports of IH with *IL36RN* mutations. Here we report two cases of IH with homozygous and heterozygous *IL36RN* mutations.

Cases 1 and 2 were a 23-year-old woman and a 28-year-old Japanese woman who were admitted to our hospitals for pustular lesions in the 29th week and the 20th week of their first pregnancies, respectively (Figure 1a and b). There was no family history of GPP, no IH, and no consanguinity in their families. Case 1 had no previous history of GPP. Her pustular lesions had begun to develop at the 21st week of pregnancy, and she had been hospitalized in a maternity hospital. Oral prednisolone at a dose of 15 mg per day had been administered, but the eruptions had persisted. A skin biopsy from a pustular eruption on the trunk revealed a spongiform pustule of Kogoj in the epidermis consistent with IH (Figure 1c). Case 2 had suffered from GPP from the age of 8 to 18 years. Skin biopsies from pustular eruptions on the trunk revealed spongiform pustules of Kogoj in the epidermis at the age of 8 and 28 years (Figure 1d). She had been admitted to hospitals four times for GPP flare-ups. She had been treated with cyclosporine or etretinate. In the ten years leading up to her pregnancy, her GPP had been in remission without any treatment. Both cases had erythema with pustules over the whole body and fever of over 38 °C. Blood examinations from Cases 1 and 2, respectively, revealed white blood cell counts of 12,000 μl^{-1} and 21,170 μl^{-1}, and C-reactive protein concentrations of 6.5 and 14.9 mg dl^{-1} (normal range: <0.3 mg dl^{-1}). Bacterial cultures of the pustules were negative. Thus, Cases 1 and 2 were, respectively, diagnosed as having IH and IH with a previous history of GPP.

Following ethical approval, written informed consent was obtained in compliance with the Declaration of Helsinki Principles. The entire coding regions of *IL36RN* including the exon/intron boundaries were sequenced using genomic DNA samples from the patients. Case 1 had the homozygous mutation c.115+6T>C, which was proven to result in p.Arg10ArgfsX1 in *IL36RN* by us previously, and Case 2 had the heterozygous mutation c.28C>T (p.Arg10X) in *IL36RN*. Both of these are GPP-causing founder mutations in the Japanese cohort (Sugiura *et al.*, 2013, 2014b; Figure 1e and f, and Figure 2). A search for a second *IL36RN* mutation in all intron and putative promoter regions in Case 2 revealed no other *IL36RN* mutations (Supplementary Figure S1 online and Supplementary Table S1 online). However, there is still the possibility of a second unidentified *IL36RN* mutation in Case 2.

More than 10 cases of GPP with heterozygous *IL36RN* mutations have been reported (Capon, 2013; Korber *et al.*, 2013; Li *et al.*, 2013; Setta-Kaffetzi *et al.*, 2013; Sugiura *et al.*, 2013). Moreover, in some patients, heterozygous *IL36RN* mutations are associated with palmoplantar pustulosis, a type of pustular psoriasis, and acute generalized exanthematous pustulosis, a severe cutaneous drug reaction (Navarini *et al.*, 2013; Setta-Kaffetzi *et al.*, 2013). IL-36 is absent in normal skin but is induced by inflammatory cytokines such as tumor necrosis factor-α, IL-17A, and IL-22 (Carrier *et al.*, 2011). When functional IL-36RN is absent or underproduced, overexpressed IL-36 can induce neutrophil-rich infiltration. Tumor necrosis factor-α is often elevated in the blood of pregnant women, whereby it induces various serious diseases (Mallmann *et al.*, 1991). As for skin diseases, tumor necrosis factor-α sometimes causes exacerbation of PV lesions in pregnant women (Puig *et al.*, 2010). Hence, it is very likely that a pregnant woman who has the *IL36RN* mutation

Abbreviations: GPP, generalized pustular psoriasis; IH, impetigo herpetiformis; IL-36RN, IL-36 receptor antagonist; PV, psoriasis vulgaris

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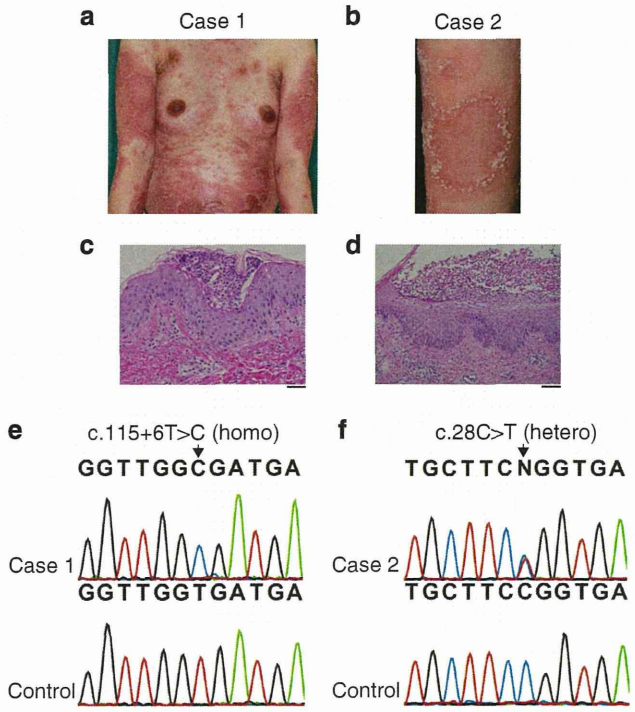

Figure 1. Clinical features, pathological findings of pustules, and mutation analysis of *IL36RN* in the patients. The clinical features of Cases 1 and 2 (**a**, **b**) are shown. Pustules on background erythema are seen on the trunk and arms. The pathology of the pustules is indicated for Cases 1 and 2 (**c**, **d**). Spongiosis of Kogoj and acanthosis are observed in the epidermis of the pustular erythematous lesions on the trunks. Scale bar = 50 μm for **c** and 100 μm for **d**. Direct sequencing reveals the homozygous mutation c.115 + 6T>C in Case 1 (**e**) and the heterozygous mutation c.28C>T in Case 2 (**f**).

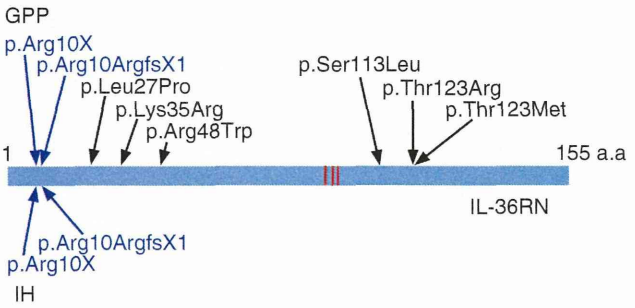

Figure 2. The second structure of IL-36RN and location of all *IL36RN* mutations ever reported in generalized pustular psoriasis (GPP) and impetigo herpetiformis (IH). All *IL36RN* mutations ever reported in GPP and IH (including in the present report) are shown (Kanazawa *et al.*, 2013). The blue characters indicate truncating mutations, and the black characters indicate missense mutations. Red lines show critical residues that mediate receptor interaction, such as Tyr89, Glu94, and Lys96 of IL36-RN (Sugiura *et al.*, 2013).

occasionally cannot produce enough IL-36RN to adequately antagonize IL-36 excessively induced by inflammatory cytokines, and this imbalance results in IH.

After longstanding controversy over whether IH is an independent disease entity from GPP, today there is the consensus that IH is not a distinct entity but is identical to GPP, i.e., IH is GPP occurring during pregnancy (Lotem *et al.*, 1989; Robinson *et al.*, 2012). However, there have been no reports with experimental or genetic evidence to bolster the assertion that IH and GPP are identical diseases. This report clearly shows IH patients with homozygous or heterozygous *IL36RN* mutations. Case 1 was affected with IH only once in the gestational period. In this context, the present case suggests that IH and GPP, especially GPP alone, are identical diseases caused by *IL36RN* mutations. Future study should elucidate the proportion of IH cases that are *IL36RN* negative; however, given that the majority of GPP alone is associated with *IL36RN* mutations, we consider that mutation analysis of *IL36RN* is a very promising method for the prediction of IH risk to prevent subsequent serious complications in the patient and the fetus.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Acta Derm Venereol 2014; 94: 589–590

SHORT COMMUNICATION

Novel *TGM1* Missense Mutation p.Arg727Gln in a Case of Self-healing Collodion Baby

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Collodion babies are newborns encased in a glistening membrane that cracks in a characteristic manner within 48 h and desquamates in large lamellae after a few days. Most collodion babies later develop one of the several types of autosomal recessive congenital ichthyoses (ARCI), such as lamellar ichthyosis (LI) or congenital ichthyosiform erythroderma; however, about 10% heal spontaneously (1). This healing condition is known as "self-healing collodion baby" or "self-improving collodion baby" (SHCB/SICB). Raghunath et al. (1) showed that this phenotype is possibly a hydrostatic pressure-sensitive phenotype of *TGM1* mutations. The SHCB/SICB phenotype was subsequently reported in patients with *ALOX12B* and *ALOXE3* mutations (2). To date, few reports on SHCB/SICB cases with *TGM1* mutations have been published (1–4).

TGM1 is the most commonly involved gene in ARCI, and encodes transglutaminase-1 (TGase-1) (1, 5–8).

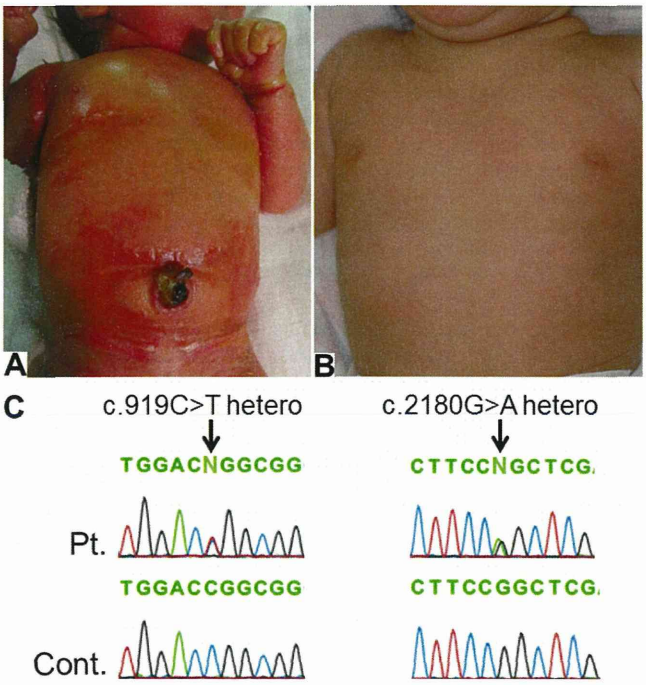

Fig. 1. Clinical features and *TGM1* sequence data of the patient. The patient showed collodion membrane at birth (A). The skin manifestations healed completely by the age of 2 months (B). (C) Sequence data of *TGM1* in the patient in exon 6 (left) and exon 14 (right). Arrows indicate c.919C>T (p.Arg307Trp) (heterozygous) and c.2180G>A (p.Arg727Gln) (heterozygous).

Here, we describe an ARCI patient with a novel *TGM1* mutation who presented at birth with a collodion membrane but spontaneously healed within 2 months without any skin manifestations.

CASE REPORT

A Japanese girl born to nonconsanguineous parents at 40 weeks gestation presented at birth with a collodion membrane but without ectropion or eclabium. Her skin spontaneously healed by the age of 2 months (Fig. 1A and B). The ethics committee of Nagoya University Graduate School of Medicine approved the present studies. The participants gave written informed consent. The coding regions of *TGM1* (GenBank accession No. 359), *ALOX12B* and *ALOXE3* were amplified from genomic DNA by PCR, as described previously (9). Direct sequencing of the patient's PCR products revealed that the patient had the compound heterozygous *TGM1* mutations p.Arg307Trp (c.919C>T) and p.Arg727Gln (c.2180G>A) (Fig. 1C), but had no mutation in *ALOX12B* or *ALOXE3*. The former mutation, p.Arg307Trp, was previously reported as a founder mutation in Japanese LI cases by our group (10, 11). The arginine residue mutated in the other mutation, p.Arg727Gln, in the present case is in the β-barrel 2 domain of TGase-1 (Fig. 2B). This arginine residue was confirmed to be highly conserved in vertebrates. p.Arg727Gln was not detected in the 100 control alleles (Fig. 2A) (data not shown). p.Arg307Trp was present in the mother and p.Arg727Gln was demonstrated as paternal (data not shown). The patient was diagnosed as having SHCB/SICB with compound heterozygous *TGM1* mutations.

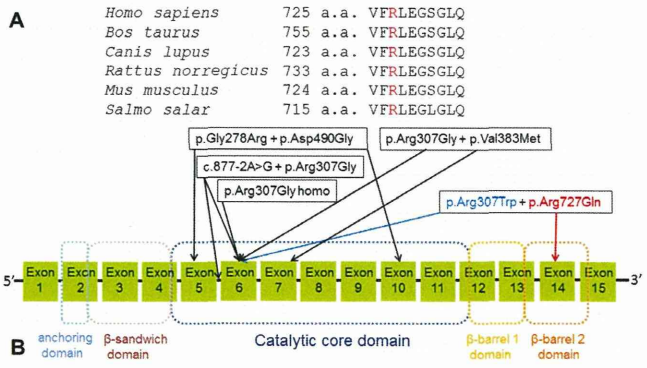

Fig. 2. Sequence alignments around the missense mutation and the summary of *TGM1* mutations in the SHCB/SICB phenotype. (A) The sequence alignment of TGase-1. Arg727 is in red. The leucine residue at codon 727 of human TGase-1 is conserved among the TGase-1 of diverse species. (B) The TGase-1 protein domain structure and *TGM1* mutations in SHCB in this study and in the literature. The present novel missense mutation p.Arg727Gln in the β-barrel domain is in red font. The other mutation in the present case, p.Arg307Trp, is in blue font. It has been reported to be a founder mutation in Japanese LI cases (10, 11).

DISCUSSION

Previously reported SHCB/SICB cases with *TGM1* mutations had the homozygous mutation p.Arg307Gly, the compound heterozygous mutations p.Arg307Gly and c.877-2A>G, the compound heterozygous mutations p.Arg307Gly and p.Val383Met, or the compound heterozygous mutations p.Gly278Arg and p.Asp490Gly (1–4). Concerning the compound heterozygous mutations p.Gly278Arg and p.Asp490Gly, p.Asp490Gly is considered to be responsible for the SHCB/SICB phenotype because p.Gly278Arg is inactive under any conditions, but p.Asp490Gly has been proven inactive under high hydrostatic water pressure, such as in the uterus, and active under the lower-pressure conditions out of the uterus (1). In the compound heterozygous for p.Arg307Gly and c.877-2A>G, and the compound heterozygous for p.Arg307Gly and p.Val383Met, p.Arg307Gly possibly contributes to the SHCB/SICB phenotype, because the homozygous mutation p.Arg307Gly is known to cause the SHCB/SICB phenotype and c.877-2A>G is considered to bring a splicing error (not analysed in detail). In light of this, the mutations causing SHCB/SICB are limited to substitutions of the 2 residues p.Arg307 and p.Asp490 in the catalytic domains. In our report, one mutation was p.Arg307Trp, which is a founder mutation always associated with typical LI in the Japanese population (10, 11). We do not know the exact reason why p.Arg307Gly is associated with SHCB/SICB, but p.Arg307Trp is associated with typical LI. The difference in the side chain of the amino acid could explain the difference in the phenotype, i.e., tryptophan is the most voluminous amino acid, but glycine has a small side chain. Hence, we attribute the present case of SHCB/SICB to the novel mutation p.Arg727Gln in the β-barrel 2 domain. Several authors suggest that β-barrel domains, which are at the carboxyl-terminus of the gene, increase TGase-1 activity but are not essential for the function of the enzyme (12, 13). Arginine is a polar basic amino acid, but glutamine is a polar neutral amino acid. The reduction of charge in p.727Arg in β-barrel domains may affect the function of TGase-1 slightly. Thus, p.Arg727Gln may contribute to the disease onset and disease healing of SHCB/SICB. Mutations in the β-barrel 1 or β-barrel 2 domains have not been reported in SHCB/SICB. Even in typical LI, mutations in the β-barrel domains have been rarely found (14). Therefore, genotype-phenotype correlations related to the β-barrel domains in TGase-1 have not been determined (14).

In conclusion, we suggest for the first time that the missense mutation in the β-barrel 2 domain of the catalytic domains may cause SHCB/SICB.

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CARD14 c.526G>C (p.Asp176His) Is a Significant Risk Factor for Generalized Pustular Psoriasis with Psoriasis Vulgaris in the Japanese Cohort

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TO THE EDITOR

Generalized pustular psoriasis (GPP) often presents in patients with existing or prior psoriasis vulgaris (PV), although cases of GPP have been known to arise without a history of PV. We recently reported that the majority of the cases of GPP without a history of PV ("GPP alone") are caused by homozygous or compound heterozygous mutations of *IL36RN* (Sugiura *et al.*, 2013). In contrast, only a few exceptional cases of GPP that were preceded or accompanied by PV ("GPP with PV") were found to have *IL36RN* mutations. Thus, GPP with PV is genetically different from GPP alone.

CARD14 encodes caspase recruitment domain family member 14 (CARD14), which is an activator of nuclear factor of κ-light-chain-enhancer of activated B cells within the epidermis. Jordan *et al.* (2012a, b) identified rare gain-of-function variants and mutations in *CARD14*, including p.Gly117Ser, in two large multiplex families affected by Mendelian forms of psoriasis and psoriatic arthritis (Figure 1). Autosomal dominant pityriasis rubra pilaris, which is phenotypically related to psoriasis, was also reported to be caused by mutations in *CARD14* (Fuchs-Telem *et al.*, 2012), including p.Glu138del and p.Leu156Pro. Moreover, *CARD14* p.Arg820Trp (rs11652075) was found to be a PV-susceptible variant in a large psoriasis cohort (Tsoi *et al.*, 2012; Jordan *et al.*, 2012a). Therefore, *CARD14* variants and mutations are closely related to various types of psoriasis and psoriasis-related diseases. Jordan *et al.* (2012b) identified the rare *de novo CARD14* gain-of-function variant p.Glu138Ala in a child with severe early-onset GPP. They found three other rare *CARD14* gain-of-function variants in large PV cohorts including p.Asp176His. There was no association of the p.Asp176His variant with PV because the rare variant was found not only in the large PV cohorts but also in the large control cohorts.

The present study aimed to investigate the presence of *CARD14* variants in GPP with PV. We analyzed the entire coding regions of *CARD14* in 19 cases of GPP with PV and in 11 cases of GPP alone. Then, we analyzed exon 4 of *CARD14* in the 100 cases of PV and the 100 healthy controls that we had previously studied (Sugiura *et al.*, 2013). All cases and controls were Japanese. It was previously reported that 9 out of 11 cases of GPP alone had homozygous or compound heterozygous *IL36RN* mutations (Sugiura *et al.*, 2013). The clinical characteristics of the 30 patients with GPP and the 100 patients with PV are indicated in Supplementary Table S1 and S2 online, respectively. Following approval from the Ethics Committee of Nagoya University, written informed consent was obtained from all participants in compliance with the Declaration of Helsinki guidelines.

The sequence primers for analysis of *CARD14* are indicated in Supplementary Table S3 online. Direct-sequencing analysis of the entire coding regions of *CARD14* with exon–intron boundaries revealed 4 of the 19 cases of GPP with PV to be heterozygous for c.526G>C (p.Asp176His) (Figure 2). We found no other mutations or variants. However, none of the 11 cases of GPP alone had any variants. Among the cases

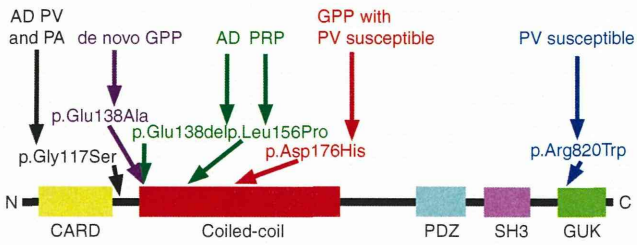

Figure 1. Caspase recruitment domain family, member 14 (CARD14) protein domain structure, locations of amino acid substitutions and deletions, and related psoriatic diseases. CARD and coiled-coil domains are essential for nuclear factor of kappa-light-chain-enhancer of activated B cells (NF-κB) activation. The PDZ, SH3, and GUK domains are proposed as being essential for membrane localization and lipid raft recruitment of CARD14 protein (Scudiero *et al.*, 2011). Rare and common variants and mutations found are indicated. p.Asp176His is the generalized pustular psoriasis (GPP) with psoriasis vulgaris (PV)-susceptible variant shown in the present study. p.Arg820Trp is the PV-susceptible variant (Tsoi *et al.*, 2012; Jordan *et al.*, 2012a). p.Gly117Ser is a mutation found in the family of Mendelian autosomal dominant (AD) PV and psoriatic arthritis (PA; Jordan *et al.*, 2012a). p.Glu138Ala was found in a patient with early-onset GPP. p.Glu138del and p.Leu156Pro indicate that the mutations found in AD pityriasis rubra pilaris (RPR; Fuchs-Telem *et al.*, 2012). CARD, caspase recruitment domain; GUK, guanylate kinase; PDZ, a domain from the initial letters of the PSD-95, Dlg, and ZO-1 proteins; SH3, src homology 3.

Abbreviations: CARD14, caspase recruitment domain family, member 14; GPP, generalized pustular psoriasis; PV, psoriasis vulgaris

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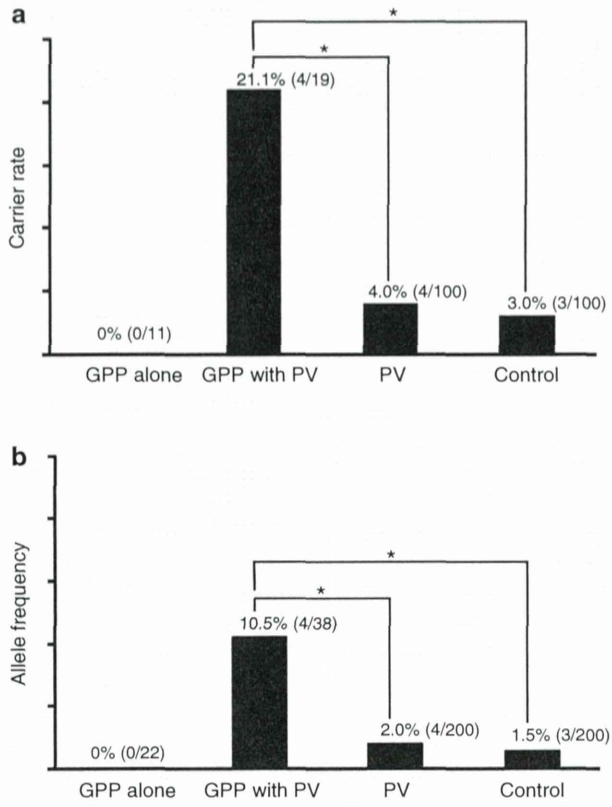

Figure 2. Carrier rate and allele frequency of p.Asp176His in generalized pustular psoriasis (GPP) with psoriasis vulgaris (PV), and in PV. The carrier rate (**a**) and allele frequency (**b**) of p.Asp176His in GPP with PV are significantly higher compared with those in PV and in healthy controls. *$P<0.05$.

with homozygous or compound heterozygous *IL36RN* mutations, no cases had the variant c.526G>C (p.Asp176His). Direct-sequencing analysis of exon 4, which encodes p.Asp176, revealed 4 out of the 100 cases of simple PV and 3 out of the 100 healthy controls to have the heterozygous variant c.526G>C (p.Asp176His). The carrier rate of the p.Asp176His variant in GPP with PV (4/19: 21.1%) was significantly higher compared with that in the controls (3/100: 3%; Fisher exact test: $P<0.0123$; odds ratio of 8.62; confidence intervals between 1.75 and 42.4; power calculation of 0.609). However, there was no significant difference in the carrier rate of p.Asp176His between GPP alone (0/11: 0%) and the healthy controls. Jordan *et al.* (2012a) reported that p.Asp176His was found in 2 out of 1609 controls in European populations. The carrier rate of p.Asp176His in the Japanese individuals in the present study is significantly higher compared with that in the European cohort ($P<0.01$; Jordan *et al.*, 2012a). The Ensembl genome browser indicates that 3 out of 178 alleles in the Japanese population have c.526G>C. This frequency is very close to the 3 out of 200 alleles of the present study. We conclude that the *CARD14* variant p.Asp176His is an important predisposing factor for GPP with PV in the Japanese population.

Because of the low number of patients, the current study is underpowered to detect variants at low frequencies, such as pGlu138Ala, in this population (Jordan *et al.*, 2012a). pGlu138Ala was reported in an early-onset GPP patient, although the mutant was not found in any of the 30 GPP patients in the present study. HLA-Cw*602 is the main genetic predisposing factor for psoriasis. We analyzed HLA-C in the 30 patients with GPP and the 100 patients with PV using Micro SSPTM HLA typing trays (One Lambda, Canoga Park, CA; Supplementary Tables S1 and S2 online). No interaction was found between the *CARD14* variant c.526G>C and HLA-Cw*602.

The haplotype block structure flanking the *CARD14* gene was constructed using genotype data from the HapMap database. We analyzed the haplotypes of 200 alleles of the controls. The haplotype block was represented by eight haplotypes (Supplementary Figure S1 online). In all, 11 individuals carrying the *CARD14* variant c.526G>C (p.Asp176His), the chromosome containing c.526G>C, had an identical haplotype, haplotype III (GGACCTC CA), which is seen in 11.5% of the Japanese population. Thus, the variant c.526G>C (p.Asp176His) in the present Japanese individuals appears to represent founder effects of the prevalent variant *CARD14* alleles among individuals in this island nation.

In this study, we found that *CARD14* p.Asp176His is a prevalent founder variant in the Japanese population and is a predisposing factor for GPP with PV. In contrast, *CARD14* p.Asp176His is not associated with PV in the Japanese population. These findings suggest that GPP with PV has a genetic background different from that of simple PV. In addition, no patient with GPP alone has had *CARD14* p.Asp176His, although the majority have had *IL36RN* mutations, as previously reported (Sugiura *et al.*, 2013). In this context, the present results further support the idea that GPP with PV is genetically different from GPP alone.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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