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Association of *IL36RN* mutations with clinical features, therapeutic response to acitretin, and frequency of recurrence in patients with generalized pustular psoriasis

Background: Previous studies have revealed that *IL36RN* mutations play a pivotal role in the pathogenesis of generalized pustular psoriasis (GPP), however, the clinical relevance is unclear. *Objective:* To investigate the correlation between *IL36RN* mutations and clinical features, recurrence frequency, and therapeutic response to acitretin in GPP patients with long-term follow-up. *Materials & Methods:* This retrospective cohort study, lasting 2-4 years, included 61 GPP and 48 psoriasis vulgaris (PV) patients. *Results:* Sequence analysis of all five exons of the *IL36RN* gene revealed two genetic variants (c.115+6 T>C and c.227C>T). The cohort was divided into three subgroups according to the c.115+6 T>C mutation (present in 52.5% of the patients): homozygous mutation group (HOMG), heterozygous mutation group (HEMG), and non-mutation group (NMG). Initially, 21/25 HOMG patients were diagnosed with GPP with provocative factors, but 13 developed erythrodermic psoriasis after the pustular phase. Patients in the HEMG (5/7) and NMG (23/29) maintained PV diagnosis before and after the pustular phase. Most patients exhibited a marked response to acitretin, but patients who were prescribed a maintenance dosage (10-30 mg/d) had mild recurrence (0-2 times/year) during follow-up. *IL36RN* mutations were strongly linked with early onset and hyponychial pustules, but not with therapeutic efficacy of acitretin or recurrence frequency. *Conclusions:* Early onset and hyponychial pustules may be specific to *IL36RN* mutation, however this alone is an insufficient biomarker for acitretin therapy. Other provocative factors play important roles in disease onset, clinical manifestations, and disease outcome. Low-dose maintenance therapy with acitretin might help reduce the recurrence of GPP.

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Key words: generalized pustular psoriasis, *IL36RN*, gene mutation

Generalized pustular psoriasis (GPP) is a rare, life-threatening systemic inflammatory disease that features sterile pustules on either the erythema or normal skin over a large area of the body [1]. Provocative factors include infection, pregnancy, hypocalcaemia, and drugs [2]. Successful systemic therapies for treating GPP include acitretin, methotrexate (MTX), cyclosporine (CsA), biologics, or a combination of these agents [3]. However, many patients relapse during the dosage reduction, which makes managing GPP a challenge. For this reason, dermatologists have sought a more reasonable and feasible maintenance therapeutic regimen.

Even though the first case of GPP was reported by Von Zumbusch in 1910 [4], the pathogenesis of GPP was elucidated by Marrakchi *et al*. [5] in 2011, who reported that *IL36RN*, which encodes the IL-36 receptor antagonist, was responsible for Tunisian familial GPP (FGPP), also known as deficiency of IL-36 receptor antagonist (DITRA). Using an exon-sequencing strategy, Onoufriadis *et al*. [6] then confirmed that *IL36RN* mutations could cause sporadic GPP. Subsequent reports documented other disease alleles in various ethnic groups.

However, *IL36RN* mutations cannot be detected in a substantial subset of GPP patients [7]. The onset age of DITRA is dramatically different, ranging from one week after birth [5] to 65 years old among patients with the homozygous *IL36RN* mutation, c.28 C>T [8]. As well as homozygous mutations of *IL36RN*, more than 10 GPP patients have been reported to be heterozygous for an *IL36RN* mutation [8-11]. Furthermore, Li *et al*. [11] reported two healthy control subjects harbouring homozygous mutations of *IL36RN* (c.115+6T>C). The frequency of this allele in the Chinese population (2.05%) indicates that approximately 1/2,400 [11] individuals carry this homozygous mutation. However, the morbidity of GPP is reported to be low among the general population. Thus, although *IL36RN* mutations contribute to the pathogenesis of GPP, the association between *IL36RN* mutations and the clinical manifestations of GPP

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is unclear. Studies investigating the correlation of *IL36RN* mutations with clinical features, therapeutic response, and recurrence frequency are lacking. Further investigations are required to understand the role of *IL36RN* in GPP pathogenesis, identify any genotype/phenotype correlations, and determine whether *IL36RN* mutations are viable indicators of therapeutic outcomes for patients with GPP.

In this study, Sanger sequencing was used to screen mutations in all five exons (including exon-intron boundaries) of *IL36RN* using DNA from 61 patients with GPP, 48 patients with psoriasis vulgaris (PV), and 186 healthy controls. The results show that GPP patients may be categorized into different groups. We compared the age at onset, the initial clinical subtype of psoriasis, possible triggering factors, disease severity, cutaneous manifestations, and responses to acitretin monotherapy, as well as the frequency of recurrence during a follow-up period ranging from two to four years among the mutation groups.

Materials and methods

Subjects

This study consisted of 61 patients with GPP who were admitted to the Department of Dermatology in Peking Union Medical College Hospital between 2012 and 2014. All patients met the Japanese diagnostic criteria for GPP, established by Umezawa [12]. A total of 48 patients with confirmed PV and 186 healthy Chinese Han individuals were also included. This study was approved by the Research Ethics Committee of Peking Union Medical College Hospital. All individuals signed informed consent forms.

Clinical data

The clinical data collected included gender, age at the first episode of GPP, previous history of PV, disease severity (based on the severity classification system) [12], laboratory parameters (*e.g.* white blood cell count, erythrocyte sedimentation rate [ESR], and hypersensitive C-reactive protein [hsCRP] levels), and treatment outcomes.

Criteria for clinical efficacy and recurrence

According to our clinical experience, the following criteria were used to determine therapeutic efficacy. A complete response to treatment was defined as a normal body temperature and the complete disappearance of any rashes, including pustules, erythema, and scales. A marked response was defined as a near-normal body temperature with the disappearance of 75% of the rashes, including pustules, erythema, and scales. Improvement was defined as a partial clearing of pustules and loss of 50-75% of the erythema and scales. No response was defined as the disappearance of a few pustules or partial disappearance of the pustules and less than 50% loss of erythema and scales, or therapy intolerance. The response rate was calculated as the number of cases with either complete responsiveness or a marked response divided by the total number of cases. The recurrence frequency during the follow-up period (two to four years) was classified as no to mild recurrence (zero to two times per year), moderate recurrence (three to four times per year), and severe recurrence (over five times per year).

Methods

The sequence of IL36RN was derived from the GenBank website (NM-012275). Amplimers were designed for all five exons, as well as exon-intron boundaries of the gene using the premier software PRIMER3 (*table S1*). Owing to the excessive size of exon 5 (which contains 2,291 base pairs), a total of seven primer pairs was designed to clone three fragments of the exon. The primers were synthesized by Bioengineering Company, with the synthetic quantity of 20D. The extracted genomic DNA from peripheral blood was treated as template, and the total volume of reaction system was 50 μL: 25 μL of 2×EasyTaq PCR Super Mix, 4 μL of DNA, 2 μL of forward primer, 2 μL of reverse primer, and 17 μL of ddH2O. The amplification conditions were as follows: one cycle of denaturation at 94 for 5 minutes, 35 cycles of denaturation at 94 for 30 seconds, annealing at 60 for 30 seconds, extension at 72 for one minute, and one cycle of extension at 72 for seven minutes. PCR products were detected using 2% agarose gel electrophoresis (AGE), and the results were observed under an ultraviolet lamp. The amplified PCR products were collected, purified, and subjected to DNA sequencing by Shanghai Meiji Company.

Statistical analysis

Data were analysed using the SPSS package, version 13 (SPSS Inc., Chicago, IL, USA) and Plink1.07 software. For normally distributed data, intra-group comparison was performed using either a t-test or one-way ANOVA. For abnormally distributed data, the Mann-Whitney U rank sum test was applied for intra-group comparisons, and the significance level was set at $p<0.05$. For numeric data, the χ^2 test and Fisher's exact test were used for intra-group comparisons, and the significance level was set at $p<0.05$.

Results

Sequencing results

The sequencing results revealed two genetic variants, c.115+6T>C and c.227 C>T (*figure 1*); a single heterozygous c.115+6T>C mutation in seven GPP patients (11.5%), homozygous c.115+6T>C mutation in 25 GPP patients (41.0%), and a c.227 C>T heterozygous mutation in two patients. The overall percentage of GPP patients with the *IL36RN* c.115+6T>C mutation was 52.5%. Conversely, only one (2.1%) of the 48 patients in the PV group and eight (4.3%) of the 186 healthy subjects had a heterozygous c.115+6T>C mutation, and fewer patients had c.227 C>T mutations. Therefore, we only considered the c.115+6T>C mutation as the relevant *IL36RN* variant for the association analysis. The GPP patients were further categorized into three different groups based on their c.115+6T>C mutation status, as follows: the homozygous mutation group (HOMG; 25/61), heterozygous mutation group (HEMG; 7/61), and non-mutation group (NMG; 29/61).

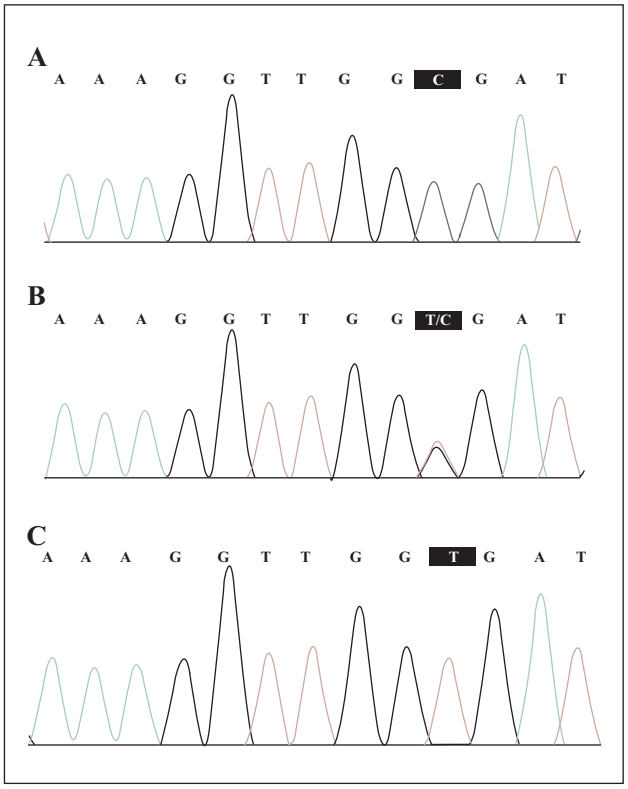

Figure 1. Sequence of *IL36RN* revealing the homozygous **(A)** and heterozygous **(B)** c.115+6T>C mutation; **(C)** wild-type.

Analysis of genotype frequencies

None of the individuals in either the healthy control group or PV group were homozygous for the c.115+6T>C mutation, therefore the genotype frequencies were statistically analysed using an additive model, which showed that this allele was in Hardy-Weinberg equilibrium for all control groups ($p>0.05$). After adjusting for gender and age in GPP and control groups, the CC and CT genotypes were more frequent in GPP patients than in the control subjects. Individuals with the C allele had a 12.72-fold increased risk of developing GPP. The OR for GPP with the combined CC and CT genotypes compared with the TT genotype was 26.1. There was no significant difference in *IL36RN* C allele between the PV group and the healthy control group (*table 1*).

Correlations between *IL36RN* mutations and phenotypes

Initial clinical psoriatic subtype and provocative factors

In the HOMG, four patients initially presented PV, which took two, five, four, and nine years to develop into GPP, respectively. The remaining 21 patients were initially diagnosed with GPP; four cases were related to glucocorticoid withdrawal (one with external and systemic use and the other three with systemic use), six cases with infection, two cases with other specific drugs (one with cephalosporin antibiotics and one with analgesic-antipyretic drugs), four cases with pregnancy, and five patients with no evident possible triggering factors. In addition, 13 patients who initially presented GPP manifested erythrodermic psoriasis after the pustular phase; among these 13 cases, six were accompanied by lymphadenectasis and two by hyperbilirubinaemia. After a short remission, the pustules locally recurred in most patients, one to five times per year, but two patients achieved long-term remission for two to three years.

In the HEMG, five patients initially had PV, which was induced by reduced systemic glucocorticoid dosage in three, external glucocorticoid withdrawal in one, and unknown factors in another. After pustular outbreak, all of these cases resolved into stable PV (*table 2*). The periods of transformation were one, seven, 14, one, and seven years, respectively. During the outbreak phase, one patient developed lymphadenectasis, and another suffered from chills and pitting oedema of the lower limbs. The other two patients in the HEMG initially presented with GPP with no evident triggering factor, however, they developed several local episodes, again without a clear cause.

In the NMG, only one patient was diagnosed with psoriatic arthritis (PsA) after a one-year history of HLA-B27 positivity, but developed GPP after systemic glucocorticoid dosage reduction. A total of 23 patients in the NMG were classified as PV; six cases were related to a reduced systemic glucocorticoid load, four to withdrawal of external glucocorticoid use, six to other specific drugs (including antineoplastic drugs, traditional Chinese medicine, and NSAIDs), one to upper respiratory tract infection, and six patients with no evident cause. Five NMG patients initially developed GPP, which was due to specific treatments in four cases and related to upper respiratory tract infection in one. After a primary outbreak of pustular psoriasis, one patient still had frequently relapsing GPP (*table 2*). There were significant differences in co-occurrence with PV among the three groups ($p<0.001$; *table 3*).

Disease severity

Among the 25 patients in the HOMG, eight (32%) had mild disease, six (24%) had moderate disease, and 11 (44%) had severe disease. In the HEMG, two patients (28.6%) had mild disease, three (42.8%) had moderate disease, and two (28.6%) had severe disease. The majority of NMG patients (58.6%) had mild disease (*figures 2, 3*). No significant differences were observed among the different mutation groups ($p = 0.154$), however, we noticed several exceptionally serious cases in the HOMG.

One patient in the HOMG developed numerous pustules on the body and limbs at the age of 21 and suffered from PsA and IgA nephropathy without obvious stimulants at the age of 28. Severe erythroderma with fusion of the toes occurred at the age of 29 (*figure 4A*). Another patient in the HOMG manifested erythema and pustules on his trunk and limbs seven days after birth. Despite negative HLA-B27 and rheumatoid factor (RF) status, he experienced frequent attacks (three to five times per month) over the years. Tibia and fibula deformities were identified when he was eight years old, and a pustule-induced ulcer was discovered on his right leg at the age of 21. Meanwhile, a biopsy revealed squamous cell carcinoma at the ulceration site on his right leg, and this leg was therefore amputated (*figure 4B, C*).

Table 1. Comparison of the genotype frequency between GPP and control groups based on an additive model.

Genotype	GPP	Healthy Control	C/T		CC+CT/TT	
	No. (%)	No. (%)	*p*	OR (95%CI)	*p*	OR (95%CI)
CC	25 (41.0)	0 (0.0)	<0.001*	12.72 (5.37,30.12)	<0.001*	26.10 (9.54,70.57)
CT	7 (11.5)	8 (4.3)				
TT	29 (47.5)	178 (95.7)				

*The homozygous mutation was not detected in subjects in the healthy control group or PV group. *p<0.05.*

Table 2. Comparison of the clinical presentation among the different mutation groups.

Group	HOMG (*n* = 25)	HEMG (*n* = 7)	NMG (*n* = 29)	*p* value
Initial presentation				
PV (with/without)	4/21	5/2	23/6	<0.001*
PsA (with/without)	0/25	0/7	1/28	–
EP (with/without)	0/25	0/7	0/29	–
GPP (with/without)	21/4	2/5	5/24	<0.001*
Secondary psoriasis after the primary pustular episode				
PV (with/without)	2/23	5/2	22/7	<0.001*
PsA (with/without)	1/24	0/7	1/28	–
EP (with/without)	13/12	0/7	0/29	–
GPP (with/without)	7/18	2/5	5/24	0.596
R (with/without)	2/23	0/7	1/28	–
Current clinical status during 2 to 4-year follow-up				
PV (with/without)	2/23	4/3	15/14	0.001*
PsA (with/without)	1/24	0/7	1/28	–
EP (with/without)	0/25	0/7	0/29	–
GPP (with/without)	6/19	0/7	1/28	–
R (with/without)	18/7	3/4	12/17	0.061

*PV: psoriasis vulgaris; GPP: generalized pustular psoriasis; EP: erythrodermic psoriasis; PsA: psoriatic arthritis; R: remission; HOMG: homozygous mutation group; HEMG: heterozygous mutation group; NMG: non-mutation group. Disease severity was based on the severity classification system; *p<0.05.*

Table 3. Phenotypes among the different mutation groups.

	HOMG (*n* = 25)	HEMG (*n* = 7)	NMG (*n* = 29)	*p* value
Age at onset (year)	22.44 ± 16.90	26.57 ± 16.05	35.45 ± 20.15	0.040*
Gender (male/female)	13/12	4/3	16/13	0.959
Severity (mild/moderate/severe)	8/6/11	2/3/2	17/7/5	0.154
Co-occurrence (without/with PV)	21/4	2/5	6/23	<0.001*
Hyponychial pustules (with/without)	11/14	2/5	1/28	0.002*
WBC	10.98 ± 2.98	12.66 ± 6.07	8.18 ± 6.80	0.067
hsCRP	86.11 ± 49.47	85.75 ± 56.06	42.34 ± 78.70	0.077
ESR	54.67 ± 46.25	60.43 ± 40.53	24.96 ± 15.73	0.001*

*HOMG: homozygous mutation group; HEMG: heterozygous mutation group; NMG: non-mutation group; disease severity is based on the severity classification system. WBC: white blood cell count; hsCRP: hypersensitive C-reactive protein. *p<0.05.*

Hyponychial pustule

Recurrent hyponychial pustules were identified in 11 (44%) patients in the HOMG, two (28.5%) patients in the HEMG, and only one patient in the NMG. There were significant differences in the incidence of hyponychial pustules among the different mutation groups ($p = 0.002$).

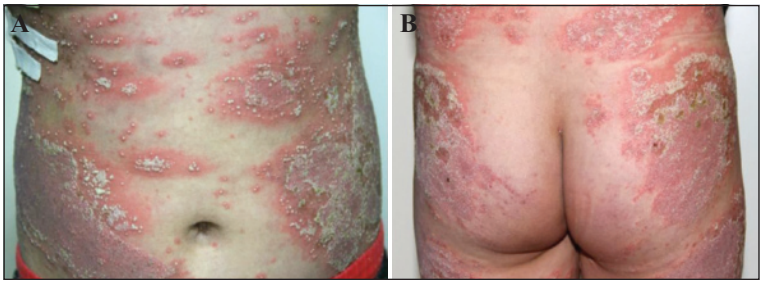

Figure 2. A, B) Extensive miliaria and sterile pustules due to psoriatic erythema on the trunk of a patient in the HOMG.

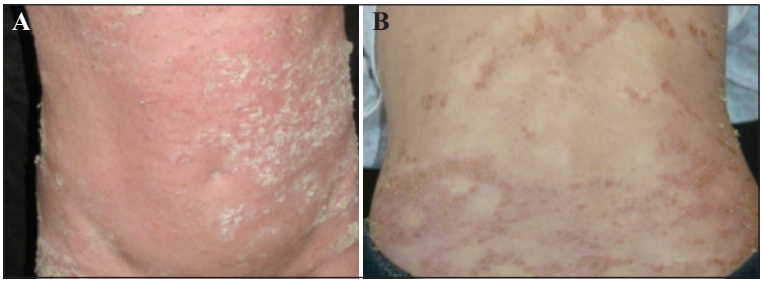

Figure 3. A) Extensive erythema and pustules in a patient in the HEMG. **B)** A patient in the NMG with erythema and pustules on the trunk.

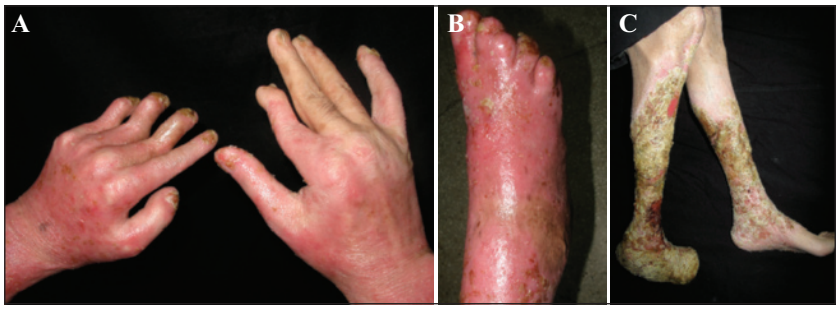

Figure 4. A, **B)** A patient in the HOMG diagnosed with IgA nephropathy with buckled fingers on both hands and a fusion of toes. **C)** A patient in the HOMG with a pustule-induced ulcer and secondary squamous cell carcinoma on the distal limb with volar contracture of the right foot.

Correlation between *IL36RN* mutations and therapeutic response to acitretin

Acitretin was administered to 46 patients (0.5 mg/kg/d), with an initial dose range of 30 mg/d to 50 mg/d. Systemic cyclosporine (100 mg/d), in combination with acitretin, was administered to one severe patient in the HOMG, and methotrexate (10 mg/w) was administered to a patient in the HEMG who developed erythrodermic psoriasis. Two patients in the HOMG and NMG who initially had PsA were treated with etanercept (50 mg/w), concomitant with acitretin. Four pregnant patients (three in the HOMG and one in the HEMG) were treated with glucocorticoids (60 mg/d), combined with intravenous immunoglobulin (20 g/d). Tripterygium is one of the most common systemic Chinese herbal medicines for psoriasis [13] and was administered to nine patients in all three groups. There was no significant difference in therapeutic efficacy of acitretin monotherapy among the three groups ($p>0.05$; *table 4*).

Correlation between *IL36RN* mutations and recurrence frequency

In our study, 61 GPP patients who received a maintenance dose of acitretin (10-30 mg/d) were followed for two to four years. No significant difference was observed in the recurrence frequency among the three groups ($p>0.05$; *table 5*). After treatments, 15 (60%), five (71%), and 16 (55%) patients in the HOMG, HEMG, and NMG, respectively, had a score of 1 at four weeks. At 12 weeks, in the HOMG, two (8%) patients had a score of 0 and 22 (88%) had a score of 1; in the HEMG, two (28.5%) patients had a score of 0 and five (71.4%) had a score of 1; and in the NMG, three patients (10.3%) had a score of 0 and 20 (69.0%) had a score of 1. At 96-144 weeks, most patients (20/25, 80%) in the HOMG had a score of 0. Five out of seven patients (71.4 %) in the HEMG had a score of 0, and 26 of 29 patients (89.7%) in the NMG had a score of 0.

Table 4. Therapeutic efficacy of acitretin.

	HOMG (n = 25)	HEMG (n = 7)	NGM (n = 29)	p value
Complete response	2	2	0	
Marked response	16	2	14	0.089
Improvement & no response	3	1	4	

HOMG: homozygous mutation group; HEMG: heterozygous mutation group; NMG: non-mutation group.

Table 5. Therapeutic efficacy based on frequency of recurrence.

	HOMG (n = 25)	HEMG (n = 7)	NGM (n = 29)	p value
No or mild recurrence	16	5	25	
Moderate recurrence	3	1	1	0.406
Severe recurrence	6	1	3	

Mild recurrence or no recurrence: 0-2 times per year; moderate recurrence: 3-4 times per year; severe recurrence: 5 times per year. HOMG: homozygous mutation group; HEMG: heterozygous mutation group; NMG: non-mutation group.

Ordinal polytomous logistic regression analysis showed that the therapeutic efficacy of acitretin was unrelated to *IL36RN* mutations and other markers (*table 6*).

Discussion

IL36RN encodes the IL-36 receptor antagonist, which can counteract the inflammatory effect of the IL-36 cytokines (*i.e*. IL-36α, IL-36β, and IL-36γ) [14]. An *IL36RN* loss-of-function mutation can lead to overactivation of the IL-36 signalling pathway and its major downstream effector NF-κB [15]. In our study, two *IL36RN* mutations were detected in the 61 GPP patients; c.115+6T>C and c.227C>T (p.Pro76Leu). Most GPP patients carried the c.115+6T>C mutation, whereas only two patients carried the c.227C>T mutation. These mutations were previously reported by Farooq *et al*. [16] and Sugiura *et al*. [17]. Studies on the correlation between GPP genotypes and phenotypes are limited. Recent investigations by Tauber *et al*. [15] suggested that null mutations are correlated with more severe clinical subtypes of psoriasis, whereas hypomorphic mutations correspond to localized clinical subtypes (such as palmoplantar pustulosis [PPP] and acrodermatitis continua), as well as disseminated subtypes. These results provide preliminary support for the correlation between the genotypes and phenotypes of IL36RN-deficient DITRA patients. These data also confirm that non-genetic factors influence the clinical features of GPP [15]. Compared with previous reports, our study reveals a more comprehensive landscape, based on analysis of a possible correlation between genotype and clinical features, acitretin response, and recurrence frequency.

In our study, 43 of 61 GPP patients had suspected inducing factors; 16 of 25 patients in the HOMG, four of seven in the HEMG, and 23 of 29 in the NMG. The primary provocative factors for GPP included sudden withdrawal of systemic corticoids, pregnancy, and infection. During the two- to four-year follow-up period, the recurrence frequency of GPP was reduced when provocative factors were limited. Therefore, we consider that, in addition to *IL36RN* mutations, provocative factors may also play a key role in disease onset, clinical manifestations, and disease outcome. Though carriers of the *IL36RN* C.115+6 CC and CT genotypes had a 26.10-fold increase in risk of developing GPP, the pathogenesis of GPP remains unclear. Identifying environmental triggers may help develop strategies that prevent recurrent GPP flares.

Moreover, our data reveals that the *IL36RN* c.115+6T>C mutation was associated with age at GPP onset, co-occurrence of PV, and hyponychial pustules, but was unrelated to gender and GPP severity. Wang *et al*. [18] also studied the correlation of *IL36RN* mutations with different clinical features of pustular psoriasis in Chinese patients and found that early onset, recurrent GPP (more than two attacks), any instance of acrodermatitis continua of Hallopeau, inverse psoriasis, and a family history of pustular psoriasis were associated with an *IL36RN* mutation. Though Korber *et al.* did not observe a significant difference in age at onset between *IL36RN* mutation carriers and non-carriers [9], a difference (p = 0.04) was identified in our study which is consistent with a study by Li *et al*. (p = 0.08) [11]. The wide range of age at onset (1-83 years of age) [5, 19] is still a mystery. Most likely, *IL36RN* mutations contribute to early onset in Chinese carriers. We also found, for the first time, that hyponychial pustules may be a manifestation specific to *IL36RN* mutation.

In our study, no significant difference was observed in the severity of disease among the different mutation groups (p = 0.154). However, we noticed some severe cases in the HOMG. There were 21 patients with initial GPP in the HOMG, 13 of whom manifested once with severe erythroderma. However, none of the patients in the HEMG or NMG had erythroderma. We also identified one GPP patient, homozygous for *IL36RN* mutation, who developed an ulceration on the pustules of his left leg when he was 21 years old, which gradually progressed to squamous cell carcinoma at the age of 24, and resulted in leg amputation. Another patient in the HOMG suffered from comorbid

Table 6. Ordinal polytomous logistic regression analysis of influencing factors for therapeutic efficacy.

	Regression coefficient (B)	*p* value	OR value	95% confidence interval
Gender	1.098	0.131	2.998	(0.721, 12.461)
Family history	-0.440	0.596	0.644	(0.127, 3.271)
Temperature	-1.708	0.001	0.181	(0.065, 0.508)
WBC	-0.039	0.620	0.962	(0.824, 1.122)
CRP	0.006	0.423	1.006	(0.991, 1.021)
ESR	0.012	0.375	1.012	(0.985, 1.040)
Age at onset	0.002	0.929	1.002	(0.964, 1.041)
Mutation	0.064	0.937	1.066	(0.219, 5.190)

WBC: white blood cell count; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

PsA and IgA nephropathy for nine years after the onset of GPP. He developed acute renal failure during hospitalisation in 2016, but successfully recovered with dialysis and systemic glucocorticoid therapy. Both of these patients experienced life-threatening conditions and were resistant to a variety of treatments. However, patients without these mutations did not present any of these complications, secondary lesions, or erythrodermic changes. Therefore, we speculate that individuals homozygous for these mutations may develop severe phenotypes.

The majority of GPP patients choose acitretin instead of biologics for economic reasons. Therefore, we evaluated the efficacy of acitretin. Through rigorous statistical analysis, no significant difference was observed in the therapeutic efficacy of acitretin and the recurrence frequencies among the three groups. Having limited possible triggering factors, most of the patients in all three groups, who were administered a maintenance dosage of acitretin (10-30 mg/d), had achieved remission with a score of 0 or 1 during the two- to four-year follow-up. This suggests that the *IL36RN* mutation is insufficient as a predictive marker for either the systemic use of acitretin or the recurrence frequency of GPP. In contrast, environmental factors play a pivotal role in preventing GPP recurrence. Arakawa *et al*. [20] reported the therapeutic efficacy of blocking IL-12/IL-23 signalling in GPP, regardless of patient *IL36RN* mutation status. Furthermore, they also showed that combining IL-12/IL-23 inhibition with acitretin may act synergistically in GPP patients by suppressing the Th17 response through the retinoid-related orphan receptors, RORγ and RORα. The mechanism by which *IL36RN* mutation leads to the progression to GPP is only partially understood. Pathogenic T-cell-mediated adaptive immune responses may also play a crucial role in GPP pathogenesis. Thus, large-scale clinical studies are required to clarify whether these IL36TN mutations affect GPP clinical manifestations. Rather than a monogenic disease, GPP might be a result of the combined effects of genetic factors, immune responses, and environmental influences. In the future, randomised controlled trials with a larger sample size are needed to provide more data in establishing a standardised therapy against GPP in clinical practice. ■

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Supplementary Material

Supplementary material (Table S1) accompanied by the online version of this article is available on http://www.sciencedirect.com and doi:10.1684/ejd.2018.3245.

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