Journal of Dermatological Science

Letter to the Editor

A newly revealed *IL36RN* mutation in sibling cases complements our *IL36RN* mutation statistics for generalized pustular psoriasis

Takuya Takeichi[1], Yaei Togawa[2], Yusuke Okuno[3,4], Rieko Taniguchi[4], Michihiro Kono[1], Hiroyuki Matsue[2], Kazumitsu Sugiura[5,*], Masashi Akiyama[1,*]

[1]Department of Dermatology, [4]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Dermatology, Chiba University Graduate School of Medicine, Chiba, Japan; [3]Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan; [5]Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan

*Correspondence: Masashi Akiyama, Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Telephone: 81-52-744-2314

E-mail: makiyama@med.nagoya-u.ac.jp

Kazumitsu Sugiura, Department of Dermatology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-1192, Japan

Telephone: 81-562-93-9256

E-mail: ksugiura@fujita-hu.ac.jp

Funding statement: None

The authors have no conflicts of interest to declare.

Running title: *IL36RN* mutation in GPP without PV

Abbreviations: GPP, generalized pustular psoriasis; IL36RN, interleukin-36 receptor antagonist; PV, psoriasis vulgaris

KEY WORDS: generalized pustular psoriasis, interleukin-36 receptor antagonist, psoriasis vulgaris

Word count: 956/1000; Number of References: 9/10; Number of Figures and Tables: 2/2

To the Editor:

Generalized pustular psoriasis (GPP) is a rare but severe form of psoriasis that is sometimes life-threatening and is characterized by sudden, repeated episodes of high-grade fever, generalized erythema and pustules. In 2011, *IL36RN* mutations were reported as causative genetic defects in several GPP cases [1, 2]. *IL36RN* encodes the interleukin 36-receptor antagonist (IL36Ra), which is an anti-inflammatory cytokine. Aberrant IL36Ra structure and function lead to the unregulated secretion of inflammatory cytokines and GPP. Subsequent reports have also implicated *IL36RN* mutations/variants in various pustular diseases, such as impetigo herpetiformis, acute generalized exanthematous pustulosis and annular pustular psoriasis [3-6]. To date, 17 pathogenic mutations in *IL36RN* have been reported in GPP or other inflammatory pustular skin disorders (www.hgmd.cf.ac.uk, as of HGMD professional, 2016.2).

We previously reported that the majority of “GPP without psoriasis vulgaris (PV)” patients had homozygous or compound heterozygous mutations in *IL36RN*, and in contrast, only a small number of “GPP with PV” patients had *IL36RN* mutations [7]. In that report, we determined that 9 out of 11 cases of “GPP without PV” had homozygous or compound heterozygous mutations in *IL36RN*. In the present study, we performed whole-exome sequencing of the other two individuals with “GPP without PV”, in whom we could not detect any mutations in *IL36RN* by the initial Sanger sequencing.

Surprisingly, a previously unreported homozygous *IL36RN* missense mutation was detected in the two siblings with “GPP without PV”.

The proband is a 51-year-old male born to related parents (Fig. 1c; the proband is II-1). He presented with recurrent episodes of localized sterile pustules with erythema over the entire body surface, but without chronic scaly erythematous plaques (Fig. 1a). He had been suffering from similar eruptions since the age of 7 years with a tendency to recurrence every spring mainly caused by common colds. A skin biopsy from a pustular eruption on the trunk revealed a spongiform pustule in the epidermis (Fig. 1d). He has been treated with systemic retinoid (20 mg/day). The younger brother of the proband, a 48-year-old man, also had recurrent episodes of generalized sterile pustules with erythema, but without chronic scaly erythematous plaques, since 2 years of age. Physical examination revealed large erythemas associated with marginal pustules over the whole body (Fig. 1b). A skin biopsy specimen from an erythematous lesion revealed moderate acanthosis with the disappearance of the granular layer, mild hyperkeratosis and neutrophilic microabscesses (Fig. 1e). Moderate neutrophilic and lymphocytic infiltration was seen in the upper dermis, suggesting GPP.

After obtaining ethics approval and informed consent, we extracted genomic DNA from peripheral blood samples from the patients and controls in compliance with

the *Declaration of Helsinki*. Using the elder brother's DNA (II-1), whole-exome sequencing was performed on the Illumina HiSeq 2500 (San Diego, CA, USA), as described elsewhere [8]. Given the disease inheritance and history of consanguinity, we focused on deleterious homozygous variants. Within these variants, a rare homozygous substitution in *IL36RN* (c.125T>A, p.Ile42Asn: rs775349262) was deemed to be of potential relevance for this pedigree. It was also validated by Sanger sequencing (data not shown). Because our initial Sanger sequencing failed to find this mutation, we designed a new primer pair (F: gtggacaggagaagccatgt, R: ctgctagggtgtcccacag) for exon 4 of *IL36RN*. The pathogenicity of this missense mutation was supported by both the 'Sorts Intolerant From Tolerant' program (SIFT; score 0, probably damaging) and the Polymorphism Phenotyping v2 program (polyphen-2; score 1, probably damaging). The global allele frequency of rs775349262 is 8.463e-06 (only one heterozygous carrier in European (non-Finnish) ethnicities was reported in the ExAC database). Next, we conducted immunohistochemical analyses with an anti-IL36RN antibody, anti-IL1F5 (R&D Systems, Minneapolis, MN), in lesional skin samples from both patients. Figures 1f and 1g show reduced or largely absent expression of IL36RN in the affected individuals' skin compared with the control skin of PV (Fig. 1h).

The mutation c.125T>A (p.Ile42Asn) in the present siblings lies on the fourth β-

strand of IL36RN, close to the pathogenic mutation p.Arg48Trp that was previously reported in a case of GPP [2]. In addition, *in silico* analysis predicted the mutation to be a “damaging” substitution. Furthermore, IL36RN protein expression is largely absent in all the epidermal layers of skin from both patients (Figs. 1f and 1g) compared with the PV control skin (Fig. 1h). These findings are consistent with immunohistochemical analyses in our previous report [7]. Thus, the present previously unreported mutation, c.125T>A, is likely to be functionally relevant.

We previously reported that whole-exome sequencing improved mutation detection in a diagnostic epidermolysis bullosa laboratory [9]. In the report, in six of nine cases, the mutations were overlooked by initial Sanger sequencing – not because of human error, but rather due to the variable peak heights associated with current Sanger sequencing chemical labelling of DNA, which could lead to imprecision in interpreting sequence traces. In the present report, the newly designed primer pair clearly confirmed the pathogenic mutation of the present cases. We also re-sequenced exon 4 of *IL36RN* in all the other 20 pedigrees of “GPP with PV” described in our previous report [7] using the newly designed primer set; however, no mutations were detected in any pedigree.

We previously analyzed *IL36RN* mutations of 11 patients from 9 pedigrees of “GPP without PV” and 20 patients from 19 pedigrees of “GPP with PV” [7]. The present

study revealed the additional pathogenic mutation of *IL36RN* in two “GPP without PV” patients, in whom we failed to detect any mutation by the initial Sanger sequencing. Altogether, among the 11 patients from 9 pedigrees of “GPP without PV” in our previous report, all have been finally found to have *IL36RN* mutations (Table 1). Thus, our findings strongly support the idea that “GPP without PV” is a distinct subtype of GPP and is etiologically distinguished from “GPP with PV”, both genetically and clinically.

Acknowledgments

This study was supported by JSPS KAKENHI Grants Numbers 15H04887, 15K15415, 15H06280, 16K19717. The work was also supported by the Japan Intractable Diseases Research Foundation. The authors acknowledge the Human Genome Center, Institute of Medical Science, the University of Tokyo (http://sc.hgc.jp/shirokane.html) for providing super-computing resources.

Figure legends

Figure 1. Clinicopathological features of the siblings of "GPP without PV"

(a) The 18-year-old proband (Patient II-1): Pustules on background erythema are seen on the chest and the upper arms. (b) The proband's 11-year-old younger brother (Patient II-2): Pustular erythema on the back and the upper arms. (c) Family tree. (d) Light microscopy of the affected skin (Patient II-1) shows acanthosis and hyperkeratosis. (e) Light microscopy of the affected skin (Patient II-2) demonstrates acanthosis, hyperkeratosis and parakeratosis in the epidermis. (f-h) Immunohistochemistry of the GPP lesion with anti-IL1F5 (IL36RN); staining is largely negative in both Patients II-1 (f) and II-2 (g) compared with the psoriasis skin (h). Scale bars = 100 μm.

References

[1] Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraitag S, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med 365:620-628,2011.

[2] Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V, et al. Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet 89:432-437,2011.

[3] Mossner R, Frambach Y, Wilsmann-Theis D, Lohr S, Jacobi A, Weyergraf A, et al. Palmoplantar Pustular Psoriasis Is Associated with Missense Variants in CARD14, but Not with Loss-of-Function Mutations in IL36RN in European Patients. J Invest Dermatol 135:2538-2541,2015.

[4] Tauber M, Bal E, Pei XY, Madrange M, Khelil A, Sahel H, et al. IL36RN Mutations Affect Protein Expression and Function: A Basis for Genotype-Phenotype Correlation in Pustular Diseases. J Invest Dermatol 2016. in press

[5] Sugiura K, Nakasuka A, Kono H, Kono M, Akiyama M. Impetigo herpetiformis with IL36RN mutations in a Chinese patient: A founder haplotype of c.115+6T>C in East Asia. J Dermatol Sci 79:319-320,2015.

[6] Hayashi M, Nakayama T, Hirota T, Saeki H, Nobeyama Y, Ito T, et al. Novel IL36RN gene mutation revealed by analysis of 8 Japanese patients with generalized pustular psoriasis. J Dermatol Sci 76:267-269,2014.

[7] Sugiura K, Takemoto A, Yamaguchi M, Takahashi H, Shoda Y, Mitsuma T, et al. The majority of generalized pustular psoriasis without psoriasis vulgaris is caused by deficiency of interleukin-36 receptor antagonist. J Invest Dermatol 133:2514-2521,2013.

[8] Kunishima S, Okuno Y, Yoshida K, Shiraishi Y, Sanada M, Muramatsu H, et al. ACTN1 mutations cause congenital macrothrombocytopenia. Am J Hum Genet 92:431-438,2013.

[9] Takeichi T, Liu L, Fong K, Ozoemena L, McMillan JR, Salam A, et al. Whole-exome sequencing improves mutation detection in a diagnostic epidermolysis bullosa laboratory. Br J Dermatol 172:94-100,2015.

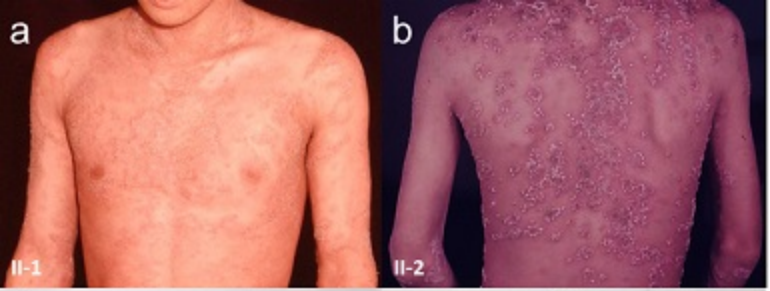
Table 1. Rates of GPP patients with *IL36RN* mutations

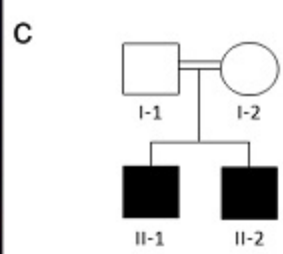
Type of GPP	Number of patients with *IL36RN* mutations/ Total number of patients analyzed for mutations in *IL36RN*[1]
GPP without PV	11/11 (100%)
GPP with PV	2/20 (10%)[2]

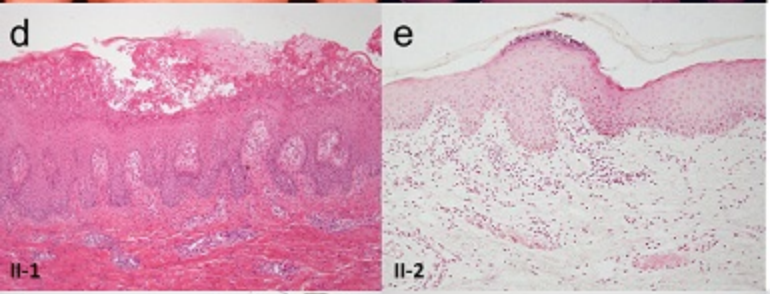
Abbreviations: GPP, generalized pustular psoriasis; PV, psoriasis vulgaris

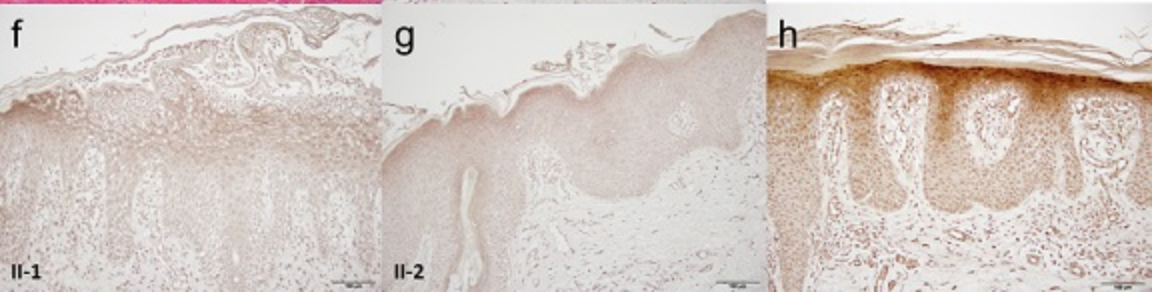
[1]We counted number of patients reported in Sugiura *et al* ., 2013.

[2]One sibling pair had one heterozygous mutant allele.


a
II-1
b
II-2


c
I-1
I-2
II-1
II-2


d
II-1
e
II-2


f
II-1
g
II-2
h