## CONFLICT OF INTEREST

Michael Sticherling supports Actelion, Biogen, and Pfizer scientifically, is a member of advisory boards of Abbott, Pfizer, MSD, and Leo, and is/was a speaker for Abbott, Pfizer, MSD, Leo, and Janssen. The other authors state no conflict of interest.

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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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*See related commentary on pg 2503*

# Prevalent and Rare Mutations in *IL-36RN* Gene in Chinese Patients with Generalized Pustular Psoriasis and Psoriasis Vulgaris

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## TO THE EDITOR

Generalized pustular psoriasis (GPP; OMIM 614204) is a severe type of psoriasis characterized by widespread erythematous skin and sterile pustules. The acute onset is typically associated with high-grade fever, rigor, and toxicity. Psoriasis vulgaris (PV; OMIM 177900) is the most common form of psoriasis, which is an immune-mediated inflammatory skin disease with scaly and well-demarcated red plaques. Although GPP is traditionally classified as a variant of PV, the common mechanisms of pathogenesis remain unclear (Setta-Kaffetzi *et al.*, 2013).

Recently, a homozygous loss-of-function mutation in *IL-36RN*, L27P, was first identified in GPP patients (Marrakchi *et al.*, 2011). On the other hand, different mutations were found by exome sequencing on unrelated individuals with GPP in Caucasians (Onoufriadis *et al.*, 2011). Of note, our previous study in 10 Chinese GPP patients showed that only one known polymorphism, c.227C>T, was identified in one patient (Li *et al.*, 2012). We also found the c.115+

*Abbreviations: AGPP, adult-onset GPP; CI, confidence interval; GPP, generalized pustular psoriasis; OR, odds ratio; PGPP, paediatric-onset GPP; PV, psoriasis vulgaris*

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**Table 1. The four *IL-36RN* mutations in GPP and PV patients**

| | c.115 + 6 T>C | | | | p.Asn47Ser | | | | p.Pro76Leu | | | | p.Arg102Gln | | | | Combined | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | Con | PGPP | AGPP | PV | Con | PGPP | AGPP | PV | Con | PGPP | AGPP | PV | Con | PGPP | AGPP | PV | Con | PGPP | AGPP | All GPP | PV |
| AA | 352 | 10 | 27 | 111 | 348 | 28 | 35 | 101 | 366 | 27 | 36 | 111 | 372 | 30 | 37 | 108 | 328 | 10 | 25 | 35 | 89 |
| Aa | 11 | 2 | 2 | 2 | 24 | 2 | 2 | 10 | 1 | 3 | 2 | 1 | 0 | 0 | 1 | 0 | 37 | 2 | 4 | 6 | 12 |
| aa | 2 | 18 | 9 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 18 | 9 | 27 | 0 |
| Total | 365 | 30 | 38 | 113 | 373 | 30 | 38 | 111 | 367 | 30 | 38 | 112 | 372 | 30 | 38 | 108 | 365 | 30 | 38 | 68 | 101 |
| *P* | – | 3.98E–22 | 1.15E–08 | 0.85 | – | 1 | 0.24 | 0.53 | – | 0.001 | 0.02 | 0.41 | – | – | 0.09 | – | – | 3.64E–21 | 1.20E–08 | 6.26E–22 | 0.74 |
| *P* (PGPP versus AGPP) | – | – | 0.004 | – | – | – | 1 | – | – | – | 0.64 | – | – | – | 1 | – | – | – | 0.008 | – | – |

Abbreviations: AGPP, adult-onset generalized pustular psoriasis; Con, control; GPP, generalized pustular psoriasis; PGPP, pediatric-onset generalized pustular psoriasis; PV, psoriasis vulgaris.

**Table 2. Comparison of the allele of the variant (115 + 6T>C) in GPP and PV patients**

| | Allele | | | | | |
|---|---|---|---|---|---|---|
| | a | A | *P* (compared with control) | OR (95% CI) | *P* (PGPP versus AGPP) | OR (95% CI) |
| Con | 15 | 715 | – | 1.00 | | |
| PGPP | 38 | 22 | 2.56E − 74 | 82.33 (39.56–171.34) | 1.46E − 05 | 4.83 (2.32–10.06) |
| AGPP | 20 | 56 | 5.29E − 23 | 17.02 (8.26–35.06) | | |
| PV | 2 | 224 | 0.387 | 0.42 (0.09–1.87) | | |

Abbreviations: AGPP, adult-onset generalized pustular psoriasis; CI, confidence interval; Con, control; GPP, generalized pustular psoriasis; OR, odds ratio; PGPP, pediatric-onset generalized pustular psoriasis; PV, psoriasis vulgaris.

6T>C variant in three GPP patients as well as in healthy controls. As the case number was so limited at that time, it was premature to identify the association between GPP and the mutation c.115 + 6T>C, while the following study conducted by Farooq *et al.* (2012) found the same mutation. Their study prompted us to further investigate *IL-36RN* mutations in Chinese patients with GPP and PV. In addition, we compared the association between *IL-36RN* mutations with paediatric-onset GPP (PGPP) and adult-onset GPP (AGPP). For detailed information of Materials and Methods see Supplementary Information online.

Four mutations, namely, c.115 + 6T>C, p.Asn47Ser, p.Pro76Leu, and p.Arg102Gln, were identified in GPP patients (Supplementary Figure S2 online). Three mutations (c.115 + 6T>C, p.Asn47Ser, and p.Pro76Leu) were identified in PV patients and in healthy controls. Mutation c.115 + 6T>C was the most common mutation in GPP. The percentage of c.115 + 6T>C was 3.6% in the normal controls. Interestingly, the c.115 + 6T>C variant was also identified in two normal controls in a homozygous state, and they are both at an age over 40.

The percentage of *IL-36RN* mutations was 48.5% in GPP patients. These variants were also carried by 10.7% of the control individuals. The associations between the most common mutation c.115 + 6T>C and GPP of either group was statistically significant comparing with controls (PGPP: $P = 3.98 \times 10^{-22}$, AGPP: $P = 1.15 \times 10^{-8}$) (Table 1). Mutation Pro76Leu was also significantly associated with GPP of either group statistically comparing with controls (PGPP: $P = 1.49 \times 10^{-3}$; AGPP: $P = 0.024$) (Table 1). The other two rarer *IL-36RN* variants (Asn47Ser and Arg102Gln) were not associated with GPP when analyzed individually. However, the combined genotype showed a highly significant association ($P = 6.26 \times 10^{-22}$) (Table 1). The frequency of *IL-36RN* mutations was 34.2 vs. 66.7% in PGPP and AGPP patients, respectively ($P = 0.008$). Twenty cases (66.7%) with PGPP and 11 cases (28.9%) with AGPP were identified to be carriers of common mutation c.115 + 6T>C ($P = 0.0035$) (Table 1). The allele of c.115 + 6T>C also demonstrated significant difference in the AGPP and PGPP groups ($P = 1.46 \times 10^{-5}$, odds ratio (OR) = 4.83, 95% confidence interval (CI): 2.32–10.06) (Table 2).

About 9% PV patients were found to be heterozygote for mutation Asn47Ser, which was higher than that for mutation c.115 + 6T>C or Pro76Leu. However, no significant relationship was found between mutation Asn47Ser and PV ($P = 0.53$) (Table 1). Mutation c.115 + 6T>C was merely found in 1.8% of PV patients, the allele of which showed no association with PV ($P = 0.387$, OR = 0.42, 95% CI: 0.09–1.87) (Table 2). A total of 12 (11.9%) patients with PV were heterozygote carriers for *IL-36RN* mutations. The association between PV and combined genotype was not significant ($P = 0.74$) (Table 1).

To date, 11 *IL-36RN* mutations (p.Arg10X, p.Leu27Pro, p.Lys35Arg, c.115 + 6T>C, p.Asn47Ser, p.Arg48Trp, p.Pro76Leu, p.Arg102Gln, p.Arg 102Trp, p.Ser113Leu, and p.Thr123Arg) have been identified in African, European, and Asian populations. In the current study, four *IL-36RN* mutations were identified in the GPP cohort. Mutation c.115 + 6T>C was the most common mutation in the GPP and normal controls. This mutation has been reported in Japanese and Malay populations, but it was not found in European and African populations (Sugiura *et al.*,

2012; Setta-Kaffetzi *et al.*, 2013). The most common mutations p.Ser113Leu in European population and p.Leu27Pro in African population were not found in the cohorts of the current study (Setta-Kaffetzi *et al.*, 2013). These results indicate that *IL-36RN* mutations in the Chinese population dramatically differed from that in the European and African population. In this study, the mutation c.227C>T was found on the same chromosome as the c.115+6T>C allele in GPP patients as Setta-Kaffetzi *et al.* reported previously in Malay and Chinese population. These results indicated that c.227C>T-c.115+6T>C allele may be the founder allele in Asian populations.

Interestingly, the c.115+6T>C variant was identified in two normal controls in homozygous state in this study, implying that multiple factors should contribute to the pathogenesis of GPP. Setta-Kaffetzi *et al.* found that six GPP cases carried heterozygous mutations in *IL-36RN*. They speculated that the GPP patients with a single *IL-36RN* variant may represent cases of tri-allelic disease inheritance. IL-38, another member of IL-1 family, specifically bound to IL-36R, had a similar biological role in the immune responses (Van de Veerdonk *et al.*, 2012). Of note, evidence showed that IL-38 could bind to IL-36R, which might be a compensative mechanism in healthy individuals with the mutant *IL-36RN*. In addition, GPP patients with no mutations in *IL-36RN* gene also had overexpressing inflammatory cytokines, which was similar to the GPP patients with specific mutation in other genes (Towne *et al.*, 2011).

Although the mutations of *IL-36RN* are strongly associated with GPP, there existed no significant association between *IL-36RN* mutations and PV in this study ($P=0.746$). The impaired function of mutant *IL-36RN* in GPP patients might attenuate the specific inhibitory effect on the proinflammatory activation of IL-36, while keratinocytes in PV patients could overexpress IL-36 lastingly through the circular induction of IL-36 and TNF, IL-17, and IL-22 (Carrier *et al.*, 2011; Johnston *et al.*, 2011) The existing data indicated that the mechanism of overexpression of IL-36 in PV differed from that of GPP.

In this study, it was remarkable that the percentage of *IL-36RN* mutations of PGPP patients was much higher than that of AGPP patients. We speculated that heritable factors might have a more important role in PGPP compared with AGPP patients. On the other hand, the age of GPP onset differed in the c.115+6T>C mutant individuals, which also suggested that the specific immunological or biological environment might lead to various pathogenical results. For example, the avoidance of the several triggering factors, such as bacterial infection, mediation, stress, and hypocalcemia, might delay the onset of GPP (Zelickson and Muller, 1991).

Taken together, the data above strongly indicated the existence of environmental factors or other regulatory genes may also have a role in the pathogenic process of GPP.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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## SUPPLEMENTARY MATERIAL

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