Genetic polymorphism of \textit{IL36RN} in Han patients with generalized pustular psoriasis in Sichuan region of China

A case–control study

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1. Introduction

Generalized pustular psoriasis (GPP) is not an uncommon skin disease, characterized by sudden episodes of generalized rash and sterile pustules with high fever and chills, neutrophilia, and elevated C-reactive protein. It could severely detrimental to the quality of life because of its frequent recurrence. GPP can occur alone or be associated with other inflammatory diseases, such as psoriasis vulgaris (PV) and palmoplantar pustulosis. Although many a research has already confirmed that the genetic susceptibility of PV was closely related to HLA gene polymorphism, the exact pathogenesis of GPP is still vague so far.

Since 2011, when Marrakchi et al. first found \textit{IL36RN} mutations in European patients with GPP alone, an increasing number of research have shown that \textit{IL36RN} mutations are extremely likely to be the main molecular genetic basis of GPP alone. The pathogenesis of GPP with PV (GPP+PV) seems to be more complex when compared with GPP alone. \textit{IL36RN} mutations may be only involved in a minority of GPP+PV patients.

In China, few studies have been conducted on the gene polymorphism of \textit{IL36RN} in GPP patients so far. In the present study, we detected the \textit{IL36RN} variant types and frequency in Han patients with GPP in Sichuan region, compared with the \textit{IL36RN} variant frequency between patients with GPP alone and GPP+PV, and tried to clarify the pathogenesis of GPP in this region.

2. Materials and methods

2.1. Subjects

In this study, a case–control design was adopted. We calculated the sample size according to the sample size calculation formulas for independent case–control designs. Ultimately, we enrolled a total number of 143 people from January 2012 to January 2016,
Table 1
Clinical data and IL36RN variants of all GPP patients in our study.

| ID            | Gender | Age, y | IL36RN variants |
|---------------|--------|--------|-----------------|
| GPP alone01   | M      | 10     | N               |
| GPP alone02   | M      | 12     | N               |
| GPP alone03   | F      | 16     | hom.c.115+6T>C  |
| GPP alone04   | F      | 13     | hom.c.115+6T>C; het.c.140A>G |
| GPP alone05   | M      | 52     | hom.c.115+6T>C  |
| GPP alone06   | M      | 12     | hom.c.115+6T>C  |
| GPP alone07   | M      | 20     | hom.c.115+6T>C; het.c.140A>G |
| GPP alone08   | M      | 5      | het.c.115+6T>C  |
| GPP alone09   | F      | 39     | hom.c.115+6T>C  |
| GPP alone10   | M      | 19     | hom.c.115+6T>C  |
| GPP alone11   | F      | 27     | hom.c.115+6T>C  |
| GPP alone12   | F      | 27     | hom.c.140A>G    |
| GPP alone13   | M      | 49     | N               |
| GPP alone14   | M      | 67     | N               |
| GPP alone15   | F      | 44     | N               |
| GPP alone16   | M      | 59     | hom.c.115+6T>C  |
| GPP alone17   | F      | 37     | hom.c.115+6T>C; het.c.227C>T |
| GPP alone18   | F      | 52     | hom.c.115+6T>C; het.c.227C>T |
| GPP alone19   | F      | 33     | hom.c.115+6T>C  |
| GPP alone20   | M      | 24     | het.c.227C>T    |
| GPP alone21   | M      | 65     | hom.c.115+6T>C  |
| GPP alone22   | M      | 42     | hom.c.115+6T>C  |
| GPP alone23   | F      | 44     | hom.c.115+6T>C  |
| GPP alone24   | F      | 52     | het.c.115+6T>C  |
| GPP + PV01    | M      | 10     | N               |
| GPP + PV02    | M      | 5      | N               |
| GPP + PV03    | F      | 48     | N               |
| GPP + PV04    | F      | 22     | het.c.115+6T>C  |
| GPP + PV05    | M      | 38     | hom.c.115+6T>C; het.c.140A>G |
| GPP + PV06    | M      | 15     | hom.c.115+6T>C  |
| GPP + PV07    | M      | 25     | hom.c.115+6T>C  |
| GPP + PV08    | F      | 24     | N               |
| GPP + PV09    | M      | 43     | N               |
| GPP + PV10    | M      | 44     | N               |
| GPP + PV11    | M      | 41     | N               |
| GPP + PV12    | M      | 29     | N               |
| GPP + PV13    | M      | 43     | N               |
| GPP + PV14    | M      | 41     | N               |
| GPP + PV15    | M      | 45     | N               |
| GPP + PV16    | F      | 69     | N               |
| GPP + PV17    | M      | 58     | het.c.115+6T>C; het.c.140A>G |
| GPP + PV18    | F      | 44     | het.c.115+6T>C; het.c.227C>T |
| GPP + PV19    | F      | 50     | hom.c.115+6T>C  |

F = female, GPP = generalized pustular psoriasis, het. = heterozygous for, hom. = homozygous for, M = male, N = no mutation, PV = psoriasis vulgaris.

Table 2
PCR primers for amplifying IL36RN.

| Amplified region | Primer sequence (5’→3’) | Annealing temperature, °C | Product size, bp |
|------------------|--------------------------|---------------------------|-----------------|
| Exon2            | F:GGTG6GTCACGGAGCTCTCC   | 57                        | 345             |
|                  | R:GAAAACACAGCAGCGCAGAATTTC |                           |                 |
| Exon3            | R:GAAAACACAGCAGCGCAGAATTTC | 57                        | 410             |
|                  | R:GAAAACACAGCAGCGCAGAATTTC |                           |                 |
| Exon4            | R:GAAAACACAGCAGCGCAGAATTTC | 57                        | 362             |
|                  | R:GAAAACACAGCAGCGCAGAATTTC |                           |                 |
| Exon5            | R:GAAAACACAGCAGCGCAGAATTTC | 57                        | 438             |
|                  | R:GAAAACACAGCAGCGCAGAATTTC |                           |                 |

PCR = polymerase chain reaction.
Table 3
The distribution of the IL36RN variants in patients and controls.

| IL36RN variants | Con n (%) | PV n (%) | Total GPP n (%) | GPP alone n (%) | GPP+PV n (%) |
|-----------------|-----------|----------|----------------|----------------|-------------|
| TT-AA-CC        | 50 (100)  | 50 (100) | 17 (39.53)     | 5 (20.83)      | 12 (36.84)  |
| TC-AA-CC, TT-AA-CT, TT-GG-CC, TC-GG-CC | 0 (0)     | 0 (0)    | 26 (60.47)     | 19 (79.17)     | 7 (20.83)   |
| CC-AA-CT, CC-GG-CC |          |          |                |                |             |
| Total           | 50        | 50       | 43             | 24             | 19          |
| P (vs Con)      |          |          |                |                |             |
| P (GPP alone vs GPP+PV) |          |          |                |                |             |

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.

2.4. Statistical analysis

The count numbering of the mutations detected in this study was on the basis of RefSeq NM_173170. Differences in frequencies of IL36RN mutations between groups were analyzed by Chi-square test by using SPSS Statistics 17.0 software (IBM SPSS, Armonk, NY). P < .05 was recognized as significant threshold.

3. Results

Three variants, c.115+6T>C (p.Arg10ArgfsX1, rs148755083), c.140A>G (p.Asn47Ser, rs28938777), and c.227C>T (p.Arg76Leu, rs139497891), were indentified in 26 out of 43 GPP patients (60.47%) (Tables 1 and 3). Among them, c.115+6T>C was the most common one, with a variant frequency of 55.81% (Table 4). None of IL36RN mutations was found in either PV patients or healthy controls. Both the separate allele frequency and total variant frequency had statistical significance when comparing GPP alone group with PV group or healthy controls (Tables 3, 5–7). GPP alone group exhibited a much higher IL36RN variant frequency than GPP+PV group (79.17% vs 36.84%, P < .05) (Table 3).

4. Discussion

IL36RN gene encodes the interleukin-36-receptor antagonist (IL-36Ra), an antagonist of 3 cytokines (interleukin-36α, interleukin-36β, interleukin-36γ), expressing primarily in the skin. IL-36Ra can competitively bind to the interleukin-36 receptor, disable the recruitment of the interleukin-1 receptor accessory protein, subsequently inhibit downstream activation of nuclear factor-κB (NF-κB) and mitogen-activated protein (MAP) kinases, and ultimately avoid exacerbated inflammatory responses.[6,13] The mutation of IL36RN could expectedly result in the deficiency of IL-36Ra and cause skin inflammation.

Table 4
The IL36RN variants in patients and controls.

| IL36RN variants | Cases | n (%) | n (%) | n (%) | n (%) | n (%) |
|-----------------|-------|-------|-------|-------|-------|-------|
| Total GPP       | 50    | 50    | 50    | 50    | 100   | 100   |
| GPP alone       | 24    | 17    | 17    | 17    | 32    | 32    |
| GPP+PV          | 19    | 7     | 7     | 7     | 11    | 11    |
| PV              | 50    | 0     | 0     | 0     | 0     | 0     |
| Con             | 50    | 0     | 0     | 0     | 0     | 0     |

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.

Table 5
The distribution of rs148755083 alleles in patients and controls.

| rs148755083 | Con n (%) | PV n (%) | Total GPP n (%) | GPP alone n (%) | GPP+PV n (%) |
|-------------|-----------|----------|----------------|----------------|-------------|
| c.115+6T    | 100 (100) | 100 (100)| 100 (100)      | 100 (100)      | 100 (100)   |
| c.115+6C    | 0 (0)     | 0 (0)    | 0 (0)          | 0 (0)          | 0 (0)       |
| P (vs PV)   | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (vs Con)  | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (GPP alone vs GPP+PV) | <.001     | <.001    | <.001          | <.001          | <.001       |

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.

Table 6
The distribution of rs28938777 alleles in patients and controls.

| rs28938777 | Con n (%) | PV n (%) | Total GPP n (%) | GPP alone n (%) | GPP+PV n (%) |
|------------|-----------|----------|----------------|----------------|-------------|
| c.140A     | 100 (100) | 100 (100)| 100 (100)      | 100 (100)      | 100 (100)   |
| c.140G     | 0 (0)     | 0 (0)    | 0 (0)          | 0 (0)          | 0 (0)       |
| P (vs PV)  | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (vs Con) | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (GPP alone vs GPP+PV) | <.001     | <.001    | <.001          | <.001          | <.001       |

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.

Table 7
The distribution of rs139497891 alleles in patients and controls.

| rs139497891 | Con n (%) | PV n (%) | Total GPP n (%) | GPP alone n (%) | GPP+PV n (%) |
|-------------|-----------|----------|----------------|----------------|-------------|
| c.227C      | 100 (100) | 100 (100)| 100 (100)      | 100 (100)      | 100 (100)   |
| c.227T      | 0 (0)     | 0 (0)    | 0 (0)          | 0 (0)          | 0 (0)       |
| P (vs PV)   | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (vs Con)  | <.001     | <.001    | <.001          | <.001          | <.001       |
| P (GPP alone vs GPP+PV) | <.001     | <.001    | <.001          | <.001          | <.001       |

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.

P < .05.
Since the first mutation was identified in GPP alone patients in 2011, an increasing number of GPP patients have been found to carry IL36RN mutations. Until now, more than 20 IL36RN variants have been reported around the world, which could be homozygous, heterozygous, and compound heterozygous.[9,10,11] c.80T > C (p.Leu27Pro), c.338C > T (p.Ser113Leu), and c.115+6T > C (p.Arg10_Arg15X) are the most common variants in Africa, Europe, and Asia, respectively.[9,10,12,13,14,15] In China, Li et al.[10] first screened IL36RN mutations in GPP patients in 2013 and found the variant frequency was 48.5%.[13] In 2014, Li et al.[10] performed a sanger sequencing in 62 Chinese patients with GPP and displayed the similar result, with a variant frequency of 46.77%. Eight IL36RN variants have been identified in Chinese GPP patients so far, that is, p.Arg10_Arg15X, p.Val57Ile, p.Pro82Leu, p.Asn47Ser, p.Thr123Met, p.Glu112Lys, p.Pro76Leu, and p.Arg102Gln. Among them, c.115+6T > C (p.Arg10_Arg15X) is the most common one.[9,10,11,12] When subgroup analysis was carried out on the basis of the clinical features, a significant difference of variant frequency has been observed between GPP alone and GPP+PV patients. Up to 46.15% to 81.82% of GPP alone patients had IL36RN mutations worldwide,[9,12,13] compared with 10% to 37.78% of GPP+PV patients.[11,12] In China, Li et al.[10] reported that the IL36RN variant frequency of 17 cases with GPP alone patients was 70.59%, while the 45 cases with GPP+PV was only 37.78%.[9,12] In this study, 3 previously reported IL36RN variants were found in Han GPP patients from Sichuan region, including c.115+6T > C (p.Arg10_Arg15X), c.140A > G (p.Asn47Ser), c.227C > T (p.Pro76Leu), c.115+6T > C was the most common IL36RN variant in both GPP alone and GPP+PV patients, with a total frequency of 55.81%. The data reinforced the argument that c.115+6T > C is a hot-spot mutation of the IL36RN gene in Chinese population, or may implicate a common ancestral variant. The total frequency of IL36RN variants was 60.47% in this study. But a closer look at the data revealed an obvious difference between GPP alone and GPP+PV patients (79.17% vs 36.84%), which corresponded well with previous reports. Our data demonstrated again that IL36RN may well be the major disease-causing gene in GPP alone patients in Chinese population, but could be only implicated in a minority of GPP+PV patients. The pathogenesis of GPP+PV seems to be more complex than GPP alone. Recently, Sugiuira et al.[17] identified that 4 of 19 patients with GPP+PV carried CARD14 heterozygous variant c.526G > C (p.Asp176His), which offered new ideas about the molecular mechanism of GPP+PV.

In conclusion, in the present study, we confirmed that the IL36RN variants had a close relation to Han patients with GPP in Sichuan region, which played a critical role in the pathogenesis of GPP alone, but only participated in the development of a minority of GPP+PV. c.115+6T > C is a possible hot-spot mutation within the IL36RN gene in Chinese population. Given that there is a significant difference between the molecular mechanism of GPP alone and GPP+PV, further studies are needed to clarify the intricate pathogenesis of GPP+PV. In this study, some limitations should not be neglected, especially the small sample size. Therefore, more works are needed to verify our findings and illustrate the detailed mechanism of these involved polymorphisms based on larger sample size in the future.

Author contributions

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