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Genetic polymorphism of *IL36RN* in Han patients with generalized pustular psoriasis in Sichuan region of China

A case–control study

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Abstract

The aim of this study was to detect *IL36RN* variant types and frequency in Han patients with generalized pustular psoriasis (GPP) in Sichuan region of China, reveal the difference of variant frequency between GPP alone and GPP + PV (psoriasis vulgaris), and preliminarily clarify the pathogenesis of GPP in this region.

Genomic DNA was extracted and subjected to polymerase chain reaction (PCR) for the amplification of the entire encoding and splice sites of the *IL36RN* gene followed by bidirectional sequencing. Differences in frequencies of *IL36RN* variants between groups were analyzed by SPSS Statistics 17.0 software. Meanwhile, the *IL36RN* variant frequency between GPP alone and GPP + PV was compared.

The total *IL36RN* variant frequency was 60.47% in Han GPP patients from Sichuan region of China. Three variant types (c.115 + 6T > C, c.140A > G, c.227C > T) were identified, among which c.115 + 6T > C exhibited the highest frequency (55.81%). All the 3 variants' frequency of GPP alone group had statistical significance when compared with PV patients and normal controls ($P < .05$). The *IL36RN* variant frequency of GPP alone group was statistically higher than that of GPP + PV group (79.17% vs 36.84%, $P < .05$).

IL36RN may be the major disease-causing gene in GPP patients in Han population in Sichuan region of China. c.115 + 6T > C is a possible hot-spot mutation within the *IL36RN* gene. In contrast to GPP + PV, *IL36RN* mutations possibly play a more important role in the development of GPP alone.

Abbreviations: GPP = generalized pustular psoriasis, GPP+PV = generalized pustular psoriasis with psoriasis vulgaris, PCR = polymerase chain reaction, PV = psoriasis vulgaris.

Keywords: GPP alone, GPP + PV, *IL36RN*, variant

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 detriment the quality of life because of its frequent recurrence.[1,2] GPP can occur alone or be associated with other inflammatory diseases, such as psoriasis vulgaris (PV) and palmoplantar pustulosis.[3,4] Although many a research has already confirmed that the genetic susceptibility of PV was closely related to *HLA* gene polymorphism,[5] the exact pathogenesis of GPP is still vague so far.

Since 2011, when Marrakchi et al[6] first found *IL36RN* mutations in European patients with GPP alone, an increasing number of research have shown that *IL36RN* mutations are extremely likely to be the main molecular genetic basis of GPP alone.[7–11] The pathogenesis of GPP with PV (GPP + PV) seems to be more complex when compared with GPP alone. *IL36RN* mutations may be only involved in a minority of GPP + PV patients.[11,12]

In China, few studies have been conducted on the gene polymorphism of *IL36RN* in GPP patients so far. In the present study, we detected the *IL36RN* variant types and frequency in Han patients with GPP in Sichuan region, compared with the *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,

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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	F	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	M	22	het.c.115+6T>C
GPP+PV05	M	38	hom.c.115+6T>C; hom.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	F	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.

including 43 patients with GPP, 50 patients with PV, and 50 healthy people as controls. GPP and PV patients were diagnosed by at least 2 senior physicians in the West China Hospital, Sichuan University, according to their medical histories, clinical and histological features. Patients with other immune diseases would be excluded. All study subjects included were Chinese Han people from Sichuan region of China. GPP patients were divided into GPP alone (24 cases) and GPP+PV (19 cases) groups. In the GPP group, there were 25 men and 18 women (male/female ratio=1.39:1) with the initial age of 0 to 66 years (mean age: 23.02±17.12 years) and the survey age of 5 to 69 years (mean age: 35.21±17.29 years) (Table 1). The healthy controls had no family history of GPP or PV.

This study protocol was approved by the ethics committee of West China Hospital, Sichuan University. Written informed consent was obtained from all the participants before their participation in the study.

2.2. Blood sample collecting

About 2.5 mL peripheral venous blood was collected in the early morning from every subject and put into blood collection tube with anticoagulant EDTA. The blood samples were stored at -20°C for next step.

2.3. Mutation detection

Genomic DNA was extracted from all of the samples' peripheral blood using Genomic DNA kit (Beijing Tiangen Biotech Co., Ltd., Beijing, China). The 4 coding exons of *IL36RN* gene including their splice sites were amplified by polymerase chain reaction (PCR) using primers. The primers of *IL36RN* (coding exons 2–5 of *IL36RN* according to RefSeq NM_173170 in NCBI website) for PCR amplification were designed by Primer Premier 5.0 software (Premier Biosoft, Palo Alto, CA) and synthesized by Shanghai Sangon Biotech Co., Ltd (Shanghai, China). The detailed information of PCR primers is listed in Table 2. PCR reaction was conducted in a volume of 25.0 μL system, containing 0.2 μg genomic DNA, 10 × buffer 2.5 μL, dNTP 0.8 mmol/L, $MgCl_2$ 1.5 mmol/L, Taq DNA polymerase 1 U, and 0.5 μmol/L of each primer. PCR procedure was completed in specific annealing temperature (Table 2) for every amplified region. Amplification products were detected for their quality and concentration using 1.5% agarose gel. The eligible PCR products were sent to Tiangen Biotech (Tiangen, Beijing, China) for sequencing. Sequence comparisons and analysis were performed using Sequencer 4.10.1 Demo software (Gene Codes, Ann Arbor, MI) so as to determine the detailed *IL36RN* mutations in all subjects.

Table 2

PCR primers for amplifying *IL36RN*.

Amplified region	Primer sequence (5'→3')	Annealing temperature, °C	Product size, bp
Exon2	F:GGTGGTACCGGAGCTCTCTC R:GTAAAACGACGGCCAGTTGAGGTGCTGGTCACAATTTC	57	345
Exon3	F:ATCCTCCTTGTAGGGCATGAG R:GTAAAACGACGGCCAGTTTGCTTAGAGCCTGGTTTGTG	57	410
Exon4	F:GTAAAACGACGGCCAGTCAGGCCGTCTTACAGCAGTC R:ATCCTTAGGGAGGAGGCAAAG	57	362
Exon5	F:GTAAAACGACGGCCAGTCAGCTTTGCCTCCTCCCTAAG R:AGGTGCCCACTAAGTCAGACG	57	438

PCR=polymerase chain reaction.

Table 3

The distribution of the *IL36RN* variants[*] in patients and controls.

	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 (63.16)
TC-AA-CC, TT-AA-CT, TT-GG-CC, TC-AG-CC, TC-AA-CT, CC-AA-CC, CC-AG-CC, CC-AA-CT, CC-GG-CC	0 (0)	0 (0)	26 (60.47)	19 (79.17)	7 (36.84)
Total	50	50	43	24	19
P (vs PV)	–	–	<.001[‡]	<.001[‡]	<.001[‡]
P (vs Con)	–	–	<.001[‡]	<.001[‡]	<.001[‡]
P (GPP alone vs GPP + PV)				.005[‡]	

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.
[*] The *IL36RN* variants include rs148755083, rs28938777, and rs139497891.
[†] TT-AA-CC represents the *IL36RN* variants, including rs148755083, rs, and rs139497891 in order.
[‡] $P < .05$.

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.Pro76Leu, 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.

	Cases	rs148755083 n (%)	rs28938777 n (%)	rs139497891 n (%)
Total GPP	43	24 (55.81)	5 (11.63)	4 (9.30)
GPP alone	24	17 (70.83)	3 (12.50)	3 (12.50)
GPP + PV	19	7 (36.84)	2 (10.53)	1 (5.26)
PV	50	0 (0)	0 (0)	0 (0)
Con	50	0 (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.

	Con n (%)	PV n (%)	Total GPP n (%)	GPP alone n (%)	GPP + PV n (%)
c.115 + 6T	100 (100)	100 (100)	43 (50)	16 (33.33)	27 (71.05)
c.115 + 6C	0 (0)	0 (0)	43 (50)	32 (66.67)	11 (28.95)
P (vs PV)	–	–	<.001[*]	<.001[*]	<.001[*]
P (vs Con)	–	–	<.001[*]	<.001[*]	<.001[*]
P (GPP alone vs GPP + PV)				.001[*]	

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.
[*] $P < .05$.

Table 6

The distribution of rs28938777 alleles in patients and controls.

	Con n (%)	PV n (%)	Total GPP n (%)	GPP alone n (%)	GPP + PV n (%)
c.140A	100 (100)	100 (100)	79 (91.86)	44 (91.67)	35 (92.11)
c.140G	0 (0)	0 (0)	7 (8.14)	4 (8.33)	3 (7.89)
P (vs PV)	–	–	.012[*]	.017[*]	.020[*]
P (vs Con)	–	–	.012[*]	.017[*]	.020[*]
P (GPP alone vs GPP + PV)				1.000	

Con = control, GPP = generalized pustular psoriasis, PV = psoriasis vulgaris.
[*] $P < .05$.

Table 7

The distribution of rs139497891 alleles in patients and controls.

	Con n (%)	PV n (%)	Total GPP n (%)	GPP alone n (%)	GPP + PV n (%)
c.227C	100 (100)	100 (100)	82 (95.35)	45 (93.75)	37 (97.37)
c.227T	0 (0)	0 (0)	4 (4.65)	3 (6.25)	1 (2.63)
P (vs PV)	–	–	.094	.033[*]	.275
P (vs Con)	–	–	.094	.033[*]	.275
P (GPP alone vs GPP + PV)				.783	

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.[11,14] c.80T > C (p.Leu27Pro), c.338C > T (p.Ser113Leu), and c.115 + 6T > C (p.Arg10ArgfsX1) are the most common variants in Africa, Europe, and Asia, respectively.[6–9,12,15,16]

In China, Li et al[9] first screened *IL36RN* mutations in GPP patients in 2013 and found the variant frequency was 48.5%.[15] In 2014, Li et al[9] 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.Arg10ArgfsX1, p.Val57Ile, p.Pro82Leu, p.Asn47Ser, p. Thr123Met, p.Glu112Lys, p.Pro76Leu, and p.Arg102Gln. Among them, c.115 + 6T > C (p.Arg10ArgfsX1) is the most common one.[9,12,15,16]

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,[11,14] compared with 10% to 37.78% of GPP + PV patients.[11,12]. In China, Li et al[9] 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%.[12]

In this study, 3 previously reported *IL36RN* variants were found in Han GPP patients from Sichuan region, including c.115 + 6T > C (p.Arg10ArgfsX1), 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, Sugiura 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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