Original Article

Tumor Necrosis Factor-alpha Induced Protein 3 Interacting Protein 1 Gene Polymorphisms and Pustular Psoriasis in Chinese Han Population

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Abstract

Background: Psoriasis is a common immune-mediated inflammatory dermatosis. Generalized pustular psoriasis (GPP) is the severe and rare type of psoriasis. The association between tumor necrosis factor-alpha induced protein 3 interacting protein 1 (*TNIP1*) gene and psoriasis was confirmed in people with multiple ethnicities. This study was to investigate the association between *TNIP1* gene polymorphisms and pustular psoriasis in Chinese Han population.

Methods: Seventy-three patients with GPP, 67 patients with palmoplantar pustulosis (PPP), and 476 healthy controls were collected from Chinese Han population. Six single nucleotide polymorphisms (SNPs) of the *TNIP1* gene, namely rs3805435, rs3792798, rs3792797, rs869976, rs17728338, and rs999011 were genotyped by using polymerase chain reaction-ligase detection reaction. Statistical analyses were performed using the PLINK 1.07 package. Allele frequencies and genotyping frequencies for six SNPs were compared by using Chi-square test, odd ratio (*OR*) (including 95% confidence interval) were calculated. The haplotype analysis was conducted by Haploview software.

Results: The frequencies of alleles of five SNPs were significantly different between the GPP group and the control group ($P \leq 7.22 \times 10^{-3}$), especially in the GPP patients without psoriasis vulgaris (PsV). In the haplotype analysis, the most significantly different haplotype was H4: ACGAAC, with 13.1% frequency in the GPP group but only 3.4% in the control group ($OR = 4.16$, $P = 4.459 \times 10^{-7}$). However, no significant difference in the allele frequencies was found between the PPP group and control group for each of the six SNPs ($P > 0.05$).

Conclusions: Polymorphisms in *TNIP1* are associated with GPP in Chinese Han population. However, no association with PPP was found. These findings suggest that *TNIP1* might be a susceptibility gene for GPP.

Key words: Association; Pustular Psoriasis; Single Nucleotide Polymorphism; Tumor Necrosis Factor-alpha Induced Protein 3 Interacting Protein 1

Introduction

Psoriasis is a common immune-mediated inflammatory dermatosis. Based on the clinical presentations, it is classified into four groups as follows: psoriasis vulgaris (PsV), arthritic psoriasis, pustular psoriasis (PP), and erythrodermic psoriasis. PP is further divided into two clinical subtypes, namely generalized pustular psoriasis (GPP) and localized PP. GPP is the severe type and is very rarely found in clinical practice. The major presentation of GPP is generalized systemic abacterial pustule. Palmoplantar pustulosis (PPP) is the most common type of localized PP in the palmoplantar regions.[1] Recent studies have discovered several susceptibility genes for psoriasis, some of which, such as interleukin 36 receptor

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antagonist (*IL36RN*) and caspase recruitment domain family member 14 (*CARD14*), are shared between PP and PsV.[2,3] Mutations of adaptor-related protein complex 1 sigma three subunits (*AP1S3*) were also detected in GPP, PPP, and acrodermatitis continua of Hallopeau (ACH).[1] These findings suggested that there might be common susceptible genes between PP and PsV. The association between tumor necrosis factor-alpha induced protein 3 interacting protein 1 (*TNIP1*) gene and PsV have been confirmed in people with multiple ethnicities.[4,5] *IL36RN*, *CARD14*, and *TNIP1* could involve in the regulation of nuclear factor kappa B (NF-κB) signaling.[2-5] GPP was responded to the tumor necrosis factor-alpha (TNF-α) inhibitors.[6] Did the variants in *TNIP1* gene was also associated with the PP? The aim of this study was to investigate the associations between *TNIP1* gene polymorphisms and PP (GPP and PPP) in Chinese Han population.

Methods

Subjects

Seventy-three Chinese Han patients with GPP (mean age 43.0 years, range: 5–88 years), with mean age at disease onset of 39.0 years (range: 0–88 years) and female to male ratio of 1.09:1, 67 PPP patients (mean age 48.2 years, range: 21–78 years), with mean age at disease onset of 44.9 years (range: 20–75 years) and female to male ratio of 2.72:1, and 476 normal controls (mean age 38.1 years, range: 17–80 years) and female to male ratio of 1.12:1 were recruited from the Affiliated Hospital of Inner Mongolia Medical University. After obtaining written informed consents, 5 ml peripheral venous blood was collected in an ethylene diamine tetraacetic acid-anticoagulant tube and stored at −80°C until use. All patients met the diagnostic criteria of GPP or PPP and were confirmed by clinical and pathological diagnoses. All subjects in the control group did not have psoriasis or other autoimmune diseases, with no family history of psoriasis. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the *Helsinki Declaration of 1975* as revised in 2000 (available at http://www.wma.net/e/policy/17-c_e.html).

Selection of single nucleotide polymorphisms

Six SNPs of the *TNIP1* gene, namely rs3805435, rs3792798, rs3792797, rs869976, rs17728338, and rs999011 were selected based on previous studies[4,5] and our previous study results (data not showed).

Genomic DNA extraction

The genomic DNA of every patients and controls was isolated from 1 ml anticoagulated peripheral blood leukocytes, using a DNA extraction kit (AxyPrep, AP-MX-BL-GDNA-25, Suzhou, China). Spectrophotometer was used to measure the optical density at 260 and 280 nm to estimate the DNA concentration. Gel electrophoresis was used to evaluate the DNA quality, which showed that all DNA samples were suitable for the experiments.

Synthesis of primers and probes

Specific primers and LDR probes were designed using Primer 3 online (version 0.4.0; http://frodo.wi.mit.edu/, USA) and Oligo (version 6.31; Molecular Biology Insights, Inc., (DBA Oligo, Inc.) USA). Two primers were designed for each SNP with identical T value. The primer sequences for PCR are listed in Supplementary Table 1. The upstream and downstream LDR probes were designed based on previous reports,[7] and the sequences are listed in Supplementary Table 2. The upstream probe was phosphorylated.

Multiplex polymerase chain reaction

The PCR reaction system (20 μl) was as follows: 1 μl of DNA template, 2 μl of buffer, 0.6 μl Mg^{2+}, 2 μl dNTP, 0.2 μl Taq DNA polymerase, 2 μl primer solution, and 12.2 μl water. PCR was performed by initial denaturation at 95°C for 2 min, denaturation at 94°C for 30 s, annealing at 56°C for 1.5 min, extension at 65°C for 30 s for 35 cycles, followed by another extension at 65°C for 10 min.

Multiplex ligase detection reaction

The LDR reaction system (10 μl) was as follows: 1 μl buffer, 1 μl probe solution (2 pmol/μl probe), 0.05 μl Taq DNA polymerase, 4 μl PCR product, and 3.95 μl water. The LDR reaction was performed by initial denaturation at 95°C for 2 min, followed by 94°C for 15 s, and 50°C for 25 s for 40 cycles.

Sequencing and genotyping

ABI 3730 DNA sequencer (Applied Biosystems, Inc., USA) was used to sequence the reaction products with technical support from Shanghai Biowing Applied Biotechnology Company (China). Gene mapper software (Applied Biosystems, Inc., USA) was used to analyze the data and determine the genotypes of the samples.

Statistical analyses

All quality control steps were performed using the PLINK 1.07 package (http://pngu.mgh.harvard.edu/purcell/plink/, USA).[8] The frequencies of the alleles of all six SNPs were calculated, and Hardy–Weinberg equilibrium was tested in the cases and controls. Chi-square test was used to compare the frequencies of the alleles and genotypes between the cases and controls, and to calculate the odds ratios (*OR*s) and the corresponding 95% confidential intervals (95% *CI*s) of the alleles. Linkage disequilibrium (LD) test was performed for the six SNPs, and the pairwise r^2 and D' values were calculated. Logistic regression was used to evaluate the associations among the other five SNPs after rs17728338 was adjusted. Stratified analyses were performed according to the status of accompanying PsV. Haploview software (Broad Institute, USA) was used for the haplotype analysis. $P < 8.33 \times 10^{-3}$ (0.05/6) was considered to be statistically significant according to Bonferroni multiple testing correction principles.

Results

Quality control of the samples and single nucleotide polymorphisms

The age and gender of the subjects were comparable between the cases and controls, with no significant differences ($P > 0.05$). All six SNPs maintained Hardy–Weinberg equilibrium in the cases as well as controls ($P > 0.05$).

Comparisons of allele frequencies between different groups

For rs17728338, the frequency of A allele was significantly higher in the GPP group (0.274) than in the control group (0.089; $P = 6.06 \times 10^{-11}$). In addition, the GPP patients without PsV had even higher frequency of A allele ($P = 2.36 \times 10^{-13}$). In the case of the other five SNPs, the frequencies of alleles were significantly different between the GPP group and the control group for four SNPs ($P \leq 7.22 \times 10^{-3}$) [Table 1]. However, no significant difference in the allele frequencies was found between the PPP group and control group for each of the six SNPs ($P > 0.05$).

Stratified analyses were performed by PsV skin lesion and showed that rs869976 was associated with GPP patients with or without PsV ($P = 4.94 \times 10^{-3}$ and 1.08×10^{-3}, respectively). The rs3792797 and rs17728338 SNPs were associated with GPP patients without PsV ($P_{rs3792797} = 5.50 \times 10^{-5}$, $P_{rs17728338} = 2.36 \times 10^{-13}$), while no significant association was found in the GPP patients with PsV. No significant association was found for rs3805435 and rs3792798 in either subgroup of GPP patients.

Comparison of the allele frequencies between the GPP patients with and without PsV showed that the alleles of rs17728338 were slightly different between the two subgroups ($P = 0.014$). No significant difference in the allele frequencies was found between these two subgroups for the other five SNPs ($P > 0.05$).

Linkage disequilibrium analyses showed mild linkage disequilibrium between rs17728338 and the other four positively associated SNPs, while the linkage disequilibrium among the four positively associated SNPs was moderate to high [Supplementary Table 3]. After rs17728338 was adjusted, the *P* value for the linkage disequilibrium among

Table 1: Association results of six SNPs in *TNIP1* region with pustular psoriasis in Chinese Han population

SNP	Position	Minor allele	Sample	Number of samples	AF*	*P*†	*OR* (95% *CI*)	*P*‡
rs3805435	150381489	G	GPP	73	0.3425	7.22×10^{-3}	0.61 (0.42–0.88)	0.549
			GPP without PsV	39	0.3205	0.02	0.55 (0.34–0.90)	
			GPP with PsV	34	0.3676	0.14	0.68 (0.41–1.13)	
			PPP	67	0.3881	0.11	0.74 (0.51–1.07)	
			Controls	476	0.4611			
rs3792798	150381958	T	GPP	73	0.3288	5.28×10^{-3}	0.59 (0.41–0.86)	0.820
			GPP without PsV	39	0.3205	0.03	0.57 (0.35–0.94)	
			GPP with PsV	34	0.3382	0.07	0.62 (0.37–1.04)	
			PPP	67	0.3731	0.09	0.72 (0.50–1.05)	
			Controls	476	0.4517			
rs3792797	150382084	T	GPP	73	0.1164	7.92×10^{-5}	3.17 (1.74–5.78)	0.321
			GPP without PsV	39	0.1410	5.50×10^{-5}	3.95 (1.93–8.08)	
			GPP with PsV	34	0.0882	0.06	2.33 (0.95–5.72)	
			PPP	67	0.0299	0.57	0.74 (0.26–2.11)	
			Controls	476	0.0399			
rs869976	150386630	G	GPP	73	0.0616	1.17×10^{-4}	4.75 (1.99–11.31)	0.895
			GPP without PsV	39	0.0641	1.08×10^{-3}	4.95 (1.72–14.26)	
			GPP with PsV	34	0.0588	4.94×10^{-3}	4.51 (1.43–14.24)	
			PPP	67	0.0224	0.43	1.65 (0.47–5.88)	
			Controls	476	0.0137			
rs17728338	150458511	A	GPP	73	0.2740	6.06×10^{-11}	3.85 (2.51–5.90)	0.014
			GPP without PsV	39	0.3590	2.36×10^{-13}	5.71 (3.42–9.55)	
			GPP with PsV	34	0.1765	0.02	2.19 (1.13–4.24)	
			PPP	67	0.1045	0.57	1.19 (0.66–2.16)	
			Controls	476	0.0893			
rs999011	150459544	T	GPP	73	0.0411	0.90	1.06 (0.44–2.56)	0.314
			GPP without PsV	39	0.0256	0.56	0.65 (0.15–2.75)	
			GPP with PsV	34	0.0588	0.42	1.55 (0.53–4.47)	
			PPP	67	0.0672	0.13	1.78 (0.84–3.78)	
			Controls	476	0.0389			

*Allele frequency; †Cases versus controls; ‡GPP without PsV versus GPP with PsV. *OR*: Odds ratio; *CI*: Confidence interval; GPPs: Generalized pustular psoriasis; *TNIP1*: Tumor necrosis factor-alpha induced protein 3 interacting protein 1; SNP: Single nucleotide polymorphism; PsV: Psoriasis vulgaris; PPP: Palmoplantar pustulosis.

the other four positively associated SNPs was statistically significant, suggesting that they represented different association signals [Supplementary Table 4].

Genotype analyses

Genotype analyses showed that the recessive model was the most significant for rs17728338 ($P = 6.61 \times 10^{-8}$), especially for GPP patients without PsV, with the *OR* of the AA genotype as high as 23.50 ($P = 6.74 \times 10^{-15}$) [Tables 2 and 3]. While for rs3792797 and rs869976, the dominant model was the most significant ($P = 5.01 \times 10^{-5}$ and 9.90×10^{-5}, respectively).

Haplotype analyses

The differences between the frequencies of four risk haplotypes and one protective haplotype were statistically significant ($P < 8.33 \times 10^{-3}$). The most significantly different haplotype was H4: ACGAAC, with 13.1% frequency in the GPP group but only 3.4% in the control group, yielding a high *OR* of 4.16 ($P = 4.46 \times 10^{-7}$) [Table 4].

Discussion

GPP is one of the most severe types of psoriasis. Its clinical characteristics include milium-sized abacterial pustule with

Table 2: Association of six SNPs in *TNIP1* region with GPP in the dominant model

SNP	Genetic model	Genotype	Cases	Controls	*P*	*OR* (95% *CI*)
rs3805435	GG+GA/AA	GPP	43/30	337/139	0.04	0.59 (0.37–0.98)
		GPP without PsV	20/19		0.01	0.43 (0.23–0.84)
		GPP with PsV	23/11		0.70	0.86 (0.41–1.82)
rs3792798	TT+TC/CC	GPP	42/31	335/141	0.03	0.57 (0.33–0.94)
		GPP without PsV	20/19		0.01	0.44 (0.23–0.86)
		GPP with PsV	22/12		0.49	0.77 (0.37–1.60)
rs3792797	TT+TG/GG	GPP	17/56	38/438	5.01×10^{-5}	3.50 (1.85–6.61)
		GPP without PsV	11/28		3.51×10^{-5}	4.53 (2.09–9.80)
		GPP with PsV	6/28		0.05	2.47 (0.96–6.34)
rs869976	GG+GA/AA	GPP	9/64	13/463	9.90×10^{-5}	5.01 (2.06–12.19)
		GPP without PsV	5/34		9.73×10^{-4}	5.24 (1.76–15.56)
		GPP with PsV	4/30		4.58×10^{-3}	4.75 (1.46–15.45)
rs17728338	AA+AG/GG	GPP	31/42	79/397	2.72×10^{-7}	3.71 (2.20–6.26)
		GPP without PsV	19/20		8.97×10^{-7}	4.77 (2.44–9.36)
		GPP with PsV	12/22		5.94×10^{-3}	2.74 (1.30–5.77)
rs999011	TT+TC/CC	GPP	6/67	37/439	0.90	1.06 (0.43–2.61)
		GPP without PsV	2/37		0.55	0.64 (0.15–2.77)
		GPP with PsV	4/30		0.41	1.58 (0.53–4.73)

SNPs: Single nucleotide polymorphisms; PsV: Psoriasis vulgaris; *OR*: Odds ratio; *CI*: Confidence interval; GPP: Generalized pustular psoriasis; *TNIP1*: Tumor necrosis factor-alpha induced protein 3 interacting protein 1.

Table 3: Association of six SNPs in *TNIP1* region with GPP in the recessive mode

SNP	Genotype	Genotype	Cases	Controls	*P*	*OR* (95% *CI*)
rs3805435	GG/GA+AA	GPP	7/66	102/374	0.02	0.39 (0.17–0.87)
		GPP without PsV	5/34		0.20	0.54 (0.21–1.41)
		GPP with PsV	2/32		0.03	0.23 (0.05–0.97)
rs3792798	TT/TC+CC	GPP	6/67	95/381	0.02	0.36 (0.15–0.85)
		GPP without PsV	5/34		0.28	0.59 (0.23–1.55)
		GPP with PsV	1/33		0.01	0.12 (0.02–0.90)
rs3792797	TT/TG+GG	GPP	0/73	0/476	NA	NA
		GPP without PsV	0/39		NA	NA
		GPP with PsV	0/34		NA	NA
rs869976	GG/GA+AA	GPP	0/73	0/476	NA	NA
		GPP without PsV	0/39		NA	NA
		GPP with PsV	0/34		NA	NA
rs17728338	AA/AG+GG	GPP	9/64	6/470	6.61×10^{-8}	11.02 (3.80–31.97)
		GPP without PsV	9/30		6.74×10^{-15}	23.50 (7.85–70.38)
		GPP with PsV	0/34		0.51	0.93 (0.91–0.96)
rs999011	TT/TC+CC	GPP	0/73	0/476	NA	NA
		GPP without PsV	0/39		NA	NA
		GPP with PsV	0/34		NA	NA

OR: Odds ratio; *CI*: Confidence interval; GPP: Generalized pustular psoriasis; SNP: Single nucleotide polymorphism; PsV: Psoriasis vulgaris; NA: Not applicable; *TNIP1*: Tumor necrosis factor-alpha induced protein 3 interacting protein 1.

Table 4: Haplotype analysis for GPP using six SNP in *TNIP1* gene region

Haplotype	Frequency		*P*	*OR* (95% *CI*)
	GPP	Controls		
H1: ACGAGC	0.377	0.435	0.18	0.78 (0.55–1.12)
H2: GTGAGC	0.231	0.399	1.16×10^{-4}	0.46 (0.31–0.69)
H3: GTGAAC	0.095	0.042	5.18×10^{-3}	2.41 (1.28–4.56)
H4: ACGAAC	0.131	0.034	4.46×10^{-5}	4.16 (2.30–7.53)
H5: ACGAGT	0.033	0.027	0.64	1.26 (0.48–3.34)
H6: ACTGGC	0.052	0.012	4.25×10^{-4}	4.50 (1.81–11.20)
H7: ACTAGC	0.017	0.015	0.60	1.39 (0.40–4.91)
H8: GCGAGC	0.014	0.013	0.92	1.09 (0.24–4.90)
H9: ACTAAC	0.032	0.009	7.29×10^{-3}	4.18 (1.35–12.94)

OR: Odds ratio; *CI*: Confidence interval; GPP: Generalized pustular psoriasis; SNP: Single nucleotide polymorphism; *TNIP1*: Tumor necrosis factor-alpha induced protein 3 interacting protein 1.

erythema, high temperature, increased white blood cell count, and hypoproteinemia. The pathogeneses of PP remain unclear. Previous studies have suggested that genetic factors, immune system, infection, drugs, and environmental factors are involved in the development of PP.[9]

Recent studies have shown that genetic factors are involved in the pathogeneses of GPP. For instance, mutations of *IL36RN*, *CARD14*, and *AP1S3* genes are associated with GPP.[1-3] The *IL36RN* mutation was also detected in PsV patients, suggesting that *IL36RN* plays a role in the development of PsV and GPP. In addition, the same diseases-related mutations including the mutations of *IL36RN, CARD14*, and *AP1S3* have been detected in GPP, PPP, ACH, and PsV patients.[1,2,10,11] These findings suggest that these diseases share some susceptibility genes. The association between *TNIP1* gene and psoriasis was first described in Europeans[5] and confirmed by several subsequent studies.[4,12]

The present study showed that multiple SNPs in the *TNIP1* gene were associated with GPP, which was more significant in patients without PsV, suggesting that *TNIP1* is a susceptibility gene for GPP. Multiple factors such as infection, glucocorticoids, abrupt discontinuation or reduction of immunosuppressant dosage, trauma, surgery, and mental stimuli could induce the conversion of PsV to GPP.[13-15] The initial presentations in some GPP patients are fever and systemic pustule, with no PsV skin lesion after the pustule alleviation. In the present study, we found that the *OR*s of the five associated SNPs were higher in the subgroup of GPP without PsV than in GPP with PsV. Especially for rs17728338, the *OR* for the A allele was 5.71 in the GPP patients without PsV, and the *OR* for AA genotype was as high as 23.50, which suggested that TNIP1 gene could play a critical role in the pathogeneses of GPP. In contrast, previous studies on PsV showed that the *OR* for A allele of rs17728338 was 1.50–1.59.[4,5,12]

LD analyses showed mild LD between rs17728338 and the other five SNPs. After controlling rs17728338 in the logistic regression model, statistical significance was seen for four other SNPs, which suggested the presence of different association signals in the *TNIP1* gene. However, such association was not found in PPP patients, suggesting that the variants in *TNIP1* gene are not associated or minimally contributed to the susceptibility of PPP.

TNIP1 gene is located at 5q32-q33.1 and its mRNA is widely expressed in the peripheral white blood cells, spleen, skeletal muscles, and kidneys. The binding of the encoded ABIN1 protein to the ligand A20 is involved in the inhibition of NF-κB. Overexpression of *TNIP1* gene could also inhibit the activities of NF-κB by inhibiting TNF-α. In addition, TNIP1 could also inhibit TNF-mediated cell apoptosis, independent of A20.[16,17] Studies have also reported that mutations of genes such as v-rel avian reticuloendotheliosis viral oncogene homolog,[18,19] nuclear factor of kappa light polypeptide gene enhancer in B-cells 1,[20,21] and TNF-α induced protein 3 (*TNFAIP3*),[5] involved in the pathological NF-κB pathway, are also associated with psoriasis. Among these, *TNIP1* and *TNFAIP3* are also susceptibility genes for multiple autoimmune diseases including rheumatoid arthritis, ulcerative colitis, Crohn's disease, psoriasis, and systemic lupus erythematosus.[22,23] Serum TNF-α level increased in patients with GPP.[24] Treating GPP with TNF-α inhibitor was demonstrated to be effective.[25] In contrast, other studies have reported that GPP or PPP is induced by TNF-α inhibitors.[26] These genetic and functional studies showed that *TNIP1* could play an important role in the development of GPP.

However, there are several limitations in the present study. Particularly, the lack of power to exclude an association with psoriasis with small effect sizes based on a limited sample size and from a single population. First, the conclusion is not robust enough considering the relatively small sample size, future studies with larger sample sizes are needed to verify our findings. Second, the associations were investigated based on SNPs, and the gene-gene interactions were not examined. Given the complex and widespread gene-gene interactions in the gigantic gene-network, whether other genes affect the associations between the genetic mutations and disease are unclear. Third, the five associated SNPs are unlikely to be the causal variants, which could affect the expression of the gene. Fine mapping of the associated regions will be required to identify all associated variants and functional ones. Functional studies are needed to elucidate the mechanisms involved in the effects of *TNIP1* gene polymorphisms on gene and immune system functions.

In conclusion, our study confirmed the association of SNPs in *TNIP1* gene with GPP in Chinese Han population. However, these variants were not associated with PPP. These findings suggest that TNIP1 might be a common susceptibility gene for PsV and GPP and play a critical role in the pathogenesis of psoriasis.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Sequence of primers for the PCR

Primer name	Sequence (5'-3')	PCR length (bp)
rs3805435	ACTCCCACTCCCTGGGTAGA	99
	ACCCAGCATGCTTATCTCCT	
rs3792798	CCAAAACTGAAGGTTGGGAC	90
	CAACATTGAGGTTTGGGGTG	
rs3792797	CTCTCTGAGTCCATTTATCC	98
	ACGTGGACTCCATGAATAAC	
rs869976	TTCAGTTCTGGGCCAAAGAG	95
	GAGTGGTGTGGATGAGACAG	
rs17728338	AAAATGTGGTTTGTTCAGC	100
	TAGGAAGGCAGGGTGCCATT	
rs999011	AATGGCTGGTCTGAGGTCAC	94
	TGTGAAGACAGCAACAGGCA	

PCR: Polymerase chain reaction.

Supplementary Table 2: Sequence of probe for the LDR

Probe name	Sequence (5'-3')	LDR length (bp)
rs3805435_modify	P-AGACCTCTCCTCTACCCAGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs3805435_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTATGTACTGGGGTGGGCTCTGT	101
rs3805435_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTATGTACTGGGGTGGGCTCTGC	103
rs3792798_modify	P-TGGAGTCAGTCCCAACCTTCTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs3792798_C	TTTTTTTTTTTTTTTTTTTTTTGGGCCATAGACAGGCCAGCACCG	89
rs3792798_T	TTTTTTTTTTTTTTTTTTTTTTTTGGGCCATAGACAGGCCAGCACCA	91
rs3792797_modify	P-GCACTCTGGGGAAGGATAAATTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs3792797_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATCATAGCTGTGTCTTCTCTGCC	105
rs3792797_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATCATAGCTGTGTCTTCTCTGCA	107
rs869976_modify	P-GTGGGAGTATCCTGGCGAGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs869976_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGCCAAAGAGAAAGAGCAGAT	121
rs869976_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGCCAAAGAGAAAGAGCAGAC	123
rs17728338_modify	P-GGTCCTACTAAGAGCTCGGTGCCAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTT-FAM	
rs17728338_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTGCC ATTCGGGAGCCTTTTGCAAT	166
rs17728338_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTG CCATTCGGGAGCCTTTTGCAAC	168
rs999011_modify	P-TCCCTCCCTCACTTGTGACCTTTTTTTTTTTTTTTTTT-FAM	
rs999011_C	TTTTTTTTTTTTTTTTTTGCAGGTGAGAGAGTCTGGTCTCG	81
rs999011_T	TTTTTTTTTTTTTTTTTTTTGCAGGTGAGAGAGTCTGGTCTCA	83

LDR: Ligase detection reaction.

Supplementary Table 3: LD analysis for six SNP (r^2/D')

SNP	rs3805435	rs3792798	rs3792797	rs869976	rs17728338	rs999011
rs3805435						
rs3792798	0.938/0.989					
rs3792797	0.042/1.000	0.041/1.000				
rs869976	0.016/1.000	0.016/1.000	0.388/1.000			
rs17728338	0.000/0.041	0.000/0.016	0.026/0.252	0.000/0.003		
rs999011	0.014/0.650	0.012/0.616	0.004/0.073	0.000/0.014	0.005/0.931	

LD: Linkage disequilibrium; SNP: Single nucleotide polymorphism.

Supplementary Table 4: Logistic analysis after control the rs17728338

SNP	Minor allele	*P**	*OR* (95% *CI*)*	*P*†	*OR* (95% *CI*)†	*P*‡	*OR* (95% *CI*)‡
rs3805435	G	6.23E-03	0.58 (0.39–0.86)	0.015	0.52 (0.31–0.88)	0.109	0.65 (0.38–1.10)
rs3792798	T	2.90E-03	0.55 (0.37–0.81)	0.017	0.52 (0.31–0.89)	0.046	0.57 (0.33–0.99)
rs3792797	T	2.94E-03	2.76 (1.41–5.40)	6.59E-03	3.15 (1.38–7.22)	0.119	2.15 (0.82–5.61)
rs869976	G	2.24E-04	5.82 (2.28–14.83)	3.76E-03	5.81 (1.77–19.12)	6.45E-03	5.27 (1.59–17.44)
rs17728338	A	NA	NA	NA	NA	NA	NA
rs999011	T	0.606	1.28 (0.51–3.20)	0.825	0.85 (0.19–3.76)	0.335	1.72 (0.57–5.20)

*All GPP patients versus controls; †GPP without PsV versus controls; ‡GPP with PsV versus controls. *OR*: Odds ratio; *CI*: Confidence interval; GPP: Generalized pustular psoriasis; SNP: Single nucleotide polymorphism; PsV: Psoriasis vulgaris; NA: Not applicable.