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

Jian-Wen Han¹, Yong Wang², Chulu Alateng³, Hong-Bin Li², Yun-Hua Bai³, Xin-Xiang Lyu¹, Rina Wu¹

¹Department of Dermatology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia 010050, China
²Department of Rheumatology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia 010050, China
³Department of Dermatology, Hulunbeier People’s Hospital, Hulunbeier, Inner Mongolia 021000, China

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 ≤ 7.22 × 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 × 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 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. Recent studies have discovered several susceptibility genes for psoriasis, some of which, such as interleukin 36 receptor

Address for correspondence: Dr. Jian-Wen Han, Department of Dermatology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia 010050, China
E-Mail: hanjianwen1981@hotmail.com

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antagonist (IL36RN) and caspase recruitment domain family member 14 (CARD14), are shared between PP and PsV.\[^{[4,5]}\] 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.\[^{[1]}\] 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 2.72:1, and 476 normal controls (mean age 38.1 years, range: 17–80 years) and female to male ratio of 2.72:1 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 Mg²⁺, 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 (ORs) and the corresponding 95% confidential intervals (95% CIs) of the alleles. Linkage disequilibrium (LD) test was performed for the six SNPs, and the pairwise r² 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. Haplovew 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 ($P \leq 7.22 \times 10^{-15}$) [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 $1.08 \times 10^{-3}$, respectively). The rs3792797 and rs17728338 SNPs were associated with GPP patients without PsV ($P_{rs3792797} = 5.50 \times 10^{-3}$, $P_{rs17728338} = 2.36 \times 10^{-13}$), 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* | $P^1$ | OR (95% CI) | $P^2$ |
|-----------|----------|--------------|-------------------------|-------------------|-----|-------|------------|-------|
| rs3805435 | 150381489 | G            | GPP                     | 73                | 0.3425 | $7.22 \times 10^{-3}$ | 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 | $5.28 \times 10^{-3}$ | 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 |       |             |       |
| rs3792977 | 150382084 | T            | GPP                     | 73                | 0.1164 | $7.92 \times 10^{-3}$ | 3.17 (1.74–5.78) | 0.321 |
|           |          |              | GPP without PsV         | 39                | 0.1410 | $5.50 \times 10^{-4}$ | 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 | $1.17 \times 10^{-4}$ | 4.75 (1.99–11.31) | 0.895 |
|           |          |              | GPP without PsV         | 39                | 0.0641 | $1.08 \times 10^{-3}$ | 4.95 (1.72–14.26) |       |
|           |          |              | GPP with PsV            | 34                | 0.0588 | $4.94 \times 10^{-5}$ | 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 | $6.06 \times 10^{-11}$ | 3.85 (2.51–5.90) | 0.014 |
|           |          |              | GPP without PsV         | 39                | 0.3590 | $2.36 \times 10^{-13}$ | 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 × 10\(^{-8}\)), especially for GPP patients without PsV, with the OR of the AA genotype as high as 23.50 (P = 6.74 × 10\(^{-15}\)) [Tables 2 and 3]. While for rs3792797 and rs869976, the dominant model was the most significant (P = 5.01 × 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 × 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 × 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 | 337/139  | 0.01| 0.43 (0.23–0.84)  |
|           |               | GPP with PsV   | 23/11 | 79/393   | 0.07| 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 | 335/141  | 0.01| 0.44 (0.23–0.86)  |
|           |               | GPP with PsV   | 22/12 | 13/541   | 0.49| 0.77 (0.37–1.60)  |
| rs3792797 | TT+TG/GG      | GPP      | 17/56 | 38438    | 5.01×10\(^{-4}\)| 3.50 (1.85–6.61)  |
|           |               | GPP without PsV | 11/28 | 38438    | 3.51×10\(^{-4}\)| 4.53 (2.09–9.80)  |
|           |               | GPP with PsV   | 6/28  | 13/541   | 0.05| 2.47 (0.96–6.34)  |
| rs869976  | GG+GA/AA      | GPP      | 9/64  | 13463    | 9.90×10\(^{-5}\)| 5.01 (2.06–12.19) |
|           |               | GPP without PsV | 5/34  | 13463    | 9.73×10\(^{-4}\)| 5.24 (1.76–15.56) |
|           |               | GPP with PsV   | 4/30  | 13463    | 4.58×10\(^{-3}\)| 4.75 (1.46–15.45) |
| rs17728338| AA+AG/GG      | GPP      | 31/42 | 79397    | 2.72×10\(^{-3}\)| 3.71 (2.20–6.26)  |
|           |               | GPP without PsV | 19/20 | 79397    | 8.97×10\(^{-3}\)| 4.77 (2.44–9.36)  |
|           |               | GPP with PsV   | 12/22 | 79397    | 5.94×10\(^{-3}\)| 2.74 (1.30–5.77)  |
| rs999011  | TT+TC/CC      | GPP      | 6/67  | 37439    | 0.90 | 1.06 (0.43–2.61)  |
|           |               | GPP without PsV | 2/37  | 37439    | 0.55 | 0.64 (0.15–2.77)  |
|           |               | GPP with PsV   | 4/30  | 37439    | 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  | 102374   | 0.02| 0.39 (0.17–0.87)  |
|           |          | GPP without PsV | 5/34  | 102374   | 0.20| 0.54 (0.21–1.41)  |
|           |          | GPP with PsV   | 2/32  | 102374   | 0.03| 0.23 (0.05–0.97)  |
| rs3792798 | TT/TC+CC | GPP      | 6/67  | 95381    | 0.02| 0.36 (0.15–0.85)  |
|           |          | GPP without PsV | 5/34  | 95381    | 0.28| 0.59 (0.23–1.55)  |
|           |          | GPP with PsV   | 1/33  | 95381    | 0.01| 0.12 (0.02–0.90)  |
| rs3792797 | TT/TG+GG | GPP      | 0/73  | 0/476    | NA  | NA                |
|           |          | GPP without PsV | 0/39  | 0/476    | NA  | NA                |
|           |          | GPP with PsV   | 0/34  | 0/476    | NA  | NA                |
| rs869976  | GG/GA+AA | GPP      | 0/73  | 0/476    | NA  | NA                |
|           |          | GPP without PsV | 0/39  | 0/476    | NA  | NA                |
|           |          | GPP with PsV   | 0/34  | 0/476    | NA  | NA                |
| rs17728338| AA/AG+GG | GPP      | 9/64  | 6470     | 6.61×10\(^{-8}\)| 11.02 (3.80–31.97) |
|           |          | GPP without PsV | 9/30  | 6470     | 6.74×10\(^{-5}\)| 23.50 (7.85–70.38) |
|           |          | GPP with PsV   | 0/34  | 6470     | 0.51| 0.93 (0.91–0.96)  |
| rs999011  | TT/TC+CC | GPP      | 0/73  | 0/476    | NA  | NA                |
|           |          | GPP without PsV | 0/39  | 0/476    | NA  | NA                |
|           |          | GPP with PsV   | 0/34  | 0/476    | 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.
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, PsV, 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[9] 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.[1-3,13] 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 ORs 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 power 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.

REFERENCES

1. Setta-Kaffetzi N, Simpson MA, Navarini AA, Patel VM, Lu HC, Allen MH, et al. AP1S3 mutations are associated with psoriasis and impaired toll-like receptor 3 trafficking. Am J Hum Genet 2014;94:790-7. doi: 10.1016/j.ajhg.2014.04.005.
2. Li M, Han J, Lu Z, Li H, Zhu K, Cheng R, et al. Prevalent and rare mutations in IL-36RN gene in Chinese patients with generalized pustular psoriasis and psoriasis vulgaris. J Invest Dermatol 2013;133:2637-9. doi: 10.1038/jid.2013.267.
3. Sugiura K, Muto M, Akiyama M. CARD14 c.526G>C (p.Asp176His) is a significant risk factor for generalized pustular psoriasis with psoriasis vulgaris in the Japanese cohort. J Invest Dermatol 2014;134:1755-7. doi: 10.1038/jid.2014.46.
4. Sun LD, Cheng H, Wang ZX, Zhang AP, Wang PG, Xu JH, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. Nat Genet 2010;42:1005-9. doi: 10.1038/ng.690.
5. Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, et al. Genome-wide scan of association with psoriasis vulgaris and impaired toll-like receptor 3 trafficking. Am J Hum Genet 2011;89:432-7. doi: 10.1016/j.ajhg.2011.07.022.
6. Fiallová J, Vojáčková N, Han J, Lu Z, Zhu K, Cheng R, et al. Development of a PCR/LDR/capillary electrophoresis assay with potential for the detection of a beta-thalassemia fetal mutation in maternal plasma. J Matern Fetal Neonatal Med 2010;23:920-7. doi: 10.1111/j.1651-2227.2009.02370.x.
7. Yi P, Chen Z, Liu G, Xie H, et al. Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet 2011;89:432-7. doi: 10.1016/j.ajhg.2011.07.022.
**Supplementary Table 1: Sequence of primers for the PCR**

| Primer name | Sequence (5'-3')                  | PCR length (bp) |
|-------------|-----------------------------------|-----------------|
| rs3805435   | ACTCCCACTCCCTGGGTAGA               | 99              |
|             | ACCCAGCATGCTATTCTCTT             |                 |
| rs3792798   | CAAAAACTGAAGGTGTTGGGAC            | 90              |
|             | CAACATTGAGGGTTGGGTGGA            |                 |
| rs3792797   | CTCTCTGAGTCCATATTAC              | 98              |
|             | ACGTGGACTCCATGAAATAC            |                 |
| rs869976    | TTCAGTTCTGGGCACAAAGGAG          | 95              |
|             | GAGTTGGTGGATGAGACAGGCA          |                 |
| rs17728338  | AAAATGTGGTTTCTTTCAGC             | 100             |
|             | TAGGAAGCCAGGTCGCTATT              |                 |
| rs999011    | AATGCTGTCTGATGAGTCAC             | 94              |
|             | TGTGAAGACAGCAACAGGCA             |                 |

**Supplementary Table 2: Sequence of probe for the LDR**

| Probe name | Sequence (5'-3')                                                                 | LDR length (bp) |
|------------|---------------------------------------------------------------------------------|-----------------|
| rs3805435_modify | P-AGACCTCTTCCTCTACCCAGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
| 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.