Supplemental Data

Supplementary Materials and Methods

A total of 236 psoriasis cases of all Chinese Han origin, including 62 with GPP, 174 with PsV and 365 controls, were recruited in our study (Table 1s). Individuals in the PsV group had at least two psoriatic scales or a single scale occupying at least 1% of the total body surface outside the scalp, and all had a history of psoriasis at least 10 years. All patients were diagnosed based on clinical and histopathological findings. Controls were unaffected individuals with no family history of psoriasis, recruited from the Health Examination Center.

This study was approved by the human medical and ethics committee of Shandong Provincial Institute of Dermatology and Venereology, and was conducted according to Declaration of Helsinki Principles. After informed consent, genomic DNA was extracted from patients' peripheral blood samples using QuickGene DNA whole blood kit L (Kurabo Industries LTD, Japan). All exons of *CARD14* gene were amplified by polymerase chain reaction(PCR) and the products were sequenced on an ABI 3130xl Genetic Analyser (Applied Biosystems ABI, Carlsbad, CA, USA).

The primers of *CARD14* (1-15 exons according to RefSeq NM_001257970.1; the last primer according to RefSeq NM_024110.4 block 18 for researching the SNP rs11652075) for PCR amplification were designed by Primer3 (http://primer3.ut.ee/) (Table 2s) and synthesized by BGI (Shenzhen, China). The sixteen fragments were amplified by PCR. The genotyping was done by using SNaPshot (ABI Prism SNaPshot multiplex kit; Applied Biosystems) on an ABI PRISM 3130xl genetic analyser and analyzed by ABI GeneScan software. The numbering of the

variants detected in this study based on RefSeq NM_024110.4.

Differences in allele frequencies between groups were analyzed by χ^2 and Fisher's exact tests using SPSS 17.0. Specifically, the five rare variants (p.Met119Val, p.Arg166His, p.Ala216Thr, p.Thr591Met, p.Arg682Trp) were analyzed by Fisher's exact tests (Table 1) and the four other variants (p.Asp176His, rs34367357, rs2066964, rs11652075) using χ^2 tests (Table 5s). And the p-values were corrected by using false discovery rate (FDR) with software R3.0.3. The burden test was performed using the r-project SNP-set (sequence) kernel association test (SKAT). Corrected and nominal p-values were both presented in our test . And the significance level is $P \leq 0.05$.

Table 1s. Baseline characteristics of case patients and controls

Type	Male %	Age (mean ± SD)	Age at onset (mean ± SD)	Number
GPP	61.70%	36.65±17.59	33.73±19.15	62 (41[1])
PsV	50.57%	46.30±20.70	29.62±21.77	174
Pediatric-onset PsV	35.42%	29.03±9.45	10.08±3.59	94
Late-onset PsV	68.75%	66.58±7.83	52.57±5.91	80
Psoriasis	53.39%	43.76±20.34	30.54±21.27	236
Controls	52.60%	43.35±17.68		365

PsV: psoriasis vulgaris (including Pediatric-onset PsV and Late-onset PsV); GPP: generalized pustular psoriasis.1: 41 patients had a preceding history of PsV.

Table 2s. Primer sequences of PCR amplification for *CARD14*

CARD14 Exon	Forward primer	Reverse primer	Annealing temperature(℃)	PCR production size(bp)
1	5'-GGTTGTGACCGAACCTTGAG-3'	5'-GAGAGTCGTGTGTGCAGGAG-3'	58	298
2	5'-GGGTTCTGACTGGCAAAGAG-3'	5'-CCCTGAAGACAGAGGCAGAC-3'	60	438
3	5'-CAGAAAACCGCTTTCACCTC-3'	5'-GTAGGGCAAATCGGCAAGTA-3'	62	377
4[1]	5'-CTACCCACCTGCCCACCTAT -3'	5'-CTCATGGAAGTGTGCGCTAA-3'	58	286
4[1]	5'-GAGGAGCTGAACCAGGAAAA -3'	5'-ACAAGGAAGAGGGGAAAGGA -3'	60	371
5	5'-GGAGGAAGGGTGAGAAATGC-3'	5'-AGAAAGTGGGTGAGATGAGTCAC-3'	62	396
6	5'-GGGATTCTGCTTGCCTAGGG-3'	5'-CGGCGTGGATGAAGGAATCT-3'	62	286
7	5'-AGAACTGTCTCCCTCCCTCC-3'	5'-AGAGACAGCATGTGGACAAC-3'	66	413
8	5'-AGTGGATGTCAGATGAGCGC-3'	5'-TGTCCTCCCAGTGCCTCCAT-3'	64	357
9	5'-GGTGAAATGGGTGGTGCTGA-3'	5'-GGCTCACTTTCGTCCTGACA-3'	62	295
10	5'-CTCAGCGCATGTGACCCCAT-3'	5'-CCCTGAGGTCTAGGTTGGTG-3'	66	386
11	5'-AGGGCATCTGGGTATTGCAG-3'	5'-TTCCCAAAGTGCAAGGTCGA-3'	62	400
12	5'-AAGCATTGAGGAAGGGGTGG-3'	5'-GACACTCACCCTCTGCTCTG-3'	68	377
13	5'-CAGAGGGAAGCAATGGGGA-3'	5'-AGTGGATCTGCATCGTCTGG-3'	60	382
14	5'-CTCTCTGGCCAGCACTATGT-3'	5'-TAAGGGTCATGGAGTCTGGC-3'	58	399
15	5'-CTGAGACTGGGAGGGTCCTT-3'	5'-ATTTCCCTGTGGGCCTCTCT-3'	64	400
X[2]	5'-CTGCAGTGAGCAAAGCAGAC-3'	5'-CAGGTGAGTGTGGGAATGTG-3'	64	399

1: exon 4 of *CARD14* was divided into two fragments with two primers for its size. 2: x is the block 18 of *CARD14* according to RefSeq NM_024110.4 for the SNP rs11652075.

Table 3s. The known variants of *CARD14* associated with psoriasis

variants	Population	Type of Ps	Reference
p.Glu138Ala	European	GPP	Jordan *et al.,*2012a; Jordan *et al.,*2012b
p.Gly117Ser	Haiti/European	GPP/PsV/PsA	Jordan *et al.,*2012a; Jordan *et al.,*2012b; Körber *et al.,*2013
c.349+5G>A	Taiwan	PsV	Jordan *et al.,*2012a
p.Arg38Cys	European	PsV	Jordan *et al.,*2012b
p.Glu142Lys		PsV	
p.Glu142Gly		PsV	
p.His171Asn		PsV/PsA	
p.Arg179His		PsV	
p.Val191Leu		PsV	
p.Asp285Gly		PsV	
p.Ile593Asn		PsV	
p.Arg62Gln (rs115582620)		Ps	
p.Leu150Arg (rs146214639)		Ps	
p.Asp176His (rs144475004)		Ps	
p.Arg682Trp (rs117918077)		Ps	
p.Gly714Ser (rs151150961)		Ps	
p.Asp973Glu (rs144285237)		Ps	
p.Arg547Ser (rs2066964)		Ps	
p.Val585Ile (rs34367357)		Ps	
p.Arg69Gln	European	GPP	Körber *et al.,*2013
p.Arg826Trp		GPP /PsV	
p.Ser200Asn	European	GPP /PsV	Jordan *et al.,*2012b; Körber *et al.,*2013
p.Arg820Trp (rs11652075)	European	PsV/PsA	Jordan *et al.,*2012b

There were 23 variants in *CARD14* according to the former research. Ps: psoriasis; PsV: psoriasis vulgaris; PsA: psoriasis arthropathica; GPP: generalized pustular psoriasis. Jordan CT, Cao L, Roberson ED *et al.* (2012a) PSORS2 is due to mutations in CARD14. *Am J Hum Genet* 90(5):784-95; Jordan CT, Cao L, Roberson ED *et al*. (2012b) Rare and common variants in CARD14, encoding an epidermal regulator of NF-kappa B, in psoriasis. *Am J Hum Genet* 90(5):796-808; Körber A, Mössner R, Renner R *et al.* (2013) Mutations in IL36RN in patients with generalized pustular psoriasis. *J Invest Dermatol* 133(11):2634-7

Table 4s. Characteristics and frequencies of rare variants of *CARD14*

CARD14 exon	cDNA mutation	Corresponding protein change	Group	Protein domain	Predicted effect on protein function (score)	Risk allele frequency in cases	Risk allele frequency in controls
4	c.355A>G	p.Met119Val	GPP	none	Tolerated(0.55)	0.002	0
4	c.497G>A	p.Arg166His	GPP	coiled-coil	Tolerated(0.14)	0.002	0
4	c.646G>A	p.Ala216Thr	PsV	coiled-coil	Tolerated(0.45)	0.002	0
13	c.1772C>T (rs200102454)	p.Thr591Met	PsV	PDZ	Damaging(0.03)	0.002	0
15	c.2044C>T [a] (rs117918077)	p.Arg682Trp	GPP	SH3	Damaging	0.002	0

The protein domain was identified according to the NCBI database (http://smart.embl-heidelberg.de/smart/ show_motifs.pl; http://www.ncbi.nlm.nih.gov/protein/296434421). The effects of variants were predicted by SIFT. a: The characteristic of the variant has been examined in previous studies (Jordan *et al*., 2012b).

Table5s. Analysis of one low frequency variant and three common SNPs in *CARD14* by χ^2 test

	p.Asp176His				rs2066964				rs34367357				rs11652075			
	AA	Aa	aa	allele frequency(a)	AA	Aa	aa	allele frequency(a)	AA	Aa	aa	allele frequency(a)	AA	Aa	aa	allele frequency(a)
P-PsV	89	5	0	0.026	26	51	17	0.452	81	13	0	0.069	23	52	19	0.479
L-PsV	78	2	0	0.012	25	37	18	0.456	69	11	0	0.069	23	45	12	0.431
PsV	167	7	0	0.02	51	88	35	0.454	150	24	0	0.069	46	97	31	0.457
GPP	60	2	0	0.016	23	28	11	0.403	51	11	0	0.086	24	26	12	0.403
Psoriasis	227	9	0	0.019	74	116	46	0.441	201	35	0	0.074	70	123	43	0.443
Control	352	13	0	0.018	113	168	84	0.460	324	41	0	0.056	106	166	93	0.482
P(Psoriasis vs Control)				0.872 (0.918)				0.918 (0.918)				0.195 (0.561)				0.87 (0.918)
P(PsV vs GPP)				1 (1)				0.256 (0.438)				0.453 (0.647)				0.069 (0.438)
P(PsV vs Control)				0.810 (1)				0.764 (1)				0.399 (0.998)				0.541 (1)
P(GPP vs Control)				1 (1)				0.377 (0.539)				0.205 (0.342)				0.137 (0.29)
P(P-PsV vs L-PsV)				0.578 (1)				0.604 (1)				0.988 (1)				0.523 (1)

P-PsV: pediatric-onset psoriasis vulgaris; L-PsV:late-onset psoriasis vulgaris; PsV: psoriasis vulgaris (including P-PsV and L-PsV); GPP: generalized pustular psoriasis; Ps: psoriasis (including PsV and GPP). In the table, there were two types of p-values—the nominal and the corrected, of which the latter were in the braces calculated by false-discovery rate.

Figure 1s.**The sequencing figures of variants in *CARD14* in PsV and GPP patients**

A:p.Met119Val (heterozygous); B:p.Arg166His (heterozygous); C:p.Arg682Trp (heterozygous); D:p.Thr591Met (heterozygous); E:p.Asp176His (heterozygous); F:p.Ala216Thr (heterozygous); G:p.Arg547Ser (rs2066964) (homozygous); H:p.Arg547Ser (rs2066964) (heterozygous); I:p.Arg820Trp (rs11652075) (homozygous); J:p.Arg820Trp (rs11652075) (heterozygous); K:p.Val585Ile (rs34367357) (heterozygous)