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RESEARCH NOTE

Updating and identifying three novel variants of the *CARD14* gene in Chinese Han patients with psoriasis

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Abstract. Psoriasis-2 (PSORS2) is caused by the heterozygous mutation of the caspase recruitment domain 14 (*CARD14*) gene on chromosome 17q25. To evaluate the contribution of *CARD14* variants in psoriasis of the Chinese Han population, we performed deep sequencing of the *CARD14* gene in 372 Chinese Han patients with psoriasis. The exonic nucleotide variants were confirmed by Sanger sequencing in the affected individuals and 1114 controls. In 27 patients with psoriasis, we identified 15 variations, including three novel variants: c.381C>G (p.Cys127Trp), c.712A>G (p.Met238Val) and c.2260_2261delinsGG (p.Gln754Gly). These findings could enrich and update the Human Gene Mutation Database of *CARD14* variants for psoriasis.

Keywords. psoriasis; *CARD14* gene; sequence analysis; novel variants.

Introduction

Psoriasis is a chronic, immune-mediated, inflammatory disease that results from a polygenic predisposition combined with environmental triggers (Elder *et al.* 2010; Lowes *et al.* 2014). It is estimated to affect about 0.47% of the Chinese population as reported by a study in six cities in China (Ding *et al.* 2012). According to the cutaneous manifestations and affected organs, psoriasis is clinically classified into psoriasis vulgaris (PsV), pustular psoriasis (PPs), erythrodermic psoriasis and psoriatic arthritis (PsA) (Deng *et al.* 2016). The aetiology of psoriasis has not yet been fully understood.

In 2012, several common and rare variants in the caspase recruitment domain family member 14 (*CARD14*; OMIM: 607211), a gene located in the psoriasis susceptibility locus 2 (*PSORS2*; OMIM: 602723) have been found which can induce psoriasis and familial pityriasis rubra pilaris (Jordan *et al.* 2012a). CARD14 protein, encoded by *CARD14* and specifically expressed in skin, is the second member of the caspase recruitment domain CARD (CARMA) and membrane-associated guanylate kinase (MAGUK) family, which also includes CARD11/CARMA1 and CARD10/CARMA3. Proteins in the CARMA family contain a uniform domain structure consisting of a CARD domain, a coiled–coil domain, a linker region and a MAGUK domain comprising PDZ, SH3 and GUK subdomains (Bertin *et al.* 2001). In response to stimuli, CARD14 recruits and associates with B-cell lymphoma/leukaemia 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) to form the CARD–BCL10–MALT1 (CBM) signalling complex, which subsequently activates the nuclear factor-kappa B (NF-κB). CARD14 alterations may act as an inflammatory trigger inducing

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excessive or decreased activation of NF-κB in keratinocytes. The abnormal level of NF-κB can initiate an inflammatory reaction via regulating the transcription of many proinflammatory chemokines, cytokines and antimicrobial peptides associated with psoriasis (Van Nuffel *et al.* 2016).

It has been observed that different ethnic groups show genetic diversity of *CARD14* (Sugiura *et al.* 2014; Ammar *et al.* 2016). Here, we performed a sequence analysis of *CARD14* in 372 Chinese Han patients with psoriasis to evaluate its contribution to the disease.

Materials and methods

Subjects

This study followed the guidelines of the Helsinki Declaration and was approved by both the ethics committee and the Scientific Ethical Committee of Fudan University. Study participants provided informed consent for genetic testing. Each patient was diagnosed by at least two experienced dermatologists at the out-patient department, Huashan hospital. From January to December 2015, peripheral blood samples were collected from 372 patients with psoriasis (mean age 41 ± 15; 65% males). Besides PsV, 34.1% of cases had other forms of psoriasis that included 75 cases with PPs, 50 cases with PsA and two cases with concomitant PPs and PsA. All patients with PPs or PsA were concomitant with PsV. In parallel, peripheral blood samples of 1114 controls were recruited from one community in Shanghai, thus representing the same ethnical groups and geographic area as the patients. Genomic DNA was extracted from peripheral blood using the QIAamp DNA blood mini kit (Qiagen, Germany). The data from the East Asian control population in gnomAD (v2.1) were also used as control (Lek *et al.* 2016).

CARD14 gene sequencing

PCR primers were designed for the *CARD14* gene, covering all the exons and the immediately adjacent intron sequences. Genomic DNA 20–50 ng isolated from peripheral blood were amplified in a multiplex PCR reaction, and products were cleaned up by AMPure XP Beads (Beckman Coulter, Pasadena, USA). Then barcoding was performed in a PCR reaction. The barcoded PCR products from various samples were cleaned up and quantified by Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, USA). According to the quantitation, the PCR product was pooled together with equal mole. Purified pooled PCR products were routinely sequenced on an Illumina NextSeq 500 sequencer using 2 × 150 bp end sequencing protocol. For all successful sequencing runs, read depth was 30x at any given position, with 100x mean coverage across the entire targeted sequence, and a Q30 greater than 75% of reads. The variant calling and the coverage of each captured region were analysed by an in-house developed bioinformatics pipeline, based on the general analysis algorithm methods. Briefly, the reads were mapped to the hg19 version of the human reference genome, filtered to remove off-target and poor-quality reads. Variants were identified and annotated. Exonic nucleotide variants were confirmed by Sanger sequencing in the affected individuals with psoriasis and 1114 controls (Applied Biosystems, Foster City, USA). The primer sequences of the three novel variants for sanger validation is shown in table 1 in electronic supplementary material at https://www.ias.ac.in/jgenet. The numbering of *CARD14* variants in this manuscript was based on RefSeq NM_024110.4.

Statistical analysis and functional protein prediction

All statistical analyses were performed using SPSS v. 20.0 software (SPSS, Chicago, USA). The association between familial psoriasis and exonic variants of *CARD14* was analysed by Fisher's exact tests. The minor allele frequencies (MAF) of each variant was compared between patients and two control groups by Fisher's exact tests. The effects of the exonic variants on protein function were predicted by SIFT (http://provean.jcvi.org/index.php/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEN (http://provean.jcvi.org/index.php/), MutationTaster (http://www.mutationtaster.org/), and MutationAssessor (http://

Table 1. Characteristics and frequencies of three novel *CARD14* exonic variants.

			MAF					
			Cases					
Variant	Amino acid change	Exon	PsV (n = 245)	PPs (n = 75)	PsA (n = 52)	Controls (n = 1114)	P value (controls)	Predicted effect on protein function
c.381C>G	p.Cys127Trp	4	0.204%	0	0	0	PsV vs C 0.180	Benign
c.712A>G	p.Met238Val	5	0	0	0.962%	0	PsA vs C 0.045*	Benign
c.2260_2261delinsGG	p.Gln754Gly	16	0	0	0.962%	0	PsV vs C 0.045*	Benign

PsV, psoriasis vulgaris; PPs, pustular psoriasis; PsA, psoriasis arthritis; C, control.
*$P < 0.05$.

mutationassessor.org/r3/). The variant is considered as 'damaging' when it is predicted to be damaged by at least four algorithms. The variant is considered as 'probably damaging' when it predicted to be damaged by three algorithms. Otherwise, the variant is considered as 'benign'.

Results

In total, 15 heterozygous missense variants of the *CARD14* were identified in 27 Chinese Han psoriasis cases (i.e. 15 PsV, eight PsA, and four PPs). The three novel exonic variants are indicated in table 1 and others are listed in table 2 in electronic supplementary material. The corresponding locations of all variants in the protein domains are shown in figure 1. The predicted effects of the exonic variants on protein function is shown in table 3 in electronic supplementary material.

Three novel rare variants, c.381C>G (p.Cys127Trp), c.712A>G (p.Met238Val) and c.2260_2261delinsGG (p.Gln754Gly) were identified in one sporadic PsV and two familial PsA patients, respectively. MAF of *CARD14* c.712A>G and c.2260_2261delinsGG in PsA were both higher than our control population ($P = 0.045$). The three novel variants were considered as 'benign'. In addition, four known *CARD14* variations detected in our patients, including c.185G>A (p.Arg62Gln), c.349G>A (p.Gly117Ser), c.526G>C (p.Asp176His) and c.646G>A (p.Ala216Thr).

Discussion

Recently, the role of CARD14 is emphasized in the pathogenesis of psoriasis. It is well known that interleukin-23 (IL-23) / interleukin-17 (IL-17A) axis is critical in the pathogenesis of psoriasis and antagonists of IL-23 and IL-17A display great curative effects to psoriasis (Sofen *et al.* 2014; Yiu and Griffiths 2016). Two recent studies show that *CARD14* gain-of-function variants drive an inflammatory cascade via IL-23/IL-17A mediated by NF-κB pathway leading to psoriasiform skin inflammation (Mellett *et al.* 2018; Wang *et al.* 2018). Conversely, *CARD14*$^{-/-}$ mice hardly develop psoriasiform skin inflammation in response to imiquimod treatment owing to defective IL-17A signalling in keratinocytes (Wang *et al.* 2018). In this study, three novel missense variants in *CARD14* were detected (table 1). The c.381C>G (p.Cys127Trp) variant was detected in a single patient with PsV, but not found in two control groups. As indicated in table 1, significant differences in the MAF between patients with PsA and two control groups were detected in the c.712A>G (p.Met238Val) and c.2260_2261delinsGG (p.Gln754Gly) variants. It suggested that these variants might be risk factors for PsA.

There were four known *CARD14* variations detected in our patients, including c.185G>A (p.Arg62Gln), c.349G>A (p.Gly117Ser), c.526G>C (p.Asp176His) and c.646G>A (p.Ala216Thr). Their frequencies and characteristics showed obvious ethnic differences (Jordan *et al.* 2012b; Körber *et al.* 2013; Ammar *et al.* 2016; Eskin-Schwartz *et al.* 2016). The c.185G>A (p.Arg62Gln) variant reported in 0.15% of European cases and 0.8% of controls (Jordan *et al.* 2012b), but observed in 0.735% Tunisian cases of PV and no controls (Ammar *et al.* 2016) was seen in one pustular psoriasis patient in our samples. Gain-of-function variant c.349G>A (p.Gly117Ser), which upregulates activation of NF-κB (3.7-fold) via enhancing CBM complex formation was identified in one sporadic case with PsV (Afonina *et al.* 2016; Ammar *et al.* 2016). The c.526G>C (p.Asp176His) variant was first identified in Caucasian, known as an important predisposing factor for PPs with PsV in the Japanese population, predicted to be damaging by a number of prediction programmes (Jordan *et al.* 2012b; Sugiura *et al.* 2014). The previous variant analysis of *CARD14* in a Chinese Han population showed that c.526G>C (p.Asp176His) was detected with MAF 1.9% in psoriasis patients ($n = 236$) and 1.8% in controls ($n = 365$) (Qin *et al.* 2014). In our study, this variant was detected with MAF 1.34% in patients with psoriasis, and 1.83% in East Asian controls from gnomAD, which is higher than other ethnic populations (table 4 in electronic supplementary material). Further, c.646G>A (p.Ala216Thr) that downregulates activation of NF-κB (0.6-fold) was detected in one patient with PsV and one patient with PsA (Ammar *et al.* 2016). Since the activation level of

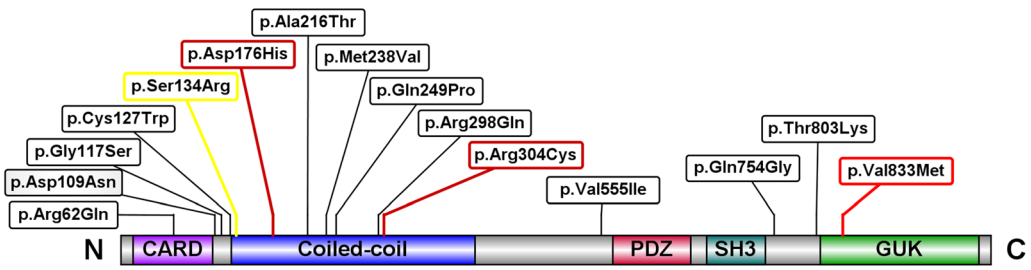

Figure 1. CARD14 protein domains and locations of amino acid substitutions. Missense variants identified in *CARD14* in our psoriasis samples are shown relative to critical protein domains. Red outlining indicates predicted 'damaging' effect on protein function by variant. Gold outlining indicates predicted 'probably damaging' effect on protein function by variant. CARD, caspase recruitment domain; PDZ, postsynaptic density 95/disk large/zona occludens 1; SH3, Src homology 3; GUK, guanylate kinase.

NF-κB influenced by pathogenic *CARD14* variants might have a 'pathogenic threshold', the more diverging from the threshold the more trigger factors were needed to initiate psoriasis.

Conclusion

In this study, three novel variants were identified in the Chinese Han patients with psoriasis, showing the ethnic difference in genetic diversity. Further functional studies will be necessary to clarify their effects on *CARD14* and the pathogenesis of psoriasis.

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