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Variant Analysis of *CARD14* in a Chinese Han Population with Psoriasis Vulgaris and Generalized Pustular Psoriasis

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TO THE EDITOR

Psoriasis is a common, chronic, inflammatory, organ-specific autoimmune skin disease with a complex genetic background (Nestle *et al.*, 2009; Zhang *et al.*, 2013). Psoriasis vulgaris (PsV) is the most common type, accounting for approximately 85–90% of all psoriasis patients, and characterized by raised, well-demarcated, erythematous oval plaques with adherent silvery scales (Nestle *et al.*, 2009). Generalized pustular psoriasis (GPP) is the least prevalent form of psoriasis and is considered to be a potentially life-threatening, multi-systemic disease (Körber *et al.*, 2013), characterized by the sudden eruption of generalized sterile pustules in a wave-like manner (Mengesha and Bennett, 2002).

Genetic factors have been shown to have a critical role in the pathogenesis of psoriasis, and several genes and genomic regions have been reportedly associated with some clinical forms of this disease (http://omim.org/), including the gene for caspase recruitment domain family member 14 (*CARD14*), which was identified by fine-mapping of psoriasis susceptibility locus 2 (*PSORS2*) in European and Taiwanese patients (Hwu *et al.*, 2005; Jordan *et al.*, 2012a). To date, 23 variants have been reported in the *CARD14* gene (Supplementary Table S3 online), mainly in patients with PsV (Jordan *et al.*, 2012a; 2012b; Körber *et al.*, 2013). However, most studies of *CARD14* have been carried out in European populations, and studies in Chinese patients are rare.

We performed direct DNA sequencing in 236 psoriasis patients (174 PsV and 62 GPP patients) and 365 controls to investigate the prevalence of *CARD14* variants in the Chinese Han population. We also compared *CARD14* variants between patients with GPP and PsV and between pediatric- and late-onset PsV.

A total of four new, rare heterozygous missense variants (allele frequency <1%) were found in the *CARD14* exon, all with frequencies of 0.2% in psoriasis patients: p.Met119Val (c.355A>G) and p.Arg166His (c.497G>A) were only seen in GPP with PsV patients, and p.Ala216Thr (c.646G>A) and p.Thr591Met (c.1772C>T) (rs200102454) were only seen in PsV patients. We predicted the effects of the four variants on protein function using the Sorting Intolerant From Tolerant (SIFT) tool (http://sift.bii.a-star.edu.sg). Only p.Thr591Met was predicted to have damaging effects (score = 0.03), whereas the other three were predicted to be tolerated (Supplementary Table S4 and Supplementary Figure S1 online).

An additional known rare heterozygous variant p.Arg682Trp (c.2044C>T) (rs117918077), which was reported in 1.3% of 2169 European psoriasis patients (Jordan *et al.*, 2012b), was only found in one GPP with PsV patient (0.2% in psoriasis patients) in this study (Supplementary Table S4 and Supplementary Figure S1 online).

The overall frequency of these five rare variants was 1.1% in psoriasis patients (0.6% in PsV patients and 2.4% in GPP patients) but 0% in controls. A significant association between *CARD14* rare variants and GPP was observed using Fisher's exact test (corrected-$P = 0.03$), but there was no significant association with either psoriasis or PsV (corrected-$P = 0.09$, 0.998, respectively). We also conducted a gene-burden test using the sequence kernel association test package to compare the distributions of the five rare missense variants in psoriasis patients and controls, which showed a *P*-value of 0.00172. Subtype analysis of PsV versus GPP and pediatric- versus late-onset PsV revealed no significant differences in the frequencies of the five rare variants (corrected-$P = 0.438$, 1, respectively; Table 1).

One known low-frequency variant, p.Asp176His (c.526G>C) (rs144475004), was detected with frequencies of 1.9% in psoriasis patients and 1.8% in controls. Three common single-nucleotide polymorphisms (SNPs), rs2066964, rs34367357, and rs11652075, were also detected in our samples, with frequencies of 44.1 vs. 7.4 vs. 44.3% in psoriasis patients and 46.0 vs. 5.6 vs. 48.2% in controls, respectively. There were no differences between the groups in terms of these four variants (Supplementary Table S5 and Supplementary Figure S1 online).

CARD14 is located within PSORS2 and encodes a nuclear factor (NF)-κB activator. Variants in *CARD14* have recently been detected in association with psoriasis, mostly in patients with PsV (Jordan *et al.*, 2012b). Particular rare variants within *CARD14* could lead to psoriasis by upregulating psoriasis-associated genes in keratinocytes (Jordan *et al.*, 2012a).

The present study identified five rare variants that, in combination, were more common in psoriasis patients

Abbreviations: CARD14, caspase recruitment domain family member 14; GPP, generalized pustular psoriasis; late-onset PsV, late-onset psoriasis vulgaris; pediatric-onset PsV, pediatric-onset psoriasis vulgaris; PsV, psoriasis vulgaris

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Table 1. Analysis of five rare *CARD14* variants using Fisher's exact test

	p.Met119Val				p.Arg166His				p.Ala216Thr				p.Thr591Met				p.Arg682Trp				Combination of five variants			
	AA	Aa	aa	Allele frequency (a)	AA	Aa	aa	Allele frequency (a)	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	94	0	0		94	0	0		93	1	0	0.005	94	0	0		94	0	0		93	1	0	0.005
L-PsV	80	0	0		80	0	0		80	0	0		79	1	0	0.006	80	0	0		79	1	0	0.006
PsV	174	0	0		174	0	0		173	1	0	0.003	173	1	0	0.003	174	0	0		172	2	0	0.006
GPP	61	1	0	0.008	61	1	0	0.008	62	0	0		62	0	0		61	1	0	0.008	59	3	0	0.024
Psoriasis	235	1	0	0.002	235	1	0	0.002	235	1	0	0.002	235	1	0	0.002	235	1	0	0.002	231	5	0	0.011
Control	365	0	0		365	0	0		365	0	0		365	0	0		365	0	0		365	0	0	
P (Psoriasis versus control)				0.393 (0.561)				0.393 (0.561)				0.393 (0.561)				0.393 (0.561)				0.393 (0.561)				0.009 (0.09)
P (PsV versus GPP)				0.263 (0.438)				0.263 (0.438)				1 (1)				1 (1)				0.263 (0.438)				0.115 (0.438)
P (PsV versus control)				1 (1)				1 (1)				0.322 (0.998)				0.322 (0.998)				1 (1)				0.104 (0.998)
P (GPP versus control)				0.145 (0.29)				0.145 (0.29)				1 (1)				1 (1)				0.145 (0.29)				0.003 (0.03)
P (P-PsV versus L-PsV)				1 (1)				1 (1)				1 (1)				0.46 (1)				1 (1)				1 (1)

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

compared with controls. Regarding the predicted effects of these rare variants on protein function, p.Thr591Met and p.Arg682Trp were predicted to be damaging, possibly by regulating the activation of NF-κB, leading to inflammation and epidermal hyperplasia. The other variants were not predicted to be damaging, and their functions remain unknown.

GPP is considered to differ from PsV in terms of its etiology, especially regarding variants in the *IL36RN* gene (Körber *et al.*, 2013; Li *et al.*, 2013; Sugiura *et al.*, 2013). In our study, variants within *CARD14* significantly associated with GPP, compared with controls. However, we failed to find any difference between GPP and PsV, which might be attributed to the insufficient sample size. To elucidate whether the gene *CARD14* is a specific susceptibility gene for PsV or GPP, further study with a large sample size is needed.

Different genes have been reported to be responsible for diseases with different onset ages (Swanbeck *et al.*, 1995). Cheng *et al.* (2014) performed an association analysis in patients with early-onset psoriasis (onset age <40 years) and identified significant associations with the SNP rs4649203 in *IL28RA* and rs2303138 in *LNPEP* (rs4649203, $P=0.0191$, odds ratio (OR) $=0.87$; rs2303138, $P=0.00391$, OR $=1.15$). However, the current study failed to distinguish between pediatric- and late-onset PsV in terms of *CARD14* variants. We therefore conclude that the *CARD14* gene variant cannot explain age of onset in this cohort of psoriasis patients.

In conclusion, we performed an association analysis between *CARD14* variants and psoriasis in a Chinese Han population. The results suggest that rare *CARD14* variants may have an important role in the pathogenesis of GPP.

This study was approved by the human medical and ethics committee of Shandong Provincial Institute of Dermatology and Venereology and was conducted according to the Declaration of Helsinki Principles. After informed consent, genomic DNA was extracted from patients' peripheral blood samples

using a QuickGene DNA whole blood kit L (Kurabo Industries, Osaka, Japan). All exons of the *CARD14* gene were amplified by PCR, and the products were sequenced on an ABI 3130xl Genetic Analyser (Applied Biosystems ABI, Carlsbad, CA). (For specific details about materials and methods see Supplementary Data online).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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An Actin-Binding Protein Espin Is a Growth Regulator for Melanoma

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TO THE EDITOR

Effective therapies for melanoma are limited despite the fact that the incidence is increasing at a greater rate than that of any other cancers (Chen *et al.*, 1996). Identification of key molecules regulating growth, progression, and metastasis in melanoma is essential to provide novel therapeutic strategies. We previously developed *RET*-transgenic mice of line 304/B6 carrying oncogenic *RET* (*RFP/RET*) under regulation of the metallothionein-I promoter (RET-mice), in which skin melanoma develops spontaneously (Kato *et al.*, 1998). As melanoma in RET-mice histopathologically resembles human melanoma, RET-mice have been used worldwide as a standard model for melanoma (Kato *et al.*, 1998; Kumasaka *et al.*, 2010).

The *Espin* gene encodes an actin filament–binding protein (Bartles *et al.*, 1996; Sekerková *et al.*, 2006). Espin affects the actin cytoskeleton, resulting in a special association with microvillar specializations of sensory cells (Sekerková *et al.*, 2004). Our recent study showed that Espin expressed in melanoma cells in mice and humans affects metastasis through the regulation of invasion via lamellipodia formation (Yanagishita *et al.*, 2014). That was the first report showing a correlation between Espin and cancer cells. However, there has been no study showing whether Espin regulates the proliferation of cancer cells. In this study, we examined the effect of Espin on anchorage-dependent and -independent growth of melanoma cells.

Anti-Espin rabbit polyclonal antibody (Yanagishita *et al.*, 2014), murine

Abbreviations: GFP, green fluorescent protein; RET-mice, RET-transgenic mice

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