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Lack of association between *CARD10*/*CARMA3* tag SNPs and psoriasis vulgaris in the southern Chinese population

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ABSTRACT. Previously, we determined that the *CARD11* rs4722404 single nucleotide polymorphism (SNP) increases risk of early-onset psoriasis vulgaris (PsV). Moreover, the *CARD14* gene polymorphism c.C2458T (p.Arg820Trp) is associated with clinical features of this disease. CARMA1/CARD11, CARMA2/CARD14, and CARMA3/CARD10 are conserved across many species and constitute a family of proteins, all of the members of which contain various functional domains characteristic of this group. The NF-κB signaling pathway, regulated by the CARMA family of scaffold proteins and its eponymous component, is a crucial mediator in the pathogenesis of psoriasis. However, little is known about the association between CARMA3/CARD10 and PsV. The aim of this study was to evaluate the relationship between the gene encoding this protein and risk of PsV in the southern Han Chinese population. Genomic DNA from 568 individuals of southern Chinese origin, including 355 patients with PsV and 213 control subjects, was analyzed. We selected seven tag SNPs in the *CARMA3*/*CARD10* gene and genotyped them by the SNaPshot assay. Our results identified no

significant association between these SNPs and PsV in the Chinese population examined. Future studies should focus on the potential function of the *CARMA3/CARD10* gene in the pathogenesis of PsV.

Key words: Psoriasis vulgaris; Single nucleotide polymorphism; *CARD10*; *CARMA3*; Clinical features; Chinese population

INTRODUCTION

Psoriasis is an immune-mediated, genetic skin disorder (Wu et al., 2011; Boehncke and Schön, 2015). Abundant evidence shows that single nucleotide polymorphisms (SNPs) of the caspase recruitment domain (CARD) family member 14 (*CARD14*) gene are implicated in this condition (Jordan et al., 2012a,b; Tsoi et al., 2012; Qin et al., 2014; Aizu et al., 2015). In a previous study, we also determined that the rs4722404 SNP of *CARD11* can increase risk of early-onset psoriasis vulgaris (PsV; Shi et al., 2016). In addition, the *CARD14* gene polymorphism c.C2458T (p.Arg820Trp) has been associated with clinical features of PsV in a Chinese cohort (Feng et al., 2016). The *CARD14* gene encodes a member of the CARD- and membrane-associated guanylate kinase (MAGUK)-like domain-containing protein family (CARMA2), as does the *CARD11* gene (CARMA1). The CARMA protein family comprises CARMA1 (*CARD11*), CARMA2 (*CARD14*), and CARMA3 (*CARD10*). The CARD-containing proteins share high degrees of sequence, structural, and functional homology (Scudiero et al., 2014). Structurally, all CARMA proteins contain a CARD domain at their amino terminus, followed sequentially by a coiled-coil domain, an SH3 domain, and a carboxy-terminal MAGUK domain (Causton et al., 2015). CARMA proteins belong to the MAGUK superfamily, members of which can function as molecular scaffolds to facilitate recruitment and assembly of signal transduction complexes (Stilo et al., 2004; Causton et al., 2015). However, the three CARMA proteins exhibit tissue-specific distributions, indicating that they serve distinct biochemical functions in different cell types (Stilo et al., 2004).

Previous studies have determined that the CARMA family of scaffold proteins, including CARMA2 (*CARD14*), mediates activation of the nuclear factor κB (NF-κB) pathway, which, along with NF-κB itself, is a crucial mediator in the pathogenesis of psoriasis (Blonska and Lin, 2011; Jiang and Lin, 2012; Goldminz et al., 2013).

Nevertheless, little is known about the association between *CARMA3*/*CARD10* and PsV. We hypothesized that variations in this gene alter PsV risk; therefore, we decided to investigate the relationship between *CARMA3/CARD10* SNPs and this disease. The present study explored the influence of *CARMA3*/*CARD10* tag SNPs on the risk of PsV in a southern Han Chinese population.

MATERIAL AND METHODS

Study population

A total of 355 patients with PsV and 213 age- and gender-matched control subjects were recruited, all of whom were genetically unrelated Han Chinese individuals from southern China. The subjects' clinical information was collected during a full medical examination, and additional demographic data concerning both case and control groups were obtained from a

structured questionnaire, as described previously (Zhu et al., 2014a,b; Zhang et al., 2015; Shi et al., 2016). After participants signed informed consent forms, demographic and clinical data, including age, gender, family history (a family history was considered to be positive if at least one relative of the probands had psoriasis among first- and second-degree relatives) of PsV, age at onset (early onset being defined as ≤40 years, and late onset as >40 years), and psoriasis area and severity index (PASI) score, were collected. Control subjects had no personal or family history of psoriasis, autoimmune disorders, or systemic disorders. This study was approved by the relevant Ethics Committees of the Affiliated Hospital of Guangdong Medical College.

DNA isolation and genotyping

Genomic DNA was prepared from peripheral blood cells in accordance with standard protocols. Seven tag SNPs ($r^2 > 0.9$; rs5750441, rs5750446, rs5756708, rs6000759, rs738304, rs742152, and rs8140025) providing full coverage of the gene of interest were identified in the Han Chinese in Bejing, China, from HapMap dataset on chromosome 22 (36216347-36245156), using the pairwise tagging algorithm Tagger. These SNPs were selected from across the *CARMA3*/*CARD10* region, including 10 kb up- and down-stream, using the Tagger application in Haploview (Barrett et al., 2005). This panel of SNPs captured 97% of variants with minor allele frequencies >1%. Genotyping was carried out using the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Characteristics of PsV cases and controls were analyzed by the chi-square test and *t*-test for categorical and quantitative variables, respectively, in SPSS version 13 for Windows (SPSS Inc., Chicago, IL, USA). Results are reported as means ± standard deviations. For allele and genotype comparisons, the statistical significance of differences between groups was calculated by either the chi-square test or the Fisher exact test. Deviation from Hardy-Weinberg equilibrium was assessed according to the method described by Shan (2013). All tests were two-sided, and the threshold for statistical significance was set as $P < 0.05$.

RESULTS

Demographic and clinical data

Table 1 presents the characteristics of patients with PsV and control subjects. For patients, age at onset (30.30 ± 15.76), PASI score (8.57 ± 7.44), and family history of PsV (ratio of familial:sporadic cases = 74:281) were recorded. There were no significant differences in terms of age and gender between the patients and controls.

Association between *CARMA3*/*CARD10* polymorphisms and PsV risk

SNP genotype distributions did not deviate from Hardy-Weinberg equilibrium in the control group ($P > 0.05$). The frequency and distribution of each genotype, as well as odds ratios and 95% confidence intervals for their association with PsV, are shown in Table 2. Comparisons of allele and genotype distributions revealed no significant relationships ($P > 0.05$) between the SNPs tested and risk of PsV.

Table 1. Characteristics of patients with psoriasis vulgaris and control subjects.

Variable	Cases (N = 355)	Controls (N = 213)	P value
Age (mean ± SD, years)	37.53 ± 15.20	35.97 ± 14.57	0.24
Women, N	150	78	0.19
Men, N	205	135	
PASI score (mean ± SD)	8.57 ± 7.44	-	-
Mean age at onset (mean ± SD, years)	30.30 ± 15.76	-	-
Early onset (≤40 years), N	256	-	-
Late onset (>40 years), N	99	-	-
Familial cases, N	74	-	-
Sporadic cases, N	281	-	-

PASI = psoriasis area and severity index, SD = standard deviation. Based on the median, a PASI score <6.0 was defined as mild psoriasis, and 6.0 as severe psoriasis. The chi-square test and the *t*-test were performed for categorical and quantitative variables, respectively.

Table 2. Odds ratio (OR) and confidence interval (95%CI) estimates of the effect of *CARD10* tag single nucleotide polymorphisms (SNPs) on risk of psoriasis vulgaris.

CARD10 tag SNP	Comparison	Minor allele and genotype frequency		OR (95%CI)	P value
		Cases (N = 355)	Controls (N = 213)		
rs5750441	C (*vs* T)	24.93%	22.30%	1.157 (0.871-1.538)	0.315
	CC (*vs* CT+TT)	7.04%	4.69%	1.538 (0.724-3.268)	0.260
	CC+CT (*vs* TT)	42.82%	39.91%	1.128 (0.798-1.593)	0.496
rs5750446	A (*vs* G)	37.46%	34.27%	1.149 (0.894-1.477)	0.279
	AA (*vs* AG+GG)	13.52%	11.27%	1.231 (0.730-2.076)	0.435
	AA+AG (*vs* GG)	61.41%	57.28%	1.187 (0.840-1.677)	0.331
rs5756708	A (*vs* G)	49.30%	48.36%	1.038 (0.816-1.320)	0.759
	AA (*vs* AG+GG)	28.73%	27.70%	1.052 (0.721-1.536)	0.791
	AA+AG (*vs* GG)	69.86%	69.01%	1.041 (0.720-1.504)	0.832
rs6000759	G (*vs* T)	29.30%	29.34%	0.998 (0.766-1.299)	0.987
	GG (*vs* GT+TT)	6.20%	6.10%	1.016 (0.501-2.063)	0.964
	GG+GT (*vs* TT)	52.39%	52.58%	0.992 (0.706-1.395)	0.965
rs738304	C (*vs* T)	13.38%	13.62%	1.021 (0.716-1.455)	0.910
	CC (*vs* CT+TT)	1.69%	2.35%	0.715 (0.216-2.373)	0.582
	CC+CT (*vs* TT)	25.07%	24.88%	1.010 (0.682-1.496)	0.960
rs742152	C (*vs* T)	41.97%	42.96%	0.960 (0.753-1.225)	0.745
	CC (*vs* CT+TT)	16.62%	16.90%	0.980 (0.622-1.544)	0.931
	CC+CT (*vs* TT)	67.32%	69.01%	0.925 (0.642-1.333)	0.676
rs8140025	A (*vs* G)	50.99%	52.11%	0.956 (0.752-1.216)	0.713
	AA (*vs* AG+GG)	27.61%	25.82%	1.095 (0.745-1.610)	0.643
	AA+AG (*vs* GG)	74.37%	78.40%	0.799 (0.533-1.197)	0.276

DISCUSSION

The present study demonstrated that *CARMA3*/*CARD10* polymorphisms might not play a major role in susceptibility to PsV in the Chinese population investigated. We failed to identify significant differences in allele or genotype frequencies between the patient and control groups. Further studies should focus on the potential function of the *CARMA3*/*CARD10* gene in PsV pathogenesis.

The CARMA family is conserved across species and has three members, CARMA1, CARMA2, and CARMA3 encoded by the genes *CARD11*, *CARD14*, and *CARD10*, respectively

(Blonska and Lin, 2011). From a functional perspective, all CARMA protein isoforms are able to activate the transcription factor NF-κB when overexpressed in mammalian cells (Scudiero et al., 2014). The NF-κB family of transcription factors plays a crucial role in cell activation, survival, and proliferation. The aberrant activity of its members results in cancer, immunodeficiency, or autoimmune disorders such as psoriasis (Lippens et al., 2011; Jiang and Lin, 2012; Jordan et al., 2012a). *CARMA3*/*CARD10* on chromosome 22q13.1 encodes a novel CARMA protein that activates NF-κB, a well-characterized transcription factor with multiple physiological and pathological functions, via interaction with B-cell lymphoma/leukemia 10 (BCL10), a component of the apoptosis and NF-κB signaling pathway (Wang et al., 2001; Khor et al., 2011). As a carrier of the CARD domain, CARMA3/CARD10 is intimately involved in the regulation of caspase activation and apoptosis. CARMA1 and CARMA3/CARD10 physically associate with Iκ kinase γ/NF-κB essential modulator (IκKγ/NEMO) in lymphoid and non-lymphoid cells. *In vitro* expression of constructs containing the NEMO-binding region of CARMA3/CARD10 exerts a dominant negative effect on BCL10-mediated activation of NF-κB (Wang et al., 2001). In addition, CARMA3/CARD10 belongs to the MAGUK protein superfamily responsible for assembly of membrane-associated signaling complexes.

Our findings show that *CARMA3*/*CARD10* tag SNPs are not likely to be associated with PsV, at least in the southern Chinese population. However, as allele frequencies differ according to ethnicity, this relationship should be tested in other populations and functional studies should be performed to clarify the contribution of genetic background to the development of this disease. The pathogenicity of such sequence variants needs to be further validated in large independent cohorts and subsequent pathophysiological and therapeutic studies.

Conflicts of interest

The authors declare no conflict of interest.

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