



Association between atopic dermatitis-related single nucleotide polymorphisms rs4722404 and psoriasis vulgaris in a southern Chinese cohort

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Genet. Mol. Res. 15 (2): gmr.15028356

Received December 8, 2015

Accepted February 26, 2016

Published July 15, 2016

DOI <http://dx.doi.org/10.4238/gmr.15028356>

ABSTRACT. Genome-wide association studies have identified a single nucleotide polymorphism (SNP), rs4722404, in the caspase recruitment domain family member 11 (*CARD11*) gene, which is associated with atopic dermatitis. Previous genetic studies have also reported genomic similarities between psoriasis and atopic dermatitis. However, little is known regarding the association between rs4722404 and psoriasis vulgaris (PsV). The aim of this study was to evaluate the relationship between rs4722404 and the risk and clinical features of PsV in a southern Chinese Han cohort. This hospital-based case-control study included 355 patients with PsV and 213 control subjects (N = 568); the samples were analyzed using a standard SNaPshot assay. We identified no association between the SNP and risk of PsV. However, a stratified analysis according to the age of onset, family history, and

psoriasis area and severity index sub-phenotypes revealed a significant correlation between the C allele and CC+CT genotype of rs4722404 and an increased risk of early-onset PsV (≤ 40 years) compared to that of late-onset PsV (> 40 years) (odds ratio, OR = 1.486; $P = 0.026$ for C allele and OR = 1.718, $P = 0.023$ for CC+CT genotype). The results of this study suggested that the SNP rs4722404 in *CARD11* could increase the risk of early-onset PsV. Further studies must analyze the potential function of *CARD11* in the pathogenesis of PsV.

Key words: *CARD11*; Single nucleotide polymorphism (rs4722404); Psoriasis vulgaris; Clinical features

INTRODUCTION

Psoriasis is an etiologically complex heterogeneous skin disease with a strong genetic component (Parisi et al., 2013). Psoriasis and atopic dermatitis (AD) share common pathological backgrounds, such as barrier dysfunction, as well as similar clinical features, including epidermal hyperplasia and altered terminal differentiation of keratinocytes (Miyagaki and Sugaya, 2015). Genome-wide association (GWA) studies have also demonstrated a high degree of genetic overlap between these two common skin disorders (Baurecht et al., 2015). GWA studies have identified an association between the single nucleotide polymorphism (SNP) rs4722404, in the caspase recruitment domain family member 11 gene (*CARD11*) on chromosome 7, and AD (Hirota et al., 2012; Weidinger et al., 2013). The identification of shared genetic components highlights the key molecular pathways involved in chronic inflammatory skin diseases; therefore, we further explored the association between the SNP rs4722404 and psoriasis vulgaris (PsV) in a Chinese Cohort.

Prior to the start of this study, we also analyzed the level of expression of *CARD11* in 180 skin biopsies (one in involved and one in uninvolved skin of 58 psoriatic patients and 64 controls) uploaded to the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>; GSE13355) (Nair et al., 2009). Two sample *t*-tests performed on three groups of samples (psoriatic skin from patients and healthy skin from controls, involved and uninvolved skin from affected individuals, and uninvolved skin from patients and normal skin from controls) revealed a contrasting genetic expression among the different groups (Nair et al., 2009). The results revealed a significant upregulation of the *CARD11* in the skin of psoriasis patients compared to that in the skin of the control subjects ($P < 0.01$) and the uninvolved skin of psoriasis patients ($P < 0.01$).

Based on the results of previous research and pre-analyses, we hypothesized that variations in the *CARD11* across various pathways may alter PsV risk. Therefore, this study was conducted to the relationship between the *CARD11* SNP rs4722404 and the risk and clinical features of PsV in a southern Chinese Han population.

MATERIAL AND METHODS

Sample collection

A total of 355 patients with PsV and 213 controls were included in this study. Both

cases and controls were age- and gender-matched genetically unrelated ethnic Chinese Han subjects. All patients with PsV were recruited from Zhanjiang city and the surrounding regions in southern China between 2009 and 2014. The participants were asked to sign informed consent forms, and were subsequently requested to complete a standard questionnaire to collect relevant clinical data, including their demographic information, age, gender, family history, age of onset (early onset, ≤ 40 years; late onset, >40 years of age), severity of psoriasis lesions (psoriasis area and severity index; PASI score). The patients were considered to have a family history of psoriasis if at least one first- or second-degree relative were afflicted with the disease. Based on median distribution, a PASI score <6.0 was defined as mild psoriasis (a score of 6.0 and above was classified as severe psoriasis). Simultaneously, normal controls were selected from among people who received routine annual health examinations at an examination center located in the same geographic area. All control subjects had no personal or family history of psoriasis, and no autoimmune or systemic disorders. This study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical College.

DNA isolation and genotyping

Genomic DNA was prepared in accordance with standard protocols from peripheral blood cells. The SNP rs4722404 in the *CARD11* on chromosome 7 was genotyped using the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer protocols.

Statistical analysis

The differences in allele frequencies of the polymorphism between case and control subjects were analyzed using the Pearson χ^2 test and odds ratio (OR) with the Statistical Package for Social Scientists (SPSS v.13.0; SPSS Inc., Chicago, IL, USA). The allelic test of association was performed, and the genetic model was calculated. Cases with a particular sub-phenotype were compared to cases that did not have this sub-phenotype, to determine the sub-phenotype-related association. The P values, OR and 95% confidence intervals were determined for each sub-phenotype by the Pearson χ^2 test. All tests were two-sided, and the statistical significance was set at $P < 0.05$.

RESULTS

Basic characteristics of the cohort

A total of 355 patients (205 men and 150 women; mean \pm SD age: 37.53 ± 15.20 years) and 213 controls (135 men and 78 women; mean \pm SD age: 35.97 ± 14.57 years) were investigated. The distribution of selected characteristics between patients with PsV and control subjects is summarized in Table 1. We observed no significant difference in age and gender between the patients and controls. The PASI score (mean \pm SD: 8.57 ± 7.44), age of onset (mean \pm SD: 30.30 ± 15.76 years), and family history (familial:sporadic cases = 74:281) of psoriasis was recorded for all patients.

Table 1. Characteristics of psoriasis vulgaris (PsV) cases and controls.

Variables	Cases (N = 355)	Controls (N = 213)	P value
Age (mean \pm SD, years)	37.53 \pm 15.20	35.97 \pm 14.57	0.24
Females (N)	150	78	0.19
Males (N)	205	135	
PASI score (mean \pm SD)	8.57 \pm 7.44	-	-
Mean age of onset (mean \pm SD, years)	30.30 \pm 15.76	-	-
Early-onset (\leq 40 years, N)	256	-	-
Later-onset ($>$ 40 years, N)	99	-	-
Familial cases (N)	74	-	-
Sporadic cases (N)	281	-	-

PASI, psoriasis area and severity index. χ^2 test for categorical variables. *t*-test for quantitative variables.

Association of the *CARD11* variant rs4722404 and risk of PsV

The genotype distribution of the SNP did not deviate from the Hardy-Weinberg equilibrium in the control subjects ($P > 0.05$). The comparison of allele distributions revealed no significant association ($P = 0.669$) between the C allele and risk of PsV. The minor allele C was present in 36.76% of the patients and 38.97% of the controls. In addition, we observed no significant differences between the dominant and recessive models ($P > 0.05$). The genotype and allele frequencies of the SNP are summarized in Table 2.

Table 2. Frequency distribution of genotype and allele in patients and controls.

CARD11 (rs4722404)	Minor allele and genotype frequency		OR (95%CI)	P value
	Cases (N = 355)	Controls (N = 213)		
C (vs T)	36.76%	38.97%	0.947 (0.739-1.214)	0.669
CC (vs CT+TT)	13.52%	15.02%	0.884 (0.545-1.434)	0.618
CC+CT (vs TT)	60.00%	61.03%	0.958 (0.676-1.356)	0.807

Genotype distribution in the clinical phenotype of PsV

We performed a stratified analysis according to the age of onset, family history of psoriasis, and PASI sub-phenotypes. The C allele of rs4722404 and the (CC+CT) genotype were found to be significantly associated with an increased risk of early-onset (\leq 40 years) PsV, compared to late-onset ($>$ 40 years) PsV (OR = 1.486, $P = 0.026$ for C allele and OR = 1.718, $P = 0.023$ for the CC+CT genotype) (Table 3). However, we observed no significant differences in the other sub-phenotypes of family history or PASI scores between the patients who are positive and negative for a particular phenotype.

Table 3. Association of rs4722404 with PsV analyzed by sub-phenotype stratification.

Comparisons	C (vs T)		CC (vs CT+TT)		CC+CT (vs TT)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95%CI)	P value
Early-onset cases (\leq 40 years) vs controls	1.053 (0.809-1.372)	0.700	0.986 (0.592-1.642)	0.957	1.119 (0.769-1.628)	0.557
Later-onset cases ($>$ 40 years) vs controls	0.709 (0.494-1.016)	0.061	0.636 (0.299-1.351)	0.236	0.651 (0.403-1.054)	0.080
Early-onset cases (\leq 40 years) vs later-onset cases ($>$ 40 years)	1.486 (1.046-2.112)	0.026	1.551 (0.741-3.248)	0.241	1.718 (1.074-2.746)	0.023
Familial cases vs controls	1.175 (0.803-1.719)	0.406	1.438 (0.728-2.839)	0.293	1.111 (0.643-1.922)	0.705
Sporadic cases vs controls	1.104 (0.829-1.470)	0.499	0.985 (0.565-1.717)	0.958	1.236 (0.818-1.866)	0.314
Familial cases vs sporadic cases	1.064 (0.722-1.569)	0.753	1.46 (0.726-2.935)	0.287	0.899 (0.512-1.581)	0.712
Severe cases (PASI $>$ 6) vs controls	1.005 (0.751-1.343)	0.975	0.974 (0.556-1.706)	0.926	1.023 (0.680-1.541)	0.912
Mild cases (PASI \leq 6) vs controls	0.893 (0.666-1.196)	0.447	0.798 (0.445-1.43)	0.447	0.897 (0.598-1.346)	0.601
Severe cases (PASI $>$ 6) vs mild cases (PASI \leq 6)	1.125 (0.829-1.527)	0.449	1.221 (0.663-2.248)	0.521	1.141 (0.746-1.744)	0.544

DISCUSSION

GWA studies have identified a strong relationship between the common SNP rs4722404 in *CARD11* and AD (Hirota et al., 2012; Weidinger et al., 2013). Genetic evidence has demonstrated a higher level of genomic similarities between AD and psoriasis (Weidinger et al., 2013). However, the effect of the SNP rs4722404 on the risk and clinical features of psoriasis, as well as the biological mechanism underlying this role, remain unclear. To this end, we first conducted a case-control study evaluating the relationship between this SNP and the clinical parameters and genetic risk factors of PsV, in a prospective southern Chinese cohort. We identified no association between the allele and genotypes of this SNP and PsV risk. However, we discovered that the rs4722404 genotype was significantly associated with an increased risk of early-onset PsV. This finding may provide further insight into the effect of *CARD11* on psoriasis.

CARD11 encodes a member of the caspase recruitment domain-containing scaffold (CARD) protein, also called membrane-associated guanylate kinase-like domain-containing protein (CARMA1) family. CARMA1 is a membrane-associated guanylate kinase required for T cell receptor- and B cell receptor-induced NF- κ B activation, and which is mostly expressed in the lymphoid tissues (Lin and Wang, 2004). CARMA1 uses its N-terminal caspase activation and recruitment domain (CARD) to interact with the CARD region in the downstream adaptor Bcl-10. Deletion of the entire *CARMA1* in mice results in impaired T and B cell NF- κ B activation, associated with low immunoglobulin serum levels of all isotypes (Hara et al., 2003). The CARMA family is conserved among species and has three members: CARMA1 (*CARD11*), CARMA2 (*CARD14*), and CARMA3 (*CARD10*), encoded by three different genes (Blonska and Lin, 2011; Shinohara et al., 2014). From the functional point of view, all CARMA protein isoforms can activate the NF- κ B transcription factor when overexpressed in mammalian cells (Chang et al., 2010; Scudiero et al., 2014). The NF- κ B family of transcription factors plays a crucial role in cell activation, survival, and proliferation. Aberrant NF- κ B activity results in cancer, immunodeficiency, or autoimmune disorders (such as psoriasis) (Lippens et al., 2011; Jiang and Lin, 2012). Recent studies have reported an association between common SNPs and several rare mutations in the *CARD14* and psoriasis (Fuchs-Telem et al., 2012; Jordan et al., 2012a,b). *CARD14* gain-of-function mutations induce unopposed NF- κ B activation as well as inflammatory mediator production in keratinocytes (Jordan et al., 2012a). Therefore, we decided to investigate the possible association between the SNP rs4722404 in *CARMA1/CARD11* and PsV.

In this study of 568 subjects, we did not find any significant differences (allele/genotype frequencies) between the patients and controls. However, the rs4722404 genotype was significantly associated with an increased risk of early-onset PsV in our cohort. Therefore, SNPs in *CARD11* may have significant effects on heritability. A previous study reported a significant association between the genotype of SNP rs4722404 and the risk of atopic dermatitis in the Japanese population (Hirota et al., 2012).

However, there are several caveats in our study. A relatively small number of psoriasis patients and controls were recruited to this study. However, the result could still provide useful information for follow-up function studies. Moreover, the study illustrates this phenomenon at the level of genetic epidemiology. Before our study, we only know that SNP is associated with AD. Now, the SNP rs4722404 was analyzed in PsV and determined the AD-related SNP associated with PsV. The associations of other SNPs within the susceptibility loci remain

unclear, and further studies are required to better understand the genetic components of PsV.

Our study suggests that the SNP rs4722404 in *CARD11* may have significant effects on heritability in the Chinese population. Therefore, *CARD11* and its pathway may be important in the pathogenesis of PsV. The pathogenicity of variants must be further validated in independent large cohorts; and subsequently, in pathophysiological and therapeutic studies.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are extremely grateful to the study participants. Research supported by grants provided by the China Natural Science Foundation (#81301351, #81201239, #31371377); the Natural Science Foundation of Guangdong Province (#S2013040012415); the Science Foundation of Guangdong Medical College (#B2012083); and the Foundation for Distinguished Young Talents in Higher Education of Guangdong Province (#2012LYM_0068, #2012LYM_0070). Additionally, this project was sponsored by SRF for ROCS, SEM (#20131792).

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