Variants of CARD14 gene and psoriasis vulgaris in southern Chinese cohort*

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DOI: http://dx.doi.org/10.1590/abd1806-4841.20164016

Abstract: Background: Recent mutation analysis identified several missense mutations in CARD14 in psoriasis. Objectives: We performed the genomic sequence analysis on CARD14 in southern Chinese Han Cantonese with Psoriasis Vulgaris (PsV) to reveal more causative missense mutations.
Methods: A total of 131 patients with PsV and 207 matched controls were included. We conducted sequence analysis of all the exon and exon-intron boundaries of CARD14 in the group of PsV patients and subsequent case control analysis of potential sequence variants of significance.
Results: We found five rare mutations and four of them are annotated or reported. Only the variant (c.1291C>G) has not been reported and annotated, but the variant was also found in controls. No significant difference was detected among all rare variant allele frequencies of patients and controls.
Conclusion: None of the new definite variants were pathogenic. The other pathogenic mutations for PsV are still elusive in our cohort.
Keywords: Mutation; Psoriasis; Sequence analysis, DNA

INTRODUCTION

Psoriasis is an inflammatory skin disorder with a genetic background. [1] Psoriasis vulgaris (PsV) is the most common subtype form of the disorder and affected approximately 90% of adult patients. [2] Recently rare variants and common single nucleotide polymorphisms (SNPs) of the gene [caspase recruitment domain family member 14 (*CARD14*)] have been reported to be related to PsV and Generalized Pustular Psoriasis (GPP). [3-6] The *CARD14* gene encodes a member of the family of CARD- and membrane-associated guanylate kinase–like domain–containing protein caspase recruitment domain–containing scaffold proteins (CARMA2), mediates activation of the nuclear factor kappa B (NF-κB) pathway. [7] The NF-κB is a crucial mediator involved in the pathogenesis of psoriasis. [8]

It is well recognized that genetic differences between different geographic populations account for significant variances observed in psoriasis populations. [9-10] We also found major differences between European and Asian populations genetic background, even differences between east and south regions of China. [9] Even though the variant analysis of *CARD14 in a Chinese Han Population* with PsV and GPP has been conducted recently, it is worth screening for *CARD14 in* a group of PsV patients and subsequent case control

Received on 08.09.2014
Approved by the Advisory Board and accepted for publication on 09.03.2015
* Work performed at the Department of Dermatology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong, 524001, China.
Financial support: We are most grateful to the members of all the families who have so willingly participated in this study. This work was supported by grants from The China Natural Science Foundation (81301351); The Natural Science Foundation of Guangdong Province (S2013040012415); the Science Foundation of Guangdong medical colloge (B2012083). Foundation for Distinguished Young Talents in Higher Education of Guangdong (2012LYM_0068,2012LYM_0070).
Conflict of interest: None.

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analysis of potential sequence variants of significance in southern Chinese Han Cantonese. [5] Here, we conducted sequence analysis of all the exon and exon-intron boundaries of *CARD14 in 131 unrelated cases of typical PsV*.

METHODS

Subjects

All PsV subjects of this study were recruited in our hospital (Affiliated Hospital of Guangdong Medical College) from December 2010 to September 2012. The clinical diagnosis was confirmed by at least two dermatologists. Clinical data included demographic information, medical history, clinical checkup, and partly histopathologic examination at the time of first diagnosis. The controls used in the study were individuals without personal and family history of psoriasis, autoimmune disorders, and systemic disorders simultaneously enrolled from the Health Examination Centre. Both cases and controls were Chinese Han Cantonese in southern China, and matched regarding age and sex. The study received approval from the relevant ethics committees according to Declaration of Helsinki Principles and every study subject provided informed consent.

Sanger Resequencing and Genotyping

Genomic DNA was extracted from the peripheral blood lymphocytes using the Takara DNA Blood Kit. The DNA was then used to amplify the exons of *CARD14* along with intronic flanking sequences by polymerase chain reaction (PCR) with previously described primers. [11] PCR products were subsequently purified using a QIAquick PCR Purification kit (Qiagen, Germany). DNA sequencing was performed using ABI PRISM®3730 automated sequencer (Applied Biosystems). As a first pass, all coding exons of *CARD14* were resequenced in 131 PsV cases. Exons with missense mutations identified were resequenced in 207 controls. Sequence comparisons and analyses were performed using the Phred-Phrap-Consed Version 12.0 program.

Statistical analysis and protein function prediction

Comparisons of characteristics between patients with psoriasis and controls were made using t-test, the Pearson's chi-square test and odds ratios (OR) by using the Statistical Package for Social Scientists (SPSS) version 13.0. Differences in allele frequencies between groups were analyzed by Fisher's exact tests. Statistical significance was defined at $p < 0.05$. We also predicted the effects of the variants on protein function using the Polyphen-2 (Polymorphism Phenotyping v2), available as software and via a Web server. [12] The software could predict the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations.

RESULTS

Baseline Characteristics

A total of 131 patients (range from 15 to 81 years) and 207 controls (range from 14 to 82 years) were investigated. The distribution of cases and controls according to age, sex, age of onset (mean±sd: 32.409±17.825 years) and family history (Familial cases: Sporadic cases=52:75) only for cases are shown in table 1. No difference was observed between cases and controls for age and sex.

Rare-Variant Screening

The genomic sequence analysis on *CARD14* found five rare heterozygous missense variants in cases. The five missense variants were c.526G>C (rs144475004), c.646G>A, c.1291C>G, c.1414C>T (rs145876317) and c.1517C>T (rs61751630) shown in table 2 and figure 1. One of the variants (c.1291C>G) has not been reported and annotated. The amino acid changes of missense polymorphisms are mapped to the coiled-coil domain or other regions of the CARMA2 protein. The variants c.526G>C (rs144475004; p.Asp176His) and c.1291C>G (p.Leu431Val) was predicted to have damaging effects (scores=0.906 and 0.602), while the other

Table 1: The characteristics of psoriasis cases and controls

Variables	Cases (n = 131)	Controls (n = 207)	P-Value
Age (mean±SD, year)	36.552±15.978	34.126±16.309	0.536
Males, n (%)	73(56%)	126(61%)	0.349
Familial/Sporadica	52/75	-	
Early onset/Later onset	61/70	-	
Age at onset of psoriasis (years), mean±SD	32.409±17.825	-	

a Four samples were missing information on the familial or sporadic status.

Table 2: Characteristics and Frequencies of CARD14 Coding Rare Variants

CARD14 Exon	cDNA Mutation	Amino acid change	Protein Domain	Predicted Effect on Protein Function-PolyPhen-2	Allele Frequency		P-Value
					Cases N (%)	Controls N (%)	
Exon 4	c.526G>C (rs144475004)	p.Asp176His	coiled-coil damaging(0.906)	Probably	3(2.290%)	1(0.483%)	0.303
Exon 4	c.646G>A	p.Ala216Thr	coiled-coil	Benign(0.002)	1(0.763%)	0(0%)	0.388
Exon 9	c.1291C>G	p.Leu431Val	none	Possibly damaging(0.602)	1(0.763%)	1(0.483%)	1.000
Exon 10	c.1414C>T (rs145876317)	p.Arg472Cys	none	Benign(0.004)	1(0.763%)	3(1.449%)	1.000
Exon 11	c.1517C>T (rs61751630)	p.Pro506Leu	none	Benign(0)	1(0.763%)	2(0.966%)	1.000

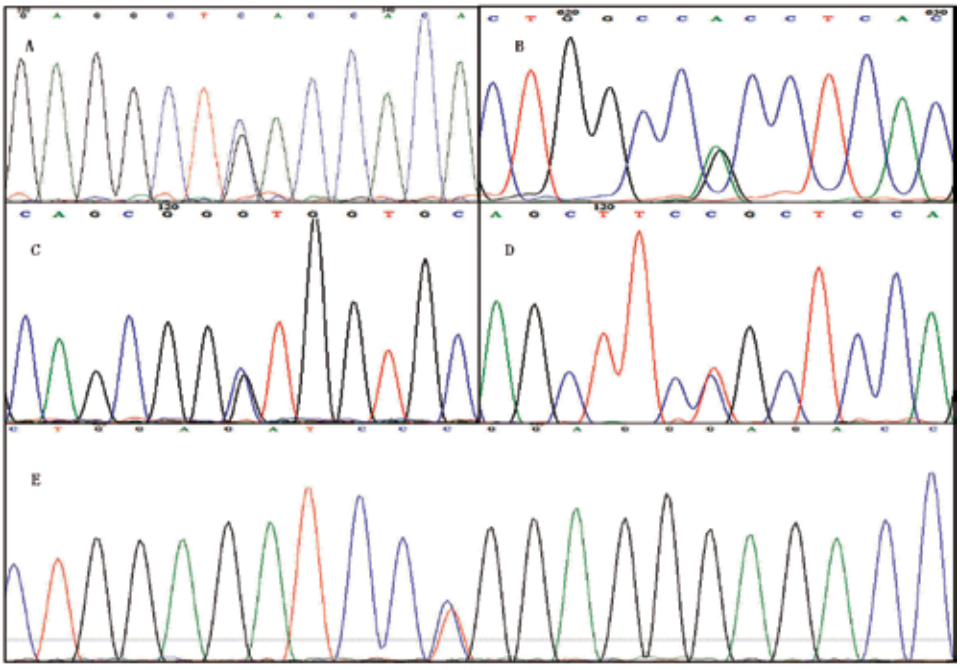

Figure 1:
The sequencing figures of variants in CARD14 in PsV. A:c.526G>C(rs144475004),B:c.646G>A,C:c.1291C>G,D:c.1414C>T(rs145876317),E:c.1517C>T(rs61751630)

three were predicted to be benign. No significant difference was detected between allele frequencies of the patients and controls (P >0.05). Phenotype genotype correlation including published cases was difficult due to different mutations in few carriers.

DISCUSSION

Past linkage and mutation analysis identified several missense mutations in *CARD14 in psoriasis patients*.[3,13] Jordan et al first describe fifteen rare missense variants in *CARD14* and their effects on NF-κB activation.[3] The gain-of-function mutations lead to unopposed NF-κB activation, and induction of inflammatory mediators from keratinocytes.[3] The NF-κB family of transcription factors plays a crucial role in cell activation, survival and proliferation and results in cancer, immunodeficiency or autoimmune disorders (e.g. psoriasis).[14] Hence, the presence of the *CARD14* mutations may result in a greater amplitude of inflammatory response upon epidermal activation.[15,16] These studies illustrate pathogenic variants as part of one of the pathways leading to common psoriasis.

We conducted this study aiming to investigate the presence of *CARD14* variants PsV in Southern Chinese. Our study found five rare mutations in patients; four of them are annotated or reported and one not reported variant (c.1291C>G), but the variant was also found in controls. We confirmed the variant c.526G>C

(rs144475004) reported in the Caucasian and it is also a risk factor for GPP with PsV in the Japanese Cohort.[3,11] Another rare variant c.646G>A (p.Ala216Thr) was also found in Chinese population with PsV.[5] Two other rare SNPs c.1414C>T (rs145876317) and c.1517C>T (rs61751630) were also detected in controls and no significant difference was detected between allele frequencies of the patients and controls. On the basis of the observation that several rare mutations were clustered in exon 4, which encodes part of the critical coiled-coil domain, we reasoned that the exon 4 might be a mutation hotspot.

CONCLUSION

In summary, none of the new definite rare variants were pathogenic. However, the result still could provide useful information for follow-up study, even with the number of psoriasis patients and controls in our study being relatively small. But the results of allele frequency are drawn from our research and results of previous studies are basically coinciding. Therefore, it is possible that others genes different mutations exist in souther Chinese Han population with PsV. The other pathogenic mutations for PsV are still elusive in our cohort . It could not be excluded that PsV in southern Chinese Han is reslated to *CARD14* mutation. The pathogenicity of the mutations and variants reported in previous studies needs to be further validated in more case-control cohorts and subsequent pathophysiologic and therapeutic studies.❑

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How to cite this article: Zhu K, Shi G, Liu H, Zhu C, Fan Y. Variants of CARD14 gene and psoriasis vulgaris in southern Chinese cohort. An Bras Dermatol. 2016;91(1):45-8.