Int J Clin Exp Pathol 2015;8(10):13465-13470
www.ijcep.com /ISSN:1936-2625/IJCEP0011137

*Original Article*

# Genetic association between CARD9 variants and inflammatory bowel disease was not replicated in a Chinese Han population

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Received June 6, 2015; Accepted July 21, 2015; Epub October 1, 2015; Published October 15, 2015

**Abstract:** *Objective:* In order to investigate whether *CARD9* gene is associated with IBD in Chinese Han population, we replicated 2 SNPs of CARD9 which have been reported to be significantly associated with IBD. *Methods:* Two SNPs were genotyped using polymerase chain reaction with sequence-specific primers in 288 patients (232 CD patients, 56 UC patients) and 274 controls. *Results:* The frequencies and distributions of alleles and genotypes of the tested SNPs were analyzed, and no significant differences were found between patients and controls. *Conclusions:* We observed no significant association between the investigated CARD9 SNPs and the susceptibility of either CD or UC. Further studies with larger sample size focusing on different ethnicities are required to elucidate the correlation between CARD9 and IBD.

**Keywords:** CARD gene, inflammatory bowel diseases, ulcerative colitis, Crohn's disease, association

## Introduction

Inflammatory bowel diseases (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD), are defined as a kind of chronic and relapsing inflammations of the gastrointestinal tract. The main symptoms of IBD are abdominal pain, diarrhea, bleeding and malabsorption [1, 2]. In the last several decades, the incidence of IBD is increasing not only in Western countries, but also in Asia. It is becoming a more and more serious global social problem because it places a heavy burden on patients [3]. Although considerable efforts have been devoted to unraveling the etiology of IBD, its precise molecular pathogenesis is not completely understood. To date, it is believed that IBD is resulted from an abnormal inflammatory response which involved both genetic and environmental factors [4, 5]. Thus, genes participated in innate immune responses are under investigation to look for variants predisposing to IBD. Combined with the development of genetic research techniques, a number of immune-related genes have been identified as risk factors for the susceptibility of IBD [6].

Caspase recruitment domain-containing protein 9 (CARD9) is a scaffold protein encoded by *CARD9* gene which located on chromosome 9q34.3. CARD9 belongs to the CARD protein family which characterized by the presence of caspase-associated recruitment domain (CARD). It is a crucial signal transducer via CARD-CARD interactions, and plays important roles in host defense and immune homeostasis through assembling multifunctional signaling complexes [7-9]. Recently, Sokol et al. reported that Card9-deficient mice are more susceptible to dextran sulfate sodium (DSS)-induced colitis and the recovery is impaired [10]. Their results suggested that CARD9 is crucial in intestinal homeostasis and may be involved in the pathogenesis of IBD. Moreover, several genetic studies also indicated that the *CARD9* locus is significantly associated with the susceptibility of IBD in different population cohorts [11-13].

In order to evaluate the correlation between *CARD9* gene and the susceptibility of IBD in the Chinese Han population, we conducted this case-control study. In this study, we genotyped 2 SNPs (rs10870077 and rs10781499) of

**Table 1.** The primer sequence used for genotyping

| SNP | Position | Primer | Sequence |
|---|---|---|---|
| rs10781499 | 139266405 | Specific primer A | GCACCTGCTCCTCATCATCA |
| | | Specific primer G | GCACCTGCTCCTCATCATCG |
| | | Internal control forward primer | GCAGGAGAGGCTGGGGGAC |
| | | Common reverse primer | AGCCCTGGCCCAGCGTCT |
| rs10870077 | 139263891 | Specific primer C | GGTTGAACACGGTTTTCCCTGAC |
| | | Specific primer G | GGTTGAACACGGTTTTCCCTGAG |
| | | Internal control forward primer | CTCAAGTGATCCGCCCGCC |
| | | Common reverse primer | TTGAGGGCAGTTGTCAGAGGATTT |

*CARD9* in 232 CD patients, 56 UC patients and 274 normal controls of Chinese Han origin and analyzed the association.

## Materials and methods

### *Patient and control subjects*

This sample set consisted of 288 unrelated IBD patients (232 CD and 56 UC) and 274 normal controls of Chinese Han population recruited from the Department of Gastroenterology of Ruijin Hospital appended to Shanghai Jiaotong University School of Medicine. All patients were diagnosed by senior physicians based on standard clinical, endoscopic, radiologic, and histological criteria. Controls were randomly selected from healthy persons under routine health screening. The detailed information of patients and controls had been previously described [14, 15].

The study was approved by the Research Ethics Committee of Ruijin Hospital, Shanghai, China. And informed consents were obtained from all subjects before blood sampling.

### *DNA extraction and genotyping of the CARD9 variants*

Genomic DNA was isolated from EDTA peripheral blood using QIAamp blood extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. All DNA samples were genotyped for CARD9 single nucleotide polymorphisms (SNP) by polymerase chain reaction with sequence-specific primers (PCR-SSP). All primers for the PCR-SSP were designed using the genomic sequences in the GenBank (http://www.ncbi.nlm.nih.gov). The primer sequences were listed in **Table 1**. The amplified products were assessed for the presence/absence of PCR amplicons specific to the particular alleles using a standard 2% agarose gel electrophoresis followed by ethidium-bromide staining. About 10% samples were then confirmed by sequencing.

### *Statistical analysis*

Hardy-Weinberg equilibrium testing (HWE), *P*-value computations [$P>0.05$], in both of the healthy control and patient groups, the allele and genotype frequency analysis were all performed on SHEsis software (http://analysis.bio-x.cn) [16, 17]. All tests were two-tailed and statistical significance was assumed at $P<0.05$.

## Results

The frequencies and distributions of alleles and genotypes at the 2 SNPs in *CARD9* were identified and compared between IBD patients and controls. Allele frequencies and genotype distributions of the polymorphisms studied were in Hardy-Weinberg equilibrium in patient and control groups.

The results of the association study are shown in **Table 2**.

For total IBD, rs10870077 ($P_{allele}$ = 0.983066, $P_{genotype}$ = 0.776959, OR and 95% CI: 1.002878 [0.769106~1.307707]), rs10781499 ($P_{allele}$ = 0.823332, $P_{genotype}$ = 0.921032, OR and 95% CI: 0.969849 [0.741285~1.268887]). The results of both allele and genotype showed no significance in the $\chi^2$ tests.

For CD subgroup, rs10870077 ($P_{allele}$ = 0.751184, $P_{genotype}$ = 0.877595, OR and 95% CI: 1.046662 [0.789536~1.387527]), rs10781499 ($P_{allele}$ = 0.306523, $P_{genotype}$ = 0.231786, OR and 95% CI: 0.859849 [0.643678~1.148619]), showed no significance in the $\chi^2$ tests.

**Table 2.** Allele and genotype frequency of the 2 SNPs of *CARD9* gene in patients and controls

| Cases | SNP ID | Allele | | OR (95% CI) | *P*-value | Genotypes | | | HWe P[b] | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| IBD | rs10870077 | C (freq) | G (freq) | | | C/C (freq) | C/G (freq) | G/G (freq) | | |
| | Case | 421 (0.731) | 155 (0.269) | 1.002878 [0.769106~1.307707] | 0.983066 | 149 (0.517) | 123 (0.427) | 16 (0.056) | 0.145782 | 0.776959 |
| | Control | 390 (0.730) | 144 (0.270) | | | 135 (0.506) | 120 (0.449) | 12 (0.045) | 0.021223 | |
| | rs10781499 | A (freq) | G (freq) | | | A/A (freq) | A/G (freq) | G/G (freq) | | |
| | Case | 146 (0.268) | 98 (0.732) | 0.969849 [0.741285~1.268887] | 0.823332 | 19 (0.070) | 108 (0.397) | 145(0.533) | 0.854954 | 0.921032 |
| | Control | 146 (0.274) | 386 (0.726) | | | 21 (0.079) | 104 (0.391) | 141 (0.530) | 0.766085 | |
| CD | rs10870077 | C (freq) | G (freq) | | | C/C (freq) | C/G (freq) | G/G (freq) | | |
| | Case | 343 (0.739) | 121 (0.261) | 1.046662 [0.789536~1.387527] | 0.751184 | 122 (0.526) | 99 (0.427) | 11 (0.047) | 0.103755 | 0.877595 |
| | Control | 390 (0.730) | 144 (0.270) | | | 135 (0.506) | 120 (0.449) | 12 (0.045) | 0.021223 | |
| | rs10781499 | A (freq) | G (freq) | | | A/A (freq) | A/G (freq) | G/G (freq) | | |
| | Case | 107 (0.245) | 329 (0.755) | 0.859849 [0.643678~1.148619] | 0.306523 | 9 (0.041) | 89 (0.408) | 120 (0.550) | 0.130949 | 0.231786 |
| | Control | 146 (0.274) | 386 (0.726) | | | 21 (0.079) | 104 (0.391) | 141 (0.530) | 0.766085 | |
| UC | rs10870077 | C (freq) | G (freq) | | | C/C (freq) | C/G (freq) | G/G (freq) | | |
| | Case | 78 (0.696) | 34 (0.304) | 0.847059 [0.542364~1.322929] | 0.465249 | 27 (0.482) | 24 (0.429) | 5 (0.089) | 0.919066 | 0.401437 |
| | Control | 390 (0.730) | 144 (0.270) | | | 135 (0.506) | 120 (0.449) | 12 (0.045) | 0.021223 | |
| | rs10781499 | A (freq) | G (freq) | | | A/A (freq) | A/G (freq) | G/G (freq) | | |
| | Case | 39 (0.361) | 69 (0.639) | 1.494342 [0.965884~2.311931] | 0.070058 | 10 (0.185) | 19 (0.352) | 25 (0.463) | 0.080998 | 0.085074 |
| | Control | 146 (0.274) | 386 (0.726) | | | 21 (0.079) | 104 (0.391) | 141 (0.530) | 0.766085 | |

Allele and genotype frequency of the two loci in IBD, CD, and UC. HWe P[b] : *P* value for Hardy-Weinberg equilibrium

For UC subgroup, rs10870077 ($P_{allele}$ = 0.465249, $P_{genotype}$ = 0.401437, OR and 95% CI: 0.847059 [0.542364~1.322929]), rs10781499 ($P_{allele}$ = 0.070058, $P_{genotype}$ = 0.085074, OR and 95% CI: 1.494342 [0.965884~2.311931]), also showed no significance in the $\chi^2$ tests.

Over all, we observed no significant association between the investigated CARD9 and the susceptibility of either CD or UC.

## Discussion

Since the intestinal tract is constitutively exposed to environmental factors including bacteria and food antigens, it is important to maintain the balance between immune tolerance to the commensal microbiota and response to pathogens. Breakdown of the intestinal homeostasis are believed to precipitate the chronic inflammatory pathology in inflammatory bowel disease, Crohn's disease and ulcerative colitis [18-20]. Studies have demonstrated that some factors which involved in intestinal homeostasis are important in the occurrence and development of IBD both in patients and animal models [21-23].

CARD9 is involved in the activation and regulation of innate immune responses to pathogens because it functions as a cytosolic signal transduction protein downstream of several pattern recognition receptors (PRR) such as dectin-1, dectin-2, and mincle [24]. Deficiency of CARD9 in patients and mouse models increased susceptibility to the enteric bacterial pathogens, as well as fungal pathogens. In 2008, it was first reported as a as a possible susceptibility gene for IBD by Zhernakova et al. They performed a multistage case-control design study in people of the Netherlands and found that CARD9 rs10870077 SNP is significantly associated with both CD and UC [13]. Some GWAS studies and subsequently replications also identified other variants of CARD9 conferred susceptibility to IBD [11, 12, 25, 26]. More importantly, CARD9-null mice are much more susceptible to DSS-induced colitis than wild-type mice [10].

In this study, we examined 2 important SNPs rs10870077, rs10781499 of CARD9 gene in 288 IBD patients (232 CD subgroups and 56 UC subgroups) and 274 normal controls in Chinese Han population. These two SNPs have been demonstrated to be significantly associated with both CD and UC risk in different population cohorts. But we found that they have no association with either CD or UC in the Chinese Han population. It is a general problem that the association of one gene with complex diseases in one population cannot be exactly replicated in others [27-29]. Since the incidence, epidemiology and phenotype are different between patients from Chinese Han population and western countries, the genetic susceptibility may be different too. Other reasons such as sample size and different endophenotypes may also lead to the inconsistency. In addition, our sample size was not very large, so more sites of SNPs for Pair-loci D'/r2 value analysis and haplotype analysis on a larger number of Chinese subjects and on other ethnicities are necessary to fully elucidate the exact role of CARD9 in the pathogenesis of IBD.

To our knowledge, this is the first case-control study to investigate the association between *CARD9* and IBD in Chinese Han population. Although we did not observe any association at the *CARD9* locus for any of the subgroups tested, our results can provide a reference for further studies based on larger sample size or other populations.

## Disclosure of conflict of interest

None.

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