Int J Clin Exp Pathol 2014;7(12):8952-8957
www.ijcep.com /ISSN:1936-2625/IJCEP0002937

Original Article

Association of *IL-27* gene three polymorphisms with Crohn's disease susceptibility in a Chinese Han population

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Received October 2, 2014; Accepted November 26, 2014; Epub December 1, 2014; Published December 15, 2014

Abstract: Objective: To investigate the association of three polymorphisms in the Interleukin-27 (*IL-27*) gene with CD risk in Chinese population. Methods: This case-control study involved 312 CD patients and 479 age- and sex-matched healthy controls. Genotyping was performed using PCR-LDR method. Data were analyzed using Haplo. Stats program. Results: There were significant differences between patients and controls in allele distributions of rs153109 (P_{allele} = 0.036). The risk for CD associated with the rs153109-G mutant allele was increased by 26% (95% CI [confidence interval]: 1.02-1.56; *P* = 0.03) under the additive model and by 45% (95% CI: 1.07-1.97; *P* = 0.02) under the dominant model. In haplotype analysis, haplotype T-T-G (in order of rs17855750, rs181206 and rs153109) increased the odds of CD by 37% (95% CI: 1.04-1.81; *P* = 0.028). Conclusions: In conclusion, genetic defects in *IL-27* gene showed remarkable associations with CD in Chinese.

Keywords: *IL-27*, polymorphism, Crohn's diseases, susceptibility, association study

Introduction

Crohn's disease (CD) is a chronic, recurrent inflammatory bowel disease (IBD) that can affect any part of the gastrointestinal tract. Although the exact etiology of CD remains unknown, it is now generally accepted that CD resulted from inappropriate immune responses in genetically susceptible individuals. Meanwhile, several association studies and linkage analyses have identified multiple candidate genes for CD, especially in the genes encoding microbe recognition, lymphocyte activation, cytokine signaling, and intestinal epithelial defense [1-5]. In view of the importance of cytokine in CD, therefore, it might be a reliable strategy to look for and indentify CD-susceptibility genes involving in cytokine signaling.

Interleukin-27 (IL-27) is a novel heterodimeric cytokine regarded as a member of IL-12 family, which secreted mainly by stimulated antigen-presenting cells such as macrophages and dendritic cells, and composed of two subunits: the Epstein-Barr virus-induced gene 3 (EBi3) and IL-27p28. Several studies have highlighted that the IL-27 played an important role in the occurrence and development of CD both in patients and animal models [6-8]. In 2009, Li et al. [8] reported that the *IL-27* rs153109 polymorphism was significantly associated with the susceptibility of CD in the Korean population. However, the role of *IL-27* polymorphisms in the Chinese CD patient's susceptibility was unknown. As the susceptibility genes in Crohn's disease may vary in different ethnic groups, in our study, we not only performed the hospital-based case-control association study analysis on the three widely-evaluated polymorphisms of *IL-27* gene, but also assessed the association between *IL-27* haplotypes and CD.

Materials and methods

Study populations

This was a hospital-based case-control study, including 312 sporadic patients with CD and 479 healthy volunteers. All patients were

Table 1. Characteristics of CD patients and healthy controls in the Chinese Han population

Characteristics	CD patients	Control subjects
Number	312	479
Male/female	205/107	308/171
Age, mean ± SD (years)	34.0 ± 13.0	36.5 ± 15.1
Age at diagnosis		
< 17 Years	42	
17-40 Years	212	
> 40 Years	58	
CD behavior		
Inflammatory	179	
Stricturing	90	
Penetrating	43	
CD location		
Ileum	194	
Colon	27	
Ileocolon	91	
Perianal lesions		
Yes	92	
No	220	
Appendectomy		
Yes	18	
No	294	
Abdominal operation		
Yes	72	
No	240	

recruited through the Outpatient Clinic at the Department of Gastroenterology at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, as part of an ongoing project to examine genetic factors that contribute to the etiology of IBD. The demographics in the study population are summarized in **Table 1**. Cases and controls were well matched by age and gender. Informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Board of the Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University.

Genotyping

Blood samples (1 mL) were collected, and genomic DNA was extracted from white blood cells using the TIANamp Blood DNA Kit (Tiangen Biotect [Beijing] Co., LTD). Genotyping was conducted using the PCR-LDR (ligase detection reactions) method by ABI 9600 system (Applied Biosystems, USA) [9]. Cycling parameters were as the following: 94°C for 2 min; 35 cycles of 94°C for 20 s; 56°C for 20 s; 72°C for 40 s; and a final extension step at 72°C for 3 min. Two specific probes to discriminate the specific bases and one common probe were synthesized (available upon request). The common probe was labeled at the 3' end with 6-carboxy-fluorescein and phosphorylated at the 5' end. The reacting conditions of LDR were: 94°C for 2 min, 30 cycles of 94°C for 30 s and 56°C for 3 min. After reaction, 1 mL LDR reaction products were mixed with 1 mL ROX passive reference and 1 mL loading buffer, and then denatured at 95°C for 3 min, and chilled rapidly in ice water. The fluorescent products of LDR were differentiated using ABI sequencer 377 (Applied Biosystems, USA).

Statistical analysis

Comparisons between CD patients and controls were conducted by unpaired t-test for continuous variables and by χ^2 test for categorical variables. To avoid gross genotyping error, all polymorphisms were checked for consistency with Hardy-Weinberg equilibrium on a contingency table of observed-versus-predicted genotype frequencies by using Pearson χ^2 test or Fisher's exact test. Genotypes were compared by Logistic regression analysis under assumptions of additive, dominant and recessive models of inheritance, respectively. Statistical significance was defined as $P < 0.05$.

Haplotype frequencies were estimated by using the haplo.em program, and odds ratio (ORs) and 95% confidence interval (CI) were estimated by haplo.cc and haplo.glm programs according to a generalized linear model [10]. Furthermore, the haplo.score was used to model an individual's phenotype as a function of each inferred haplotype, which was weighted by their estimated probability to account for haplotype ambiguity. The haplo.em, haplo.glm, and haplo.score were implemented using Haplo.stats software (version 1.4.0) developed by the R language (http://www.r-project.org/).

Results

Single-locus analysis

There were no deviations from Hardy-Weinberg equilibrium for all studied polymorphisms in

Table 2. The genotype distributions and allele frequencies of the studied polymorphisms between patients and controls, and their risk prediction for CD under three genetic models of inheritance

Polymorphism	Patients (N = 312)	Controls (N = 479)	P χ^2	Genetic models	OR; 95% CI
rs17855750					
TT	242	393		Additive	1.31; 0.95-1.80
GT	64	81	0.090	Dominant	1.32; 0.93-1.88
GG	6	5		Recessive	1.86; 0.56-6.14
G (%)	12.2	9.5	0.24		
rs153109					
AA	92	181		Additive	1.26; 1.02-1.56
GA	170	232	0.055	Dominant	1.45; 1.07-1.97
GG	50	66		Recessive	1.19; 0.08-1.78
G (%)	43.3	38.0	0.036		
rs181206					
TT	240	361		Additive	0.93; 0.68, 1.27
CT	68	112	0.92	Dominant	0.92; 0.66, 1.28
CC	4	6		Recessive	1.02; 0.29, 3.66
C (%)	12.2	12.9	0.65		

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

controls (P > 0.10). The genotype/allele distributions of the three selected polymorphisms in *IL-27* were depicted in **Table 2**. As shown in **Table 2**, significant difference between CD patients and controls was observed in allele distribution of rs153109 (P_{allele} = 0.036), In contrast, there were no statistically significant differences in rs17855750 or rs181206 alleles/genotypes between CD patients and controls (P > 0.05).

Notably, for rs153109, the risk associated with mutant G-allele or genotype was increased by 26% (95% CI: 1.02-1.56; P = 0.03) under the additive model, by 45% (95% CI: 1.07-1.97; P = 0.02) under the dominant model. No significant association was detected for rs17855750 or rs181206 under the three genetic models.

Haplotype analysis

Haplotype frequencies of the three polymorphisms examined were estimated and compared between cases and controls (**Table 3**). The frequency of haplotype T-T-G (in order of rs17855750, rs181206 and rs153109) was slightly higher (P = 0.058, $P_{simulated}$ = 0.049) in patients than that in controls. After assigning the commonest haplotype T-T-A as the reference, haplotype T-T-G increased the odds of CD by 37% (95% CI: 1.04-1.81; P = 0.028).

Discussion

IL-27 gene is an interesting candidate gene for CD for several reasons. It is a novel IL-12 family member bridging innate and adaptive immunity by playing a role in the activation of naive T cells and in development of Th1 cells. On one hand, *IL-27* not only activates JAK1, JAK2, TYK2, STAT1, STAT2, STAT3, STAT4, and STAT5 in naive CD4+ T cells [11-14], and enhances proliferation in naive CD4+ T cells, but also synergizes with IL-12 in primary IFN-γ production [11, 12, 14, 15]; one the other hand, it promotes inflammation by inducing naïve T cells to differentiate into the Th1 subset via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways [16]. Additionally, recent evidence showed that IL-27 is a strong inducer of proinflam matory cytokine and chemokine expression, including enhancement of IL-6, IP-10, MIP-1α, MIP-1β, and TNF-α expression in human primary monocytes [17]. However, several recent reports suggested that IL-27 also exerted an inhibitory effect on immune responses by inhibiting TCR/CD28-mediated IL-2 production [18, 19] and inducing IL-10-secreting T cells [20-24]. Immunosuppressive and anti-inflammatory effects of IL-27 have also been demonstrated in various experimental settings. Sasaoka et al. [6] demonstrated that in the 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced mouse acute colitis models, subcutaneous single-chain human IL-27 treatment clearly suppressed several inflammatory cytokines including IL-17, in inflamed colon, and significantly improved the colon length, extent of necrosis, and ulceration and thickened epithelium and several pathological scores in a dose-dependent manner. Moreover, a number of studies recently suggested that polymorphisms of *IL-27* are associated with susceptibility to several chronic inflammatory disorders such as asthma [25] and chronic obstructive pulmonary disease [26]. In the present study, we investigated the role of three promising polymorphisms of *IL-27*

Table 3. Haplotype frequencies of the studied polymorphisms between patients and controls, and their risk prediction for CD

Hapotype	Case (%)	Control (%)	Hapscore	*p*-Value	Psim	OR	95% CI	*p*-Value
T-T-A	56.73	61.85	-2.12	0.034	0.029	Reference		
T-C-G	11.44	12.71	-0.69	0.49	0.53	1.00	(0.72, 1.39)	0.98
G-T-G	1144	9.11	1.52	0.13	0.12	1.38	(0.99, 1.94)	0.061
T-T-G	19.65	15.94	1.89	0.058	0.049	1.37	(1.04, 1.81)	0.028

Abbreviations: OR, odds ratio; CI, confidence interval; P_{Sim}, simulated *P*. *Alleles in haplotype were presented in order of polymorphisms rs17855750, rs181206 and rs153109.

gene in the risk of CD susceptibility in Han Chinese. Our results revealed the *IL-27* gene and the haplotype association with CD. Although low-penetrance, the haplotype T-T-G (in order of rs17855750, rs181206 and rs153109) was correlated with CD and might play an important role in CD occurrence, to our knowledge, this is the pilot study exploring the genetic susceptibility of *IL-27* gene to CD in Chinese.

The rs153109, which located at the position -964 of the promoter region, was found to significantly asscciate with the occurrence of CD in Chinese population in this study. This result was in accord with previous study conducted by Li et al. in the Korean population [8]. Given the critical role of IL-27 in the activation of naive T cells and in the development of Th1 cells, we could reasonably hypothesize that the rs153109 variant might be a functional polymorphism, rs153109A allele might resulted in increasing of the transcriptional activity compared with the G allele, and causing greater IL-27 expression to promote inflammation by inducing naïve T cells to differentiate into the Th1 subset. This claim warrants further investigation.

Meanwhile, in this study, haplotype analysis of the rs17855750-rs181206-rs153109 combination revealed four main and rare haplotypes. Compared to the commonest haplotype T-T-A, the haplotype T-T-G showed a highly significant correlation between CD and control individuals (19.65% vs. 15.94%, OR = 1.37), suggesting that it might be a positive haplotype. Herein, haplotype analysis reconfirmed the results of rs153109 being the susceptible locus for CD in the Chinese population.

However, there are several drawbacks in this study. First, the sample size in our study is relatively small, which might result in a fluctuated estimation. Second, we only reveal three polymorphisms of *IL-27* gene associated with susceptibility to CD, and there might be other unidentified polymorphisms of IL-27 which influenced the development of CD. Therefore, further association studies utilizing more larger sample size from different ethnic origins and biological research should be encouraged to carry out to verify this association. Third, data on the circulating IL-27 levels are unavailable, which makes us incapable of comparing IL-27 levels across genotypes.

In conclusion, our study revealed that polymorphisms and haplotypes of the *IL-27* gene were significantly associated with susceptibility to CD in the Chinese population. This study provides supporting evidence for further investigation on pathophysiological mechanisms of *IL-27* genes in CD.

Disclosure of conflict of interest

None.

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