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RESEARCH ARTICLE

Contribution of *rs11465788* in *IL23R* gene to Crohn's disease susceptibility and phenotype in Chinese population

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Abstract

Multiple studies have shown that IL23 cytokine plays an essential role in the development of autoimmune diseases by activating IL17-producing helper T (Th17) cells. Given that the susceptibility loci in *IL23R* for Crohn's disease (CD) is present in Western population and not in Asian population; we screened the *IL23R* gene by DNA sequencing to identify susceptibility loci in a selected CD cohort and confirmed it in all our subjects (134 CD and 131 controls). A novel nonsynonymous SNP (p.Gly149Arg, c.445G>A) and 35 single nucleotide polymorphisms (SNPs) were identified. Among them, only *rs11465788* was implicated in CD susceptibility ($P = 4.9 \times 10^{-4}$, OR = 0.30). Genotype–phenotypic interaction analysis showed that *rs11465788* is associated with nonstricturing and nonpenetrating disease behaviour in CD patients ($P = 0.015$). Our results provide the evidence that *rs11465788* may influence the susceptibility and clinical features of CD in Chinese population.

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Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory disorders of the gastrointestinal tract. Although the incidence of IBD in China is still low compared with Europe and North America, it is increasing rapidly (Loftus and Sandborn 2002; Loftus 2004). The estimated prevalence of CD and UC in China is 1.4/100,000 (Loftus and Sandborn 2002; Loftus 2004) and 11.6/100,000 (Wang and Ouyang 2007), respectively.

In spite of a significant number of studies to identify the fundamental pathophysiological processes, the precise pathogenesis of IBD is still unknown; although they have been thought to be multifactorial diseases. The increased level of concordance between identical twins, elevated rates of IBD among the Ashkenazi Jewish population, and the familial risk of IBD provide strong evidence, that the genetic factors play an important role in the pathogenesis of IBD (Taylor *et al*. 2007).

The functional interleukin (IL) 23 receptor is a heterodimer of the IL12Rb1 subunit and a novel subunit named IL23R. After binding to the IL23 receptor, IL23 activates IL17-producing helper T (Th17) cells, and promotes proinflammatory cytokine IL17 secretion (Parham *et al*. 2002; Murphy *et al*. 2003). This IL23/IL17 axis has been shown to be crucially involved in immunity to infection and autoimmune disease such as rheumatoid arthritis (McKenzie *et al*. 2006). Based on these findings, the IL23/IL17 axis might also play a pivotal role in the pathogenesis of IBD. This hypothesis was supported by a recent work, where IL23 acted as a driver of both innate and T cell-mediated inflammation in two different mice colitis models (Hue *et al*. 2006).

Recently, in a genome-wide association study, Duerr *et al*. (2006) identified the interleukin 23 receptor (*IL23R*) gene as a CD susceptibility gene in North American population, which indicated that the rare *IL23R* SNP *rs11209026* (p.Arg381Gln; c.1142G>A) was associated with strong protection against CD. However, a Japanese case–control study of a CD cohort failed to confirm the association (Yamazaki *et al*. 2007). These results revealed distinct different genetic backgrounds of CD in different populations. Although it was generally accepted that the clinical profiles of CD are similar among Europeans, North Americans and Chinese, com-

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mon susceptibility variant(s) for CD have not been reported so far. Whether this candidate gene *IL23R* is also associated with Chinese CD patients is still unknown. Therefore, we sequenced the *IL23R* gene to investigate whether it is associated with CD in Chinese population. In addition, we attempted to analyse the effects of *IL23R* variants on specific CD disease subtypes in a detailed genotype–phenotype analysis.

Materials and methods

Study population

Our study was carried out in two stages. The first stage was designed to identify the candidate CD susceptibility SNPs of *IL23R* in 50 patients and 50 controls. The second stage of the research included an analysis of gentotype–phenotype interactions for the candidate SNPs with more subjects enrolled (134 CD and 131 controls).

All the CD patients and controls were from First Affiliated Hospital of Sun Yat-sen University (SYSU), Guangzhou, China. CD diagnosis was based on clinical, radiologic, endoscopic and pathology procedures according to the currently accepted Lennard-Jones (1989) criteria. Healthy controls (male 67.2%; mean age 35 ± 12.3 years) with no history of IBD, or other autoimmune disorders were recruited from people who requested annual physical examinations. This study was approved by the ethics committee of the First Affiliated Hospital of SYSU.

SNP genotyping

After informed consents were obtained, genomic DNA was isolated from 2 mL peripheral blood collected with EDTA coagulated tube according to the standard protocol of the QiAamp Midi kit (Qiagen, Hilden, Germany). SNPs were detected by direct sequencing the PCR products (table 1) covering all exons, flanking regions of the *IL23R* gene in forward or reverse directions.

Statistical analysis

Each marker was tested for Hardy–Weinberg equilibrium in both the control and CD cohort using a χ^2 goodness-of-fit test before inclusion in the following analyses ($P \geq 0.05$). The significance of differences in allele frequencies between CD patients and controls were also assessed by the χ^2 test. Within the CD cohort, χ^2 tests of contingency tables and logistic regression were used to search for possible associations between each SNP and IBD subphenotype. An interaction was considered significant if a P value of < 0.05 was attained. Statistical analysis was done using the software package SPSS 13.0 (SPSS, Chicago, USA).

Results

Demographic characteristics of the study participants are summarized in table 2. In the first stage, 35 SNPs were identified, including one unreported nonsynonymous SNP (p.Gly149Arg, c.445G>A). The *IL23R* p.Gly149Arg variant was only present in heterozygous state. The Gly/Arg genotype frequency was 0.04 in CD population as compared to 0.02 in the controls, showing no evidence for disease association ($P = 0.57$). The locations and genotype frequencies of these SNPs are shown in table 3 and figure 1. Among the 34 polymorphisms that are already present

Table 1. Primers designed for *IL23R* gene sequencing.

	Primer (forward)	Primer (reverse)	Fragment size (bp)	Optimum temperatures (°C)
Exon 1	ttcaacaaagctggaaaa	agcaacagaagccctaaa	672	60
Exon 2	ttgagttgggaggaccgcttga	tgcatttggaaggagattgggc	750	60.5
Exon 3	atcgcttgaacctggaaggcg	tgtatgaaactaatctaaaaactga	602	59.5
Exon 4	caatgagtgaggcttgatgggca	ctctggctgaactggggtggaa	618	60
Exon 5	gctggagtgaatggcgtgac	tctctaagttgtcttgggcaggtag	621	64
Exon 6	gggagtgaaaggtgggggaaag	ggaaggcaagggttgaaaagca	718	60.5
Exon 7	gcacaccacattttattattgttacc	gcaacctccacttcttgggt	408	60.5
Exon 8	ttcctcctttgagatttacc	aatccacctaagccacttc	489	60.2
Exon 9	tcagacaagccaaatgaagc	cgccgtgtagtaaaggagagaa	779	65.4
Exon 10	tggttctcccacttatcttgaatct	tcttctctgttttctttccccca	631	59.5
Exon 11	tggagggagaaaggaaagttgaa	tatgccacaggaaaacaaggc	1917	60

Table 2. Demographic characteristics of the study populations.

	CD (n = 134)	Control (n = 131)	P
Gender			
Male(%)	86 (64.2%)	90 (67.2%)	> 0.05
Age (yrs)			
Mean + SD	33.7 ± 13.4	35 ± 12.3	> 0.05
Age of diagnosis (yrs)			
Mean + SD	30.3 ± 12.6		

Table 3. Genotype frequencies of *IL-23R* SNPs in Chinese CD cohort.

SNP	Location	Allele (*a*)*A*†	Crohn's disease (*n* = 50) aa	Aa	AA	Sum	Control (*n* = 50) aa	Aa	AA	Sum	*P**	*P***
rs11465756	5′ end	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465757	5′ end	(t)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465758	5′ end	(t)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs35049319	UFR‡ of exon 1	(−)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465759	DFR§	(t)G	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs1884444	Exon 2	(g)T	8 (16%)	22 (44%)	20 (40%)	50	5 (%)	22 (%)	23 (%)	50	0.64	0.37
rs11465770	DFR of exon 2	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465778	UFR of exon 3	(−)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465779	Exon 3	(a)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465780	DFR of exon 3	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465781	DFR of exon 3	(a)G	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465786	UFR of exon 4	(g)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465787	UFR of exon 4	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465788	UFR of exon 4	(c)T	14 (28%)	21 (42%)	15 (30%)	50	5 (10%)	26 (52%)	19 (38%)	50	0.09	**0.02**
rs6687620	UFR of exon 4	(c)T	49 (98%)	1 (2%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50	1.0	1.0
Gly149Arg	Exon 4	(g)A	48 (96%)	2 (4%)	0 (0%)	50	49 (98%)	1 (2%)	0 (0%)	50	0.57	0.57
rs11465797	Exon 5	(c)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs7539625	DFR of exon 6	(g)A	13 (26%)	23 (46%)	14 (28%)	50	11 (22%)	26 (52%)	13 (26%)	50	0.82	0.64
rs11576064	DFR of exon 6	(t)G	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs7539643	DFR of exon 6	(c)T	13 (26%)	23 (46%)	14 (28%)	50	11 (22%)	26 (52%)	13 (26%)	50	0.82	0.64
rs7530511	Exon 7	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs7518660	DFR of exon 7	(g)A	33 (66%)	17 (34%)	0 (0%)	50	30 (60%)	20 (40%)	0 (0%)	50	0.53	0.53
rs11465802	DFR of exon 7	(c)A	33 (66%)	17 (34%)	0 (0%)	50	30 (60%)	20 (40%)	0 (0%)	50	0.53	0.53
rs11465804	DFR of exon 8	(t)G	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465805	DFR of exon 8	(g)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs10789229	UFR of exon 9	(t)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs10889671	UFR of exon 9	(g)A	50 (100%)	0 (0%)	0 (0%)	50	49 (98%)	1 (2%)	0 (0%)	50	1.0	1.0
rs11209026	Exon 9	(g)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465816	DFR of exon 9	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465820	UFR of exon 10	(g)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465821	UFR of exon 10	(c)T	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs10889677	3′ UTR (exon 11)	(c)A	2 (4%)	14 (28%)	34 (68%)	50	4 (8%)	15 (30%)	31 (62%)	50	0.67	0.4
rs12043902	3′ UTR (exon 11)	(g)C	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465828	3′ UTR (exon 11)	(g)A	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		
rs11465827	3′ UTR (exon 11)	(t)G	50 (100%)	0 (0%)	0 (0%)	50	50 (100%)	0 (0%)	0 (0%)	50		

*P**, comparison of all the genotype distribution of every SNP between CD patients and controls (AA vs Aa vs aa); *P***, comparison of genotype frequency of A allele carrier (AA + Aa) of every SNP between CD patients and controls [(AA + Aa) vs aa];
† 'a' represents the ancestry allele; A, represents the mutant allele; A‡ UFR, upstream-flanking region; § DFR, downstream-flanking region.
We screened all 11 exons of the *IL23R* gene, along with the flanking regions by sequencing from 50 CD patients and 50 sex-age-matched controls. There were 35 polymorphisms identified, including one unreported nonsynonymous SNP (p.Gly149Arg, c.445G>A). T allele carrier of *rs11465788* is significantly associated with susceptibility of CD ($P = 0.02$). The p.Arg381Gln *IL23R* variants were absent both in the Chinese CD cases and controls.

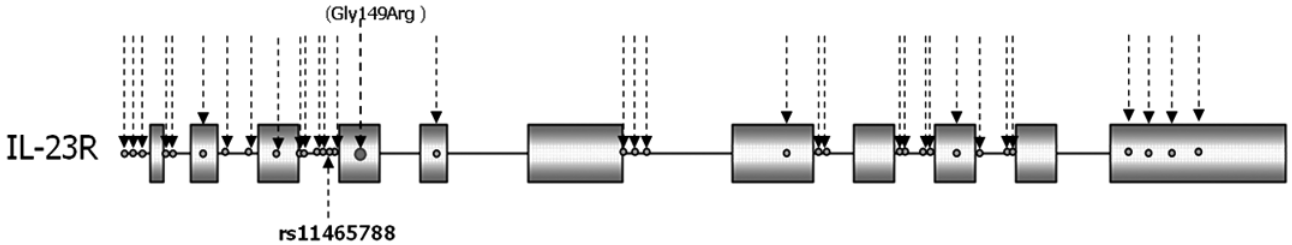

Figure 1. We screened all 11 exons, along with the flanking regions of the *IL23R* gene by direct DNA sequencing from 50 CD patients and 50 sex-age-matched controls. There were 35 polymorphisms identified, including one unreported nonsynonymous SNP located in exon 4 (p.Gly149Arg). Among them, *rs11465788* (C>T) is associated with CD protection in the Chinese Han population.

in the SNP database, except *rs11465788*, all the remaining 33 *IL23R* SNPs failed to show any significant association with CD. SNP *rs11465788* (C > T) occurring in upstream-flanking region of exon 4 is showed to be associated with CD susceptibility. The comparison of *rs11465788* T allele carriers (TT+CT) frequencies between cases and controls revealed significant difference ($P = 0.02$), suggesting that T allele carrier might have protection effects on CD development.

This association was confirmed by the second stage, with 134 CD patients and 131 controls (including the previous 50 CD patients and 50 controls). Frequencies of *rs11465788* T allele carriers were significantly lower in CD patients compared with that of controls (74.6% versus 90.9%, $P = 4.9 \times 10^{-4}$, OR = 0.30, 95%c.i.= 0.15–0.60) (table 4). Phenotypic contribution of the *IL23R rs11465788* variant was then investigated in a well characterized subgroup of CD patients. There is a higher incidence of nonstricturing and nonpenetrating disease behaviour in carriers of T allele (47.8%) compared to CC wild-type carriers (19.4%) ($P = 0.015$, OR = 3.9, 95%c.i. = 1.3–11.5). There are no differences in the frequencies of other disease characteristics such as age at diagnosis, disease location as defined by the Montreal classification (Silverberg *et al*. 2005) as well as 'use of immunosuppressive agents' (table 5). These results demonstrated some implications towards the association of the *IL23R rs11465788* variant with the disease behaviour.

Discussion

We investigated the polymorphisms of *IL23R* and their association with CD in Chinese population. This is the first study screening for all 11 exons and the flanking regions in *IL23R* gene. A total of 35 SNPs were identified, including a novel non-synonymous SNP in exon 4 (p.Gly149Arg, c.445G>A). Among them, SNP *rs11465788*(C>T) in upstream-flanking region of exon 4 was found to be associated with CD susceptibility. Our results suggest that the T allele carriers of

Table 4. Association between *IL-23R rs11465788* SNP and CD in the case–control samples.

IL23R marker	Genotype	CD *n* (%)	Controls *n* (%)	*P*	OR	95% c.i.
rs11465788	T/T	44 (32.8%)	49 (37.4%)			
	T/C	56 (41.8%)	70 (53.4%)			
	C/C	34 (25.3%)	12 (9.1%)	reference	1.00	
	T/T+T/C	100 (74.6%)	119 (90.9%)	4.9×10^{-4}	0.30	0.15–0.60

Table 5. Association between *IL23R rs11465788* genotype and CD disease characteristics ($n = 134$) for which detailed phenotypic data based on the Montreal classification (Silverberg *et al*. 2005) were available.

	(1) TT+CT ($n = 100$)	(2) CC ($n = 34$)	(1) vs (2) *P*	OR (95% c.i.)
Gender ($n = 134$)				
Male (%)	64/100 (64.0%)	22/34 (64.7%)	0.941	
Age at diagnosis ($n = 121$)				
< 16 years (A1)	6/90 (6.7%)	3/31 (9.7%)	0.794	
17–40 years (A2)	69/90 (76.7%)	22/31 (71.0%)	0.675	
> 40 years (A3)	15/90 (16.7%)	6/31 (19.4%)	0.058	
Location ($n = 121$)				
Terminal ileum (L1)	20/90 (22.2%)	11/31 (35.5%)	0.551	
Colon (L2)	18/90 (20.0%)	2/31 (6.45%)	0.210	
Ileocolon (L3)	41/90 (45.6%)	14/31 (45.1%)	0.924	
Upper GI (L4)	11/90 (12.2%)	4/31 (12.9%)	0.083	
Any ileal involvement	61/90 (67.7%)	25/31 (80.6%)	0.173	
Behaviour† ($n = 121$)				
Non-stricturing, Non-penetrating (B1)	43/90 (47.8%)	6/31 (19.4%)	0.015, 3.9 (1.3–11.5)	
Stricturing (B2)	23/90 (25.6%)	12/31 (38.7%)	0.940	
Penetrating (B3)	24/90 (26.7%)	13/31 (41.9%)	0.075	
Use of immunosuppressive agents‡ ($n = 121$)	17/90 (18.9%)	4/31 (12.9%)	0.422	

† Disease behaviour was defined according to the Montreal classification. A stricturing disease phenotype was defined as presence of stenosis without penetrating disease. The diagnosis of stenosis was made surgically, endoscopically, or radiologically. ‡ Immunosuppressive agents included azathiopurine, 6-mercaptopurine, 6-thioguanine, methotrexate, and/or infliximab.

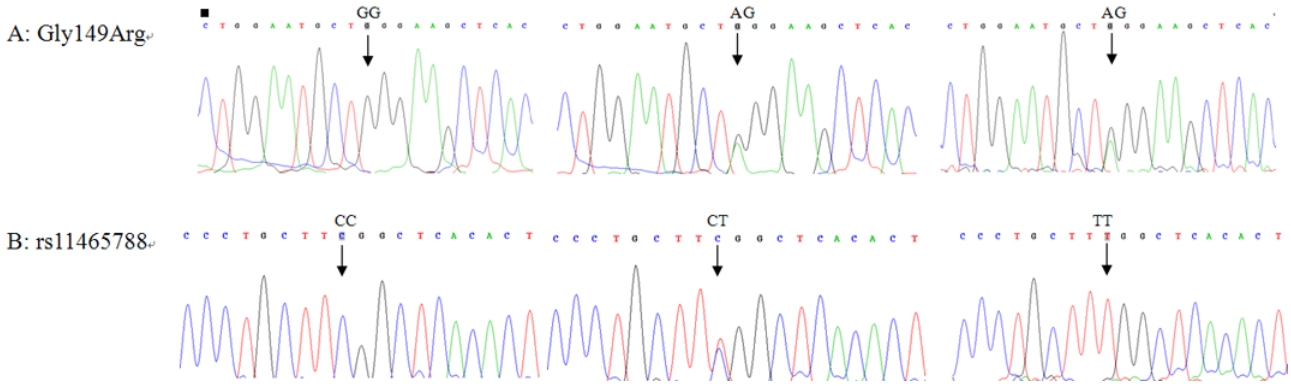

Figure 2. The sequence chromatogram of the novel SNP (p.Gly149Arg, c.445G>A) and the *rs11465788* variant that are associated with susceptibility of CD. (A) G>A transition at codon 149 position that replaces glycine to arginine (p.Gly149Arg). (B) C>T transition of *rs11465788*; the wild-type, heterozygous and homozygous mutant genotypes of *rs11465788* were CC, CT and TT, respectively.

rs11465788 may provide a protective effect against CD. Moreover, genotype–phenotype analysis demonstrated that *rs11465788* T allele carriers were likely to have some effect on clinical feature during the course of disease.

The original study by Duerr *et al*. (2006) analysed only ileal cases of CD and it has been assumed that some of the *IL23R* variants may predispose to ileal disease phenotype. A further study also revealed an interaction of the *IL23R* variant with ileal involvement and stenosis (Glas *et al*. 2007). However, in present study genotype–phenotype analysis revealed that only *rs11465788* T carriers were associated with disease behaviour of 'nonstricturing and nonpenetrating', instead of ileal involvement phenotype. This may indicate different genotype–phenotype interactions in different populations.

Our study also covered three candidate SNPs of *IL23R* identified during the recent genome-wide association and replicated studies, in which *rs11209026* (p.Arg381Gln) had the strongest association to CD (Duerr *et al*. 2006; Baldassano *et al*. 2007; Buning *et al*. 2007; Cummings *et al*. 2007; Dubinsky *et al*. 2007; Roberts *et al*. 2007; Tremelling *et al*. 2007). In another genome-wide association study from Belgium, *rs11465804* was identified as the strongest CD-associated marker (Libioulle *et al*. 2007). These studies not only confirmed *IL23R* as a CD susceptibility gene, but also demonstrated differences for certain IL23R variants regarding the strength of their disease-modifying effects in different populations. However, our study demonstrated p.Arg381Gln *IL23R* variant was absent in both the Chinese CD cases and controls, another two SNPs of *rs11465804* and *rs10889677* did not show any association with Chinese CD cases, a result similar to the large case–control study in Japan CD patients cohort, in which the author failed to replicate the association between the candidate genetic variations of *IL23R* and CD (Yamazaki *et al*. 2007). However, we identified a unique SNP *rs11465788* which was associated with CD susceptibility in Chinese population. These results revealed different genetic pathogenesis of CD among different populations.

There are increasing evidences that the IL23/IL17 axis is the key pathway in the development of chronic inflammation and in host defense against bacterial infection. It promotes local tissue inflammation by stimulating T cells, macrophages and fibroblasts as well as endothelial and epithelial cells to produce proinflammatory cytokines, metalloproteinases, and chemokines (McGovern and Powrie 2007). *IL23R* was also identified as a disease-associated gene in other chronic inflammatory diseases. Treatment with an anti-p40 IL12/23 antibody demonstrated therapeutic efficacy (Mannon *et al*. 2004). Therefore, *IL23R* potentially represents a susceptibility gene and therapeutic target in various chronic inflammatory diseases. Although the *rs11465788* variant is found in the intronic region and does not change the sequence of amino acids, it may modify the structure and function of *IL23R* by altering mRNA splicing and post-transcriptional processes (Tabor *et al*. 2002; Prokunina and Alarcon-Riquelme 2004) which consequently influences the generation and development of inflammation.

In conclusion, the direct sequencing of *IL23R* gene revealed that *IL23R rs11465788* is associated with susceptibility and clinical features of CD in the Chinese population. We identified a novel nonsynonymous SNP (p.Gly149Arg, c.445G>A), showing no association with the disease. Further, larger multi-centre replicated studies are required to confirm our findings.

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