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Polymorphisms of the TNF Gene and Three Susceptibility *Loci* Are Associated with Crohn's Disease and Perianal Fistula Crohn's Disease: A Study among the Han Population from South China

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Background: Although 90 susceptibility *loci* of Crohn's disease (CD) have been confirmed in the Asian population, susceptibility genes for perianal fistula of CD (pCD) in this population remain unknown. This study explored susceptibility genes for CD and pCD in the Han population from South China.

Material/Methods: In total, 490 patients diagnosed with CD between July 2012 and June 2016 at the Sixth Affiliated Hospital of Sun Yat-sen University were included and divided into the CD group (n=240) and the pCD group (n=250). The healthy control group was composed of 260 volunteers. Peripheral blood samples were taken, and single nucleotide polymorphism (SNP) locus sequencing was used to screen for susceptibility *loci*. SNPs were sequenced using matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Results: Nine SNPs in *TNFSF1* on chromosome 9 were associated with CD. Among them, the rs6478106 locus is a risk locus for CD. The distribution frequency of the T allele of the rs6478106 SNP was significantly different between cases and controls (32.49% versus 18.27%, $P<0.001$). Rs72553867, located in the *IRGM* gene on chromosome 5, rs4409764, located in the *NKX2-3* gene on chromosome 10, and rs3731772, located in the *AOX1* gene on chromosome 2, were susceptibility factors for pCD. Nine SNPs located in *TNFSF15* on chromosome 9 were related to CD in Han individuals from Southern China.

Conclusions: The rs6478106 T allele is associated with the risk of CD in the investigated population. SNPs rs72553867 (*IRGM* gene), rs4409764 (*NKX2-3* gene), and rs3731772 (*AOX1* gene) increase the risk of pCD.

MeSH Keywords: Crohn Disease • Fistula • Genes, vif

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2955 9 — 46

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Background

Crohn's disease (CD) is a major component of chronic idiopathic inflammatory disease, which primarily affects the terminal ileum and colon. A prospective, population-based study showed that the incidence rate for CD was as high as 1.09 per 100 000 person-years in China [1]. Although the CD prevalence in China is still lower than that in Western countries, this figure has increased rapidly over the past few decades [2]. The underlying etiology of CD is still undetermined [3], but it has long been thought as a consequence of an inappropriate mucosal immune response to antigenic stimulation from the gut microbiota in a genetically susceptible host [4]. Studies from twins suggested an approximately 50% genetic contribution in CD [5]. Therefore, the identification of the related genetic changes that are implicated in CD susceptibility would provide insights into the etiology of this disorder.

To the best of our knowledge, over 200 single nucleotide polymorphisms (SNPs) in several genes (such as *NOD2/CARD15, NOD1/CARD4*, and *ABCB1*) are related to CD in Western populations [6–9]. However, due to genetic differences, some SNPs failed to show a link to CD in the Asian population [10,11]. For example, mutations within genes from the *NOD2/CARD15, ATG16L1*, and *IL23/Th17* signaling pathways were demonstrated to confer susceptibility to CD only in Western patients and not in Chinese and Japanese patients [12–15]. In addition, studies in the Asian population have revealed some unique SNPs, e.g., c.374T>C of the *DLG1* gene in Chinese patients [16], *ATG16L2* and/or *FCHSD2* in Chinese and South Korean patients [17,18], and SNPs in the *TNFSF15* gene in East Asians [19]. These differences emphasize the importance of identifying population-specific gene variants.

Perianal fistula CD (pCD) is a subtype of CD with poor prognosis and low quality of life. According to population-based studies, the proportion of pCD ranges from 12% to 40% among CD patients, and this prevalence varies according to disease location and disease duration [20]. A European project has revealed that perianal fistula formation in CD patients might be attributed to genes including *IL23R, LOC441108, PRDM1*, and *NOD2* [21]. Another study in the Italian population suggested an association of the SNP rs4958847 in the *IRGM* gene with the susceptibility to pCD [22]. Studies in Dutch, German, and Norwegian populations found an association between rs2165047 in the *DLG5* gene and the *NOD2* haplotype with perianal development [23,24]. Furthermore, rs72796353 in *NOD2* was also reported to be significantly associated with perianal fistula development in cases devoid of SNPs rs2066844, rs2066845, and rs2066847 [25]. Among the Asian population, only 2 studies have screened potentially pathogenic SNPs in CD patients and explored their associations with perianal fistula formation. One recent study was conducted in a Japanese population and found that the AT haplotype in the *TNFRSF1B* gene might promote fistula development [26], while another study in a Korean population revealed the association of the rs4574921 CC genotype within the *TNFSF15* gene with perianal fistula formation [27]. However, susceptibility genes and SNPs have never been assessed in the Chinese population. In addition, to reveal the unique gene variants predisposing patients to pCD, it is important to identify the differences in susceptibility genes and SNPs between non-perianal CD (npCD) patients and pCD patients, which have yet to be evaluated.

Here, we extended previous findings in the Asian population by assessing the association between the CD susceptibility *loci* reported in Asians to Southern Chinese CD patients to clarify the specificity of CD susceptibility genes in the Chinese population and further compare the frequencies of those *loci* between pCD and npCD patients to explore the SNPs conferring susceptibility to pCD in the Chinese population.

Material and Methods

Patients

In total, data pertaining to 490 CD patients diagnosed between July 2012 and June 2016 were collected from the Inflammatory Bowel Disease (IBD) Center in the Sixth Affiliated Hospital of Sun Yat-sen University, including 250 patients with perianal fistula and 240 with non-perianal fistula. The CD diagnostic criteria were based on the Expert Consensus Document of IBD diagnosis and treatment in China, 2012 [28]. Demographic and clinical information, such as age, sex, race, year of diagnosis, disease location and disease behavior, were collected from all patients. CD behavior includes B1 (non-stricturing, non-penetrating), B2 (stricturing), and B3 (penetrating). In total, 260 healthy volunteers were also recruited from Guangzhou Blood Center.

All included patients and controls were of Han ethnicity and were born in Southern China, including the provinces of Guangdong, Guangxi, Fujian, Jiangxi, Jiangsu, Zhejiang, Hunan, Hubei, Sichuan, Chongqing, Yunnan, Hainan, Taiwan, Hongkong, and Macao.

This study obtained approval from the institutional Review Board of the Sixth Affiliated Hospital, Sun Yat-sen University (IRB number: 2017ZSLYEC-017). Written informed consent was obtained from each participant.

Sample collection

Approximately 2 mL of peripheral venous blood was taken from each patient after fasting. The blood sample was centrifuged

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at 1000 rpm for 10 minutes. After serum removal, the sample was stored at –80°C.

Candidate locus determination

We searched the MEDLINE, EMBASE and China National Knowledge Infrastructure (CNKI) databases to identify studies reporting the candidate *loci* and genes implicated in Asian CD patients. Finally, 90 *loci* were identified as risk *loci* candidates for screening among CD patients (Supplementary Table 1).

DNA extraction

DNA was extracted from the peripheral blood leucocytes by standard procedures with a Blood Genomic DNA Isolation Kit (Tiangen, Beijing, China; batch no., DP335). The DNA concentration was determined and then the sample was stored at –20°C.

SNP locus sequencing

Genotyping was performed with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) [29] on the MassARRAY platform (BGI Tech, Beijing, China).

A 4-μL reaction system consisted of PCR buffer with 1.5 mM $MgCl_2$ (0.625 μL), 25 mM $MgCl_2$ (0.325 μL), 25 mM dNTPs (0.1 μL), 500 nM Primer Mix (1.0 μL), 5 U/μL HotStar Taq (0.1 μL), and HPLC grade water (1.85 μL). The system was applied to a 384-well plate. Template DNA at 20 ng/μL (1 μL) was added, and a 1-minute centrifugation at 1000 rpm was performed. The amplification conditions included 94°C for 5 minutes, followed by 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds, 72°C for 1 minute, and 72°C for 3 minutes, with a final holding at 4°C.

Shrimp alkaline phosphatase (SAP) mix at 2.0 μL was prepared, which contained 1.53 μL of HPLC grade water, 0.17 μL of SAP buffer (10x), and 0.3 μL of SAP enzyme (1 U/μL). Excess dNTPs were removed from the reaction system by incubating 5 μL of the reaction with the SAP mix at 37°C for 20 minutes followed by incubating it at 85°C for 5 minutes and then at 4°C until used.

Single-based extension liquid was prepared in a final volume of 2 μL, containing 0.2 μL of iPLEX Buffer Plus (0.222×), 0.2 μL of iPLEX Termination Mix (1×), 0.94 μL of Primer Mix (7 μM: 14 μM), and 0.619 μL of HPLC grade water. The liquid was used to produce 9 μL of the single-based extension reaction system. The system was subsequently subjected to 40 cycles of 94°C for 30 seconds and 94°C for 5 seconds, 5 cycles of 52°C for 5 seconds, 45 cycles of 80°C for 5 seconds and 72°C for 3 minutes, and a final holding step at 4°C. Resin purification was performed. After centrifugation, the products were sampled onto a 384-well SpectroChip (Sequenom, USA) for MALDI-TOF MS. The obtained data were analyzed with TYPER4.0.

Statistical analysis

All analyses were performed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Comparisons of characteristics among cases with and without perianal fistula and controls were made with one-way analysis of variance (ANOVA) or the chi-squared test whichever applicable; post hoc multiple comparisons were performed by Bonferroni correction analyses. Assessment of the genetic equilibrium of two comparison sets (CD patients versus controls; CD patients with or without perianal fistula) was made using the Hardy-Weinberg equilibrium test. Genotype frequency comparisons between the aforementioned sets were performed with the chi-squared test and are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Haploview 4.2 was utilized for the linkage disequilibrium analysis. All tests were 2-sided, and $P<0.05$ was considered significant. The genome-wide association study (GWAS) involved statistical comparisons of hundreds of thousands of SNPs. To maintain a significance level of 0.05, the level of inspection for each comparison must be controlled to a certain extent, and we set a significance level for SNP comparisons at $P<10^{-7}$.

Results

Characteristics

Generally, CD patients were older than the controls. The patients with pCD were older than those with npCD (Table 1). The majority of cases were male in all 3 groups. In addition, higher percentages of patients with npCD than of those with pCD were single and had penetrating CD.

Risk genetic *loci* screening

In total, 90 genetic *loci* among 750 patients were identified (Supplementary Table 1). SNPs satisfying a detection rate >90%, MAF >5 and Hardy-Weinberg equilibrium were further screened, and 70 were obtained for further analysis (Supplementary Table 2).

Correlation analysis of genotypes in CD patients

Single SNP association analysis

The frequency comparison between risk *loci* in CD patients and controls (Supplementary Table 3) indicated that 9 SNPs (rs10114470, rs3810936, rs6478109, rs6478108, rs4263839, rs7848647, rs4246905, rs457492, and rs6478106) were significantly related to CD (all with $P<10^{-7}$ in Bonferroni multiplex

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Table 1. Clinical characteristics of CD patients and healthy controls.

Characteristics	CD with perianal fistula (n=250)	CD without perianal fistula (n=240)	Controls (n=260)	F/χ^2 value	*P* value
Age (years)	32.61±13.32	28.51±9.72	24.34±3.96	52.66	<0.001
Male (n, %)	156 (62.4)	186 (77.5)	174 (66.9)	12.931	0.002
Location (n, %)				2.72	0.26
Ileal±upper	17 (6.8)	26 (10.8)	NA		
Colonic±upper	26 (10.4)	27 (11.3)	NA		
Ileocolonic±upper	207 (82.8)	187 (77.9)	NA		
Behavior (n, %)				58.956	<0.001
B1	63 (25.2)	35 (14.6)	NA		
B2	72 (28.8)	39 (16.3)	NA		
B3	115 (46.0)	176 (73.3)	NA		

CD – Crohn's disease; B1 – non-stricturing, non-penetrating; B2 – structuring; B3 – penetrating; NA – not applicable.

Table 2. CD-related SNPs.

Chromosome no.	dbSNP	Gene	Major allele	Risk allele	Frequency among CD group	Frequency among controls	Allelic test *P*-value	OR	95% CI
9	rs10114470	*TNFSF15*	C	T	0.283	0.566	1.41E-11	0.477	(0.385, 0.593)
9	rs3810936	*TNFSF15*	C	T	0.383	0.565	2.40E-11	0.477	(0.384, 0.594)
9	rs6478109	*TNFSF15*	G	A	0.370	0.539	3.55E-10	0.504	(0.406, 0.625)
9	rs6478108	*TNFSF15*	T	C	0.274	0.543	3.71E-10	0.504	(0.406, 0.625)
9	rs4263839	*TNFSF15*	G	A	0.373	0.541	5.08E-10	0.506	(0.407, 0.628)
9	rs7848647	*TNFSF15*	C	T	0.371	0.539	5.11E-10	0.506	(0.408, 0.628)
9	rs4246905	*TNFSF15*	C	T	0.265	0.429	1.18E-09	0.499	(0.398, 0.625)
9	rs457492	*TNFSF15*	T	C	0.266	0.419	1.55E-09	0.502	(0.400, 0.628)
9	rs6478106	*TNFSF15*	C	T	0.325	0.183	4.29E-09	2.153	(1.939, 3.257)

analysis; Table 2). Among those SNPs, 5 were C>T mutations, 2 were G>A mutations, and the remaining 2 were T>C mutations. All those variants were located in the *TNFSF15* gene on chromosome 9. Rs6478106 was the only SNP that presented correlation with the pathogenicity of CD (OR=2.15, 95% CI 1.94–3.26), while the remaining SNP exhibited protective roles against CD.

We further assessed the genotype distribution of rs6478106 among different age and gender groups. The results indicated that there were no associations of rs6478106 with age (≤30 *vs.* >30, χ^2=0.386, *P*=0.824) or sex (male versus female, χ^2=2.096, *P*=0.351) (Table 3).

Haplotype analysis

Five SNPs (rs4574921, rs6478106, rs10114470, rs3810936, and rs4246905) were found in a 14-kb linkage disequilibrium region (block 1) on chromosome 9, while another 5 SNPs (rs4263839, rs6478108, rs6478109, rs7865494, and rs7848647) were found in a 17-kb linkage disequilibrium region (block 2) on chromosome 9. Haploview analysis showed that the haplotypes CCTTT, TTCCC, CCTCT and TCCTC in block 1 significantly increased the risk of CD (*P*<0.05).

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Table 3. Distribution of rs6478106 genotypes in different ages and sexes.

		Genotypes (n)			χ^2	*P* value
		CC	CT	TT		
Age (years)	≤30	112	106	24	0.386	0.824
	>30	108	115	25		
Sex	Male	77	64	14	2.096	0.351
	Female	143	157	35		

Table 4. Perianal fistula of CD-associated SNP *loci*.

Name	Chr. no	Gene or locus	Major/minor allele	Risk allele	pCD group RAF	CD group RAF	OR (95% CI)	*P* allele
rs72553867	chr5	*IRGM*	C/A	A	0.194	0.125	1.685 (1.188–2.390)	0.003
rs4958847	chr5	*IRGM*	A/G	A	0.688	0.617	1.365 (1.047–1.778)	0.021
rs4409764	chr10	*NKX2-3*	G/T	T	0.558	0.487	1.329 (1.033–1.709)	0.027
rs888208	chr10	*NKX2-3*	A/G	A	0.656	0.588	1.338 (1.032–1.735)	0.028
rs3731772	chr2	*AOX1*	T/C	T	0.681	0.615	1.335 (1.025–1.740)	0.032
rs1292053	chr17	*TUBD1*	G/A	A	0.482	0.417	1.300 (1.010–1.674)	0.041

Chr. – chromosome; RAF – risk allele frequency.

Correlation analysis between genotype distribution and perianal fistula of CD

Correlation analysis between single SNP and perianal fistula of CD

The comparison analysis between pCD and npCD patients (Supplementary Table 4) indicated that rs72553867, rs4958847, rs4409764, rs888208, rs3731772, and rs1292053 were candidate SNPs for susceptibility to CD perianal fistula (adjusted *P*<0.05, according to the Bonferroni test). Rs72553867, rs4958847, rs4409764, rs888208, rs3731772, and rs1292053 are located on chromosomes 5, 5, 10, 2, and 17, respectively. Among these candidates, rs72553867 (OR=1.685, 95% CI 1.188–2.390) and rs4958847 (OR=1.365, 95% CI 1.047–1.778) were found to be located in an *IRGM* coding region on chromosome 5. Rs4409764 (OR=1.329, 95 CI% 1.033–1.709) and rs888208 (OR=1.338, 95% CI 1.032–1.735) were found to be located in the *NKX2-3* gene. Rs3731772 is in the *AOX1* gene (OR=1.335, 95% CI 1.025–1.740), and rs1292053 was in the coding region of *TUBD1* (OR=1.300, 95% CI 1.010–1.674) (Table 4).

Adjusted analysis between single SNPs and perianal fistula of CD

We further added age and gender as covariates to the analysis (Supplementary Table 5) and found that rs72553867 located in the *IRGM* gene on chromosome 5 (OR=1.770, 95% CI 1.151–2.723), rs4409764 located in the *NKX2-3* gene on chromosome 10 (OR=1.886, 95% CI 1.181–3.012) and rs3731772 located in the *AOX1* gene on chromosome 2 (OR=2.131, 95% CI 1.150–3.949) were SNPs that conferred susceptibility to pCD.

Haplotype analysis

Haplotype analysis revealed a 54-kb monomer block in chromosome 5 that contained 4 haplotypes, namely, CTCTAG, TCCCGA, TCACAA, and CTCTAA. Compared with the CTCTAG and CTCTAA haplotypes, haplotypes TCCCGA and TCACAA were associated with pCD (*P*<0.05).

Discussion

This study showed that 9 SNPs (rs10114470, rs3810936, rs6478109, rs6478108, rs4263839, rs7848647, rs4246905, rs4574921, and rs6478106) located in *TNFSF15* on chromosome 9 are related to CD in the Han population from Southern China. Rs6478106 is the only risk SNP associated with CD. Further analysis revealed that rs72553867 (located in *IRGM* on chromosome 5), rs4409764 (located in *NKX2-3* on chromosome 10) and rs3731772 (located in *AOX1* on chromosome 2) increase the risk of pCD.

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TNFSF15 is mainly expressed in endothelial cells and can be induced in myeloid cells after the ligation of TLR and FcR by IgG ICs and the co-stimulation of T cells through the receptor DR3 [30]. Studies have confirmed the upregulated mRNA and protein levels of *TNFSF15* in macrophages and $CD4^+/CD8^+$ lymphocytes in the intestinal lamina propria of CD patients [31]. *TNFSF15* can bind to death domain receptor 3 and provide co-stimulatory signals that activate lymphocytes, inducing IFN-γ secretion and prompting participation in inflammatory responses [32,33]. Therefore, excessive expression of *TNFSF15* can initiate and aggravate mucosal inflammation in CD patients. In European populations, the association of the *TNFSF15* polymorphism with CD susceptibility has been widely reported [34,35]. Rs4979462 and rs7848647 in *TNFSF15* were reported to be related with CD in Korean and Japanese populations [18,27,36]. In China, only 1 study was conducted on the association between *TNFSF15* and CD, and the authors found that the 3 SNPs in *TNFSF15* (rs3810936, rs6478109, rs7848647) were not significantly associated with CD genetic susceptibility and clinical subtypes in the Han population [37], which contrasts with our results that found 9 SNPs (rs10114470, rs3810936, rs6478109, rs6478108, rs4263839, rs7848647, rs4246905, rs4574921, and rs6478106) in *TNFSF15* were related to CD. However, this study had a small sample size (42 CD patients and 49 healthy), which might lead to a limited power to discover significant associations [37]. Consistent with the results in the Japanese population [38], our analyses also indicated that rs6478106 was a susceptibility SNP for CD. Our analysis further revealed that this association had no relationship with age or sex. Therefore, we propose that the genetic variation of *TNFSF15*, especially rs6478106T, is related to an increased risk for CD in China. The genetic variations of *TNFSF15* in this study may provide evidence regarding the etiology of the disease and information that may be important for the development of treatments.

IRGM is widely expressed in various human cells and plays an important regulatory role in intracellular pathogen-associated immunity. IFN-γ can induce the expression of the *IRGM* mouse homologue *LRG-47* and produce auto lysosomes, while the lack of *LRG-47* results in an increased susceptibility to infection [39]. The rs13361189 and rs4958847 *loci* of IRGM were confirmed to be related to CD susceptibility in a large-scale clinical trial [40]. An Italian study showed that the polymorphisms rs1000113 and rs4958847 in the autophagy gene *IRGM* might participate in the pathogenesis of CD and that the polymorphism of rs4958847 was related to fistula behavior [22]. Another study among the Korean population suggested that rs10065172 and rs72553867 are protective factors against the development of CD [41]. Although increasing efforts have been devoted to focusing on the associations of *IRGM* mutations with CD, research on CD susceptibility genes in the Han population remains limited. In the study conducted by Zheng et al., 318 CD patients were examined, but no association between the rs13361189 polymorphism in *IRGM* and CD was observed for the Chinese population [42], consistent with our result. We also found that in addition to rs4958847, the rs72553867 polymorphism was also closely related to the formation of perianal fistula in the Southern Han population. Our results suggested that *IRGM* gene polymorphisms might affect *IRGM* expression and thus alter the severity of intestinal mucositis.

Previous studies indicated the genetic association of *NKX2-3* with pCD. Yu et al. analyzed the mRNA expression and protein level of *NKX2-3* in American patients with familial IBD and found a significant link of *NKX2-3* to CD [43]. Another Japanese study also found that the rs10883365 polymorphism of *NKX2-3* was positively correlated with CD [44]. In addition, a Korean study showed that the rs88208 locus in *NKX2-3* was also associated with CD, whereas studies in the Chinese population had the opposite conclusion [45,46]. In the southern Han population, our study showed a significant relation between the rs4409764 and rs888208 sites of *NKX2-3* and the pathogenesis of pCD. More noteworthy, this study also found that rs3731772 was significantly associated with pCD in the Han population in southern China. The results of our study may provide clues for the function of the *AOX1* gene in patients with fistula CD.

Our study suffered from several potential limitations. First, screening for selected candidate *loci* and genes instead of genome-wide sequencing might lead to missed pathogenic SNPs. However, the selection of our SNP pool was based on multiple related studies that were obtained through a systematic search in MEDLINE and 2 other comprehensive databases in China. Second, we did not perform functional genomics research in this study. Functional analysis is helpful in ascertaining the actual roles of those genes, and our analysis may lay the groundwork for further potential function analyses. Third, although this study is the first confirmative research on susceptibility *loci* associated with perianal fistula CD in the Chinese population, it is preliminary and suffers from a small sample size based on a single center. Thus, the results of this study need to be validated by future multicenter studies with a large sample.

Conclusions

In the Han population from South China, 9 SNPs in *TNFSF15* are related to CD and 3 SNPs located in *IRGM*, *NKX2-3*, and *AOX1* increase the risk of pCD. This study is the first confirmative study on susceptibility *loci* associated with perianal fistula CD in this population, and its results are helpful for the exploration of new disease-associated mechanisms in the future.

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Conflicts of interest

None.

Supplementary Data

Supplementary Table 1. Risk locus candidates for screening among CD patients.

Gene	SNP	Chr	G-position	Allele	Functional consequence
4p14	rs1487630	4	38335823	C>T	Intron variant
ATG16L1	rs2241880	2	234183368	A>G	Missense
ATG16L2	rs11235604	11	72533536	C>T	Missense
ATG16L2-FCHSD2	rs11235667	11	72863697	A>G	
BTNL2	rs28362680	6	32370816	G>A	Intron variant
CARD9	rs200735402	9	139265120	C>T	Missense
CDKAL1	rs6908425	6	20728731	T>C	Intron variant
DEFB1	rs2978880	8	6724306	G>A	Upstream variant 2KB
DNAH12	rs4462937	3	57414434	A>G	Missense
DR4	rs13278062	8	23082971	G>T	Upstream variant 2KB
DR4	rs20575	8	23059324	C>G	Missense
DR5	rs1047266	8	22900701	G>A	Intron variant
DLG1	rs527829647	3	197194534	A>G	Missense
DLG1	rs1134986	3	197138371	C>T	Missense
FUT3	rs28362459	19	5844781	A>C	Missense
FUT3	rs3745635	19	5844332	C>T	Missense
FUT3	rs3894326	19	5843773	A>T	Missense
GPR35	rs3749172	2	241570249	A>C	Missense
HLA-DQA2	rs3208181	6	32713030	T>C	Synonymous codon
IL-23R	rs11209026	1	67705958	G>A	Missense
IL-23R	rs6588248	1	67652984	T>G	Intron variant
IL-23R	rs7517847	1	67681669	T>G	Intron variant
IL-23R	rs1004819	1	67670213	G>A	Intron variant
IL-23R	rs76418789	1	67648596	G>A	Missense
IL-23R	rs11209032	1	67740092	G>A	
IL-27	rs153109	16	28507775	T>C	Intron variant
IRF5	rs2004640	7	128578301	G>T	Intron variant
IRF5	rs3807306	7	128580680	G>T	Intron variant
IRGM	rs10065172	5	150848436	C>T	Synonymous codon
IRGM	rs11741861	5	150898347	A>G	Intron variant
IRGM	rs12654043	5	150846533	A>G	Utr variant 5 prime
IRGM	rs13361189	5	150843825	T>C	
IRGM	rs4958847	5	150860025	G>A	Intron variant
IRGM	rs72553867	5	150848404	C>A	Missense
IRGM	rs9637870	5	150848053	G>A	Utr variant 5 prime

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Gene	SNP	Chr	G-position	Allele	Functional consequence
IRGM	rs9637876	5	150847863	C>T	Utr variant 5 prime
MHC	rs7765379	6	32680928	T>G	
MHC	rs9271366	6	32619077	G>A	
BTNL2	rs10947261	6	32405455	G>T	Intron variant
NFKBIA	rs2273650	14	35870798	C>T	Utr variant 3 prime
NKX2-3	rs10883365	10	101287764	G>A	Nc transcript variant
NKX2-3	rs4409764	10	101284237	T>G	
NKX2-3	rs888208	10	101284237	T>G	
NOTCH4	rs422951	6	32188383	T>C	Missense
PPP5C	rs4802307	19	46346549	G>T	Upstream variant 2KB
PTPN2	rs514000	18	12854073	C>T	Intron variant
PUS10	rs13003464	2	61186829	A>G	Intron variant
PUS10	rs7608910	2	60977721	A>G	Intron variant
RNASET2	rs2149085	6	167371110	T>C	Upstream variant 2KB
SLC22A4	rs1050152	5	132340627	C>T	Intron variant
SLC25A15-ELF1-WBP4	rs7329174	13	41558110	A>G	Intron variant
SMNDC1-DUSP5	rs11195128	10	112186148	C>T	
SOX11	rs11894081	2	5664008	G>T	
STAT3	rs1053004	17	40466092	G>A	Utr variant 3 prime
STAT3	rs9891119	17	40507980	A>C	Intron variant
STAT4	rs7574865	2	191964633	T>G	Intron variant
TBC1D1-KLF3	rs6856616	4	38325036	T>C	
TNF-α	rs1799964	6	31542308	T>C	Downstream variant 500B
TNF-α	rs1800630	6	31542476	C>A	Downstream variant 500B
TUBD1	rs1292053	17	59886176	A>G	Intron variant
TNFSF15	rs10114470	9	117547772	T>C	Utr variant 3 prime
TNFSF15	rs3810936	9	117552885	T>C	Synonymous codon
TNFSF15	rs4263839	9	117566440	A>G	Intron variant
TNFSF15	rs4574921	9	117538334	C>T	
TNFSF15	rs6478106	9	117545666	C>T	
TNFSF15	rs6478108	9	117558703	C>T	Intron variant
TNFSF15	rs6478109	9	117568766	A>G	Upstream variant 2KB
TNFSF15	rs7848647	9	117569046	T>C	Upstream variant 2KB
TNFSF15	rs7865494	9	117576479	C>T	
TNFSF15	rs4246905	9	114790969	T>C	Intron variant
TNFSF8	rs3181374	9	117665187	A>G	Intron variant
USP25	rs2823256	21	16784706	G>A	Intron variant
ZMIZ1	rs1250569	10	81045207	T>C	Intron variant
ZMIZ1	rs1250546	10	79272775	A>G	Intron variant
ZNF365	rs224143	10	64477836	G>A	
	rs1145816	6	91663151	C>T	
LOC105370520	rs1495465	14	58016414	C>A	Upstream variant 2KB
	rs10761659	10	64445564	A>G	

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Gene	SNP	Chr	G-position	Allele	Functional consequence
LOC105379031	rs7702331	5	73255307	A>G	Intron variant
	rs1819333	6	166960059	T>G	
LOC105377139	rs7282490	21	44195858	G>A	Upstream variant 2KB
NDUT15	rs186364861	13	48611934	G>A	Missense
ABCC4	rs3765534	13	95815415	C>T	Missense
AOX1	rs3731772	2	12739259	T>C	
ITPA	rs1127354	20	3193842	C>A	Intron variant, missense
MTHFR	rs1801133	1	11856378	G>A	Missense
GSTP1	rs1695	11	67585218	A>G	Missense
RANTES/CCL5	rs2107538	17	34207780	C>T	Intron variant
CCR5	rs1799987	3	46411935	A>G	Intron variant
CCR5	rs3181036	3	46412559	C>T	Intron variant

Supplementary Table 2. SNPs selected for analysis.

No.	SNP	No.	SNP	No.	SNP	No.	SNP
1	rs1004819	19	rs3765534	37	rs6478108	55	rs1801133
2	rs10065172	20	rs3810936	38	rs7608910	56	rs10883365
3	rs10114470	21	rs514000	39	rs2107538	57	rs1127354
4	rs1053004	22	rs6478109	40	rs3181374	58	rs1250546
5	rs10761659	23	rs2149085	41	rs3894326	59	rs1819333
6	rs11195128	24	rs3749172	42	rs4462937	60	rs2823256
7	rs11741861	25	rs422951	43	rs4958847	61	rs3731772
8	rs13361189	26	rs7282490	44	rs6478106	62	rs4246905
9	rs1799964	27	rs7574865	45	rs7517847	63	rs4574921
10	rs2004640	28	rs888208	46	rs7848647	64	rs72553867
11	rs2241880	29	rs11209032	47	rs7865494	65	rs7702331
12	rs3208181	30	rs11235667	48	rs11235604	66	rs1487630
13	rs3807306	31	rs1292053	49	rs1250569	67	rs1695
14	rs4409764	32	rs1799987	50	rs153109	68	rs1800630
15	rs2273650	33	rs4263839	51	rs3181036	69	rs1047266
16	rs3745635	34	rs6588248	52	rs11894081	70	rs10947261
17	rs7329174	35	rs6908425	53	rs13003464		
18	rs7765379	36	rs9637876	54	rs1134986		

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Supplementary Table 3. Analysis results for 70 SNPs related to CD.

SNP	Chr.	Gene	Frequency among the CD group	Frequency among controls	Allelic test P-value	OR	95% CI
rs10114470	9	*TNFSF15*	0.3834	0.5656	1.41E-11	0.4774	(0.3847, 0.5925)
rs3810936	9	*TNFSF15*	0.3826	0.565	2.40E-11	0.4772	(0.3835, 0.5938)
rs6478109	9	*TNFSF15*	0.3702	0.5386	3.55E-10	0.5035	(0.4058, 0.6247)
rs6478108	9	*TNFSF15*	0.374	0.5425	3.71E-10	0.5039	(0.4061, 0.6252)
rs4263839	9	*TNFSF15*	0.3729	0.5405	5.08E-10	0.5055	(0.4072, 0.6277)
rs7848647	9	*TNFSF15*	0.3714	0.5388	5.11E-10	0.5059	(0.4075, 0.628)
rs4246905	9	*TNFSF15*	0.2645	0.4189	1.18E-09	0.4989	(0.3981, 0.6252)
rs4574921	9	*TNFSF15*	0.2656	0.4189	1.55E-09	0.5015	(0.4003, 0.6285)
rs6478106	9	*TNFSF15*	0.3249	0.1827	4.29E-09	2.153	(1.661, 2.79)
rs11209032	1	*IL-23R*	0.4451	0.5598	2.35E-05	0.6307	(0.5091, 0.7814)
rs6588248	1	*IL23R*	0.3119	0.4066	0.0002632	0.6613	(0.5293, 0.8263)
rs7329174	13	*ELF1*	0.2758	0.1988	0.001074	1.535	(1.186, 1.986)
rs422951	6	*NOTCH4*	0.146	0.2115	0.00125	0.6374	(0.4843, 0.8391)
rs7517847	1	*IL23R*	0.3831	0.4692	0.001257	0.7025	(0.5667, 0.871)
rs13361189	5	*IRGM*	0.5031	0.4205	0.0024	1.395	(1.125, 1.73)
rs10065172	5	*IRGM*	0.5	0.4186	0.002739	1.389	(1.12, 1.722)
rs11235604	11	*ATG16L2*	0.1374	0.08687	0.004163	1.674	(1.173, 2.388)
rs888208	10	*NKX2-3*	0.3776	0.4537	0.004294	0.7304	(0.5886, 0.9064)
rs1487630	4	*4p14*	0.2789	0.2115	0.004379	1.442	(1.12, 1.855)
rs4958847	5	*IRGM*	0.3459	0.4187	0.006374	0.7342	(0.5879, 0.917)
rs9637876	5	*IRGM*	0.4918	0.4189	0.007165	1.342	(1.083, 1.664)
rs11235667	11	*ATG16L2-FCHSD2*	0.1381	0.09073	0.007553	1.606	(1.132, 2.278)
rs11741861	5	*ZNF300*	0.4706	0.4	0.00879	1.333	(1.075, 1.654)
rs1004819	1	*IL23R*	0.3842	0.4514	0.01217	0.7584	(0.6109, 0.9416)
rs10883365	10	*LINC01475*	0.5228	0.4554	0.01347	1.31	(1.057, 1.623)
rs3745635	19	*FUT3*	0.1701	0.1236	0.01765	1.454	(1.066, 1.982)
rs1799987	3	*CCR5*	0.3499	0.4115	0.01857	0.7696	(0.6187, 0.9573)
rs11195128	10	*SMNDC1-DUSP5*	0.1724	0.1269	0.02095	1.433	(1.055, 1.947)
rs514000	18	*PTPN2*	0.4168	0.3546	0.02101	1.301	(1.04, 1.627)
rs4409764	10	*NKX2-3*	0.4765	0.5388	0.02207	0.7792	(0.6292, 0.9649)
rs10947261	6	*BTNL2*	0.335	0.281	0.03279	1.289	(1.021, 1.628)
rs3749172	2	*GPR35*	0.3641	0.3105	0.04015	1.271	(1.011, 1.599)
rs153109	16	*IL27*	0.4074	0.3533	0.04123	1.259	(1.009, 1.57)
rs7765379	6	*MHC*	0.1068	0.07529	0.04907	1.469	(0.9997, 2.159)
rs3181374	10	*TNFSF8*	0.425	0.4764	0.06347	0.8124	(0.6523, 1.012)

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SNP	Chr.	Gene	Frequency among the CD group	Frequency among controls	Allelic test P-value	OR	95% CI
rs3181036	3	*CCR5*	0.168	0.2066	0.0651	0.7757	(0.592, 1.016)
rs1053004	17	*STAT3*	0.3476	0.3923	0.08707	0.8255	(0.6626, 1.028)
rs6908425	6	*CDKAL1*	0.1476	0.1815	0.0897	0.7811	(0.587, 1.039)
rs7865494	9	*TNFSF15*	0.2879	0.249	0.109	1.219	(0.9566, 1.554)
rs7574865	2	*STAT4*	0.3398	0.3813	0.1108	0.835	(0.669, 1.042)
rs13003464	2	*PUS10*	0.05215	0.03475	0.1269	1.528	(0.8833, 2.644)
rs2004640	7	*IRF5*	0.2945	0.2577	0.1319	1.202	(0.9459, 1.528)
rs7282490	21	*LOC105377139*	0.449	0.4884	0.1463	0.8536	(0.6894, 1.057)
rs1250546	10	*ZMIZ1*	0.4213	0.4593	0.16	0.8571	(0.6912, 1.063)
rs1292053	17	*TUBD1*	0.4483	0.4826	0.2055	0.8712	(0.7038, 1.079)
rs10761659	10	*LOC105370520*	0.2162	0.2442	0.2187	0.8537	(0.6635, 1.099)
rs7608910	2	*PUS10*	0.05263	0.03846	0.22	1.389	(0.82, 2.353)
rs2273650	14	*NFKBIA*	0.2684	0.2934	0.3062	0.8835	(0.6968, 1.12)
rs11894081	2	*SOX11*	0.4214	0.3944	0.3205	1.118	(0.897, 1.394)
rs1250569	10	*ZMIZ1*	0.4287	0.4535	0.3584	0.9044	(0.7298, 1.121)
rs2823256	21	*LOC101927745*	0.3126	0.3359	0.3598	0.8992	(0.7162, 1.129)
rs2241880	2	*ATG16L1*	0.3742	0.3514	0.3813	1.104	(0.8845, 1.378)
rs3807306	7	*IRF5*	0.1829	0.166	0.4147	1.125	(0.848, 1.491)
rs72553867	5	*IRGM*	0.1596	0.1757	0.4237	0.8908	(0.671, 1.183)
rs7702331	5	*LOC105379031*	0.1154	0.1293	0.431	0.878	(0.635, 1.214)
rs1134986	3	*DLG1*	0.06302	0.05405	0.4878	1.177	(0.7425, 1.866)
rs1801133	1	*MTHFR*	0.2957	0.2791	0.5012	1.085	(0.8561, 1.374)
rs3894326	19	*FUT3*	0.1447	0.1564	0.5467	0.913	(0.6791, 1.228)
rs2107538	17	*CCL5*	0.3418	0.3295	0.6314	1.057	(0.8432, 1.325)
rs1799964	6	*LTA*	0.1752	0.1846	0.6486	0.9379	(0.7117, 1.236)
rs1800630	6	*LTA*	0.1586	0.1673	0.6617	0.938	(0.7043, 1.249)
rs2149085	6	*RNASET2*	0.3929	0.3833	0.7181	1.041	(0.8362, 1.296)
rs1047266	8	*TNFRSF10B*	0.277	0.2857	0.7208	0.9577	(0.7555, 1.214)
rs3731772	2	*AOX1*	0.3508	0.3417	0.7247	1.041	(0.8319, 1.303)
rs4462937	3	*DNAH12*	0.3931	0.4023	0.7281	0.962	(0.7735, 1.197)
rs1819333	6	*LOC105379031*	0.3909	0.3822	0.7426	1.037	(0.8333, 1.292)
rs1127354	20	*ITPA*	0.1711	0.1647	0.7537	1.047	(0.7862, 1.394)
rs3208181	6	*HLA-DQA2*	0.1245	0.1192	0.7671	1.05	(0.7585, 1.455)
rs1695	11	*GSTP1*	0.1765	0.1712	0.7941	1.038	(0.7837, 1.375)
rs3765534	13	*ABCC4*	0.05183	0.05385	0.8676	0.9605	(0.5981, 1.543)

SNPs are ordered according to *P* values. Chr – chromosome.

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Supplementary Table 4. Analysis results for SNPs related to pCD.

Name	Chr. No.	Gene or locus	Major/minor allele	Risk allele	Case RAF	Control RAF	OR (95% CI)	*P* value allele	*P* value genotype
rs72553867	chr5	*IRGM*	C/A	A	0.194	0.125	1.685 (1.188–2.390)	0.003	0.002[a]
rs4958847	chr5	*IRGM*	A/G	A	0.688	0.617	1.365 (1.047–1.778)	0.021	0.025[c]
rs4409764	chr10	*NKX2-3*	G/T	T	0.558	0.487	1.329 (1.033–1.709)	0.027	0.007[a]
rs888208	chr10	*NKX2-3*	A/G	A	0.656	0.588	1.338 (1.032–1.735)	0.028	0.004[a]
rs3731772	chr2	*AOX1*	T/C	T	0.681	0.615	1.335 (1.025–1.740)	0.032	0.032[a]
rs1292053	chr17	*TUBD1*	G/A	A	0.482	0.417	1.300 (1.010–1.674)	0.041	0.035[c]
rs3894326	chr19	*FUT3*	A/T	A	0.880	0.836	1.438 (1.001–2.064)	0.049	0.045[c]
rs10883365	chr10	*NKX2-3*	A/G	G	0.548	0.496	1.234 (0.957–1.590)	0.105	0.033[a]
rs3181374	chr9	*TNFSF8*	A/G	A	0.687	0.642	1.226 (0.939–1.600)	0.135	0.069[a]
rs11235667	chr11	*ATG16L2-FCHSD2*	A/G	A	0.877	0.844	1.314 (0.914–1.887)	0.139	0.148[c]
rs3749172	chr2	*GPR35*	C/A	A	0.387	0.343	1.209 (0.926–1.577)	0.162	0.055[b]
rs2241880	chr2	*ATG16L1*	A/G	G	0.394	0.355	1.185 (0.915–1.534)	0.199	0.182[b]
rs153109	chr16	*IL27*	T/C	T	0.613	0.573	1.181 (0.914–1.526)	0.204	0.094[a]
rs1800630	chr6	*TNF*	C/A	C	0.857	0.828	1.242 (0.881–1.752)	0.216	0.168[b]
rs11235604	chr11	*ATG16L2*	C/T	C	0.875	0.848	1.256 (0.873–1.805)	0.219	0.225[c]
rs7282490	chr21	*ICOSLG*	G/A	A	0.468	0.429	1.168 (0.907–1.504)	0.228	0.205[b]
rs2107538	chr17	*CCL5*	C/T	T	0.360	0.325	1.168 (0.897–1.522)	0.249	0.239[c]
rs10114470	chr9	*TNFSF15*	C/T	C	0.633	0.598	1.160 (0.897–1.500)	0.259	0.128[a]
rs514000	chr18	*PTPN2*	T/C	T	0.600	0.564	1.160 (0.895–1.501)	0.262	0.106[a]
rs11209032	chr1	*IL23R-IL12RB2*	A/G	G	0.464	0.429	1.149 (0.893–1.478)	0.281	0.275[a]

SNPs are ordered according to *P* values. [a] p value for the dominant model; [b] p value for the regressive model; [c] p value for the additive model. Chr – chromosome; RAF – risk allele frequency

Supplementary Table 5. Logistic analysis results for SNPs related to pCD.

	Risk allele	Univariate		Multivariate	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs72553867[a]	AC+AA *vs.* CC	1.874 (1.246–2.817)	0.003	1.770 (1.151–2.723)	0.009
rs4958847[c]	A *vs.* G	1.366 (1.045–1.786)	0.023	–	–
rs4409764[a]	GT+TT *vs.* GG	1.780 (1.149–2.758)	0.010	1.886 (1.181–3.012)	0.008
rs888208[a]	AG+AA *vs.* GG	2.087 (1.205–3.616)	0.009	–	–
rs3731772[a]	T C+TT *vs.* CC	1.941 (1.099–3.428)	0.022	2.131 (1.150–3.949)	0.016
rs1292053[a]	AG+AA *vs.* GG	1.487 (0.992–2.230)	0.055	–	–
rs3894326[c]	A *vs.* T	1.380 (0.943–2.017)	0.097	–	–
Age (year)		/	/	0.968 (0.951–0.984)	<0.001
Male/Female		/	/	1.608 (1.059–2.442)	0.026

[a] Dominant model; [c] additive model.

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