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

Min Zhang*, Xiaoyan Wang*, Xiaodong Jiang, Xiangling Yang, Chuangyu Wen, Min Zhi, Xiang Gao, Pinjin Hu, Huanliang Liu

* Min Zhang and Xiaoyan Wang contributed equally to this work

Corresponding Authors:
Huanliang Liu, e-mail: liuhuanl@mail.sysu.edu.cn
Pinjin Hu, e-mail: pjhumd@vip.163.com

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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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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 IL23R 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 TNFRSF18 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, Hong Kong, and Macao.

This study obtained approval from the institutional Review Board of the Sixth Affiliated Hospital, Sun Yat-sen University (IRB number: 2017ZLYEC-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...
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 MgCl₂ (0.625 μL), 25 mM MgCl₂ (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 (0.222X), 0.2 μL of iPLEX Termination Mix (1X), 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 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
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, χ²=0.386, P=0.824) or sex (male versus female, χ²=2.096, P=0.351) (Table 3).

### 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/χ² 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)      |            |         |
| Location (n, %)         |                                  |                                     |                 |            |         |
| 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, %)         |                                  |                                     |                 |            |         |
| 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  | T            | C           | 0.383                    | 0.565                   | 2.40E-11             | 0.477  | (0.384, 0.594) |
| 9              | rs6478109 | TNFSF15  | T            | C           | 0.370                    | 0.539                   | 3.55E-10             | 0.504  | (0.406, 0.625) |
| 9              | rs6478108 | TNFSF15  | C            | T           | 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  | T            | C           | 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) |

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. Haplovie analysis showed that the haplotypes CCTT, TTCC, CCTC and TTCTC in block 1 significantly increased the risk of CD (P<0.05).
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 the \textit{IRGM} gene 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 \textit{NKX2-3} gene. Rs3731772 is in the \textit{AOX1} gene (OR=1.335, 95% CI 1.025–1.740), and rs1292053 was in the coding region of \textit{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 \textit{IRGM} gene on chromosome 5 (OR=1.770, 95% CI 1.151–2.723), rs4409764 located in the \textit{NKX2-3} gene on chromosome 10 (OR=1.886, 95% CI 1.181–3.012) and rs3731772 located in the \textit{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 (rs101144407, rs3810936, rs6478109, rs6478108, rs4263839, rs7848647, rs4246905, rs4574921, and rs6478106) located in \textit{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 \textit{IRGM} on chromosome 5), rs4409764 (located in \textit{NKX2-3} on chromosome 10) and rs3731772 (located in \textit{AOX1} on chromosome 2) increase the risk of pCD.
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 NKKX2-3 with pCD. Yu et al. analyzed the mRNA expression and protein level of NKKX2-3 in American patients with familial IBD and found a significant link of NKKX2-3 to CD [43]. Another Japanese study also found that the rs10883365 polymorphism of NKKX2-3 was positively correlated with CD [44]. In addition, a Korean study showed that the rs88208 locus in NKKX2-3 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 rs88208 sites of NKKX2-3 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, NKKX2-3, 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.
Acknowledgement

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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    |                        |
| BTN2            | rs28362680| 6   | 32370816      | G>S    | Intron variant          |
| CDKAL1          | rs66080425| 3   | 20728731      |        |                         |
| DEFB1           | rs2978880| 8   | 6724306       | G>A    | Upstream variant 2KB    |
| Dnah12          | rs4462937| 3   | 57414434      | A>G    | Missense                |
| DR4             | rs13278062| 8   | 23082371      | G>T    | Upstream variant 2KB    |
| DR4             | rs20575  | 8   | 23059324      | C>G    | Missense                |
| DR5             | rs1047266| 8   | 22900701      | G>A    | Intron variant          |
| Dlg1            | rs527829647| 3   | 197194354     | 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                |
| Gpr33           | rs3749172| 2   | 241570249     |        |                         |
| HLA-DQA2        | rs3208181| 6   | 32713030      | T>C    | Synonymous codon        |
| IL-23R          | rs11209026| 1   | 67705958      | G>A    | Missense                |
| IL-23R          | rs6588248| 1   | 67652984      | G>A    | Intron variant          |
| IL-23R          | rs7517847| 1   | 67681669      | T>G    | Intron variant          |
| IL-23R          | rs1004819| 1   | 67670213      | G>A    | Intron variant          |
| IL-23R          | rs7641879| 1   | 67648596      | G>A    | Missense                |
| IL-23R          | rs1120932| 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   | 15088436      | 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     |
| Gene        | SNP        | Chr | G-position | Allele | Functional consequence       |
|-------------|------------|-----|------------|--------|------------------------------|
| IRGM        | rs9637876  | 5   | 150847863  | C>T    | Utr variant 5 prime         |
| MHC         | rs7763759  | 6   | 32680928   | G>C    |                              |
| MHC         | rs9271366  | 6   | 32619077   | G>A    |                              |
| BTN2        | rs10947261 | 6   | 32405455   | G>T    | Intron variant               |
| NFKBIA      | rs2286609  | 14  | 36107079   | G>T    | Utr variant 3 prime         |
| NKK2-3      | rs10883365 | 10  | 101287764  | G>A    | Nc transcript variant        |
| NKK2-3      | rs4409764  | 10  | 101284237  | T>G    |                              |
| NOTCH4      | rs422951   | 6   | 32188383   |        |                              |
| PPP5C       | rs4802307  | 19  | 46346549   | G>T    | Upstream variant 2KB         |
| PTPN2       | rs514000   | 18  | 12854073   | C>T    | Intron variant               |
| PUS10       | rs13003464 | 2   | 5680910    | A>G    |                              |
| PUS10       | rs7608910  | 2   | 60977721   | A>G    | Intron variant               |
| RNASE1      | rs2149085  | 6   | 16737110   | T>C    | Upstream variant 2KB         |
| SLC22A4     | rs1050152  | 5   | 13240627   | C>T    | Intron variant               |
| SLC25A15-ELF1-WBP4 | rs7329174 | 13  | 415589110  | 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>G    | Intron variant               |
| STAT4       | rs7574865  | 2   | 191964633  | T>G    | Intron variant               |
| TBC1D1-KLF3 | rs685656   | 1   | 38325036   | T>G    |                              |
| TFN-        | rs1753964  | 6   | 31542308   | C>T    | Downstream variant 500B     |
| TFN-        | rs1800630  | 6   | 31542476   | C>A    | Downstream variant 500B     |
| TFN-        | rs1229204  | 2   | 59867308   |        | Intron variant               |
| TNF5F15     | rs10114470 | 9   | 117547772  | T>C    | Utr variant 3 prime          |
| TNF5F15     | rs3810936  | 9   | 117552885  | T>C    | Synonymous codon             |
| TNF5F15     | rs42639    | 9   | 117568440  | A>G    | Intron variant               |
| TNF5F15     | rs4579421  | 9   | 117538334  | C>T    | Intron variant               |
| TNF5F15     | rs6478106  | 9   | 117545666  | C>T    | Intron variant               |
| TNF5F15     | rs6478108  | 9   | 117558703  |        |                              |
| TNF5F15     | rs6478109  | 9   | 117568766  | A>G    | Upstream variant 2KB         |
| TNF5F15     | rs7848647  | 9   | 117569046  | T>C    | Upstream variant 2KB         |
| TNF5F15     | rs7865499  | 9   | 117576479  | C>T    | Intron variant               |
| TNF5F15     | rs4246905  | 9   | 114790969  | T>C    | Intron variant               |
| TNF5F8      | rs3181374  | 9   | 117665187  | A>G    | Intron variant               |
| USP25       | rs2823256  | 21  | 16784076   | A>G    | Intron variant               |
| ZMIZ1       | rs1250569  | 10  | 81045207   | T>C    | Intron variant               |
| ZMIZ1       | rs1250546  | 10  | 79272775   | A>G    | Intron variant               |
| ZNF365      | rs224143   | 10  | 64477836   | G>A    | Intron variant               |
| LOC105370520| rs1495465  | 14  | 58016414   | C>A    | Upstream variant 2KB         |
| LOC105370520| rs10761659 | 10  | 64445564   | A>G    |                              |
| Gene       | SNP     | Chr | G-position   | Allele | Functional consequence          |
|------------|---------|-----|--------------|--------|---------------------------------|
| LOC105379031 | rs7702331 | 5   | 73255307     | A>G    | Intron variant                  |
| LOC1053777139 | 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    | Missense                        |
| NDUT15     | rs186364861 | 13  | 48611934     | G>A    | Missense                        |
| ITPA       | rs1127354  | 2   | 3193842      | C>T    | 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  | rs3020181 | 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 | 70  | rs10947261 |
| 18  | rs7765379 | 36  | rs9637876 | 54  | rs1134986 | 70  | rs10947261 |
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) |
| rs7846474 | 9    | TNFSF15| 0.3714                       | 0.5388                   | 5.11E-10             | 0.5059| (0.4079, 0.6285) |
| rs6478106 | 9    | TNFSF15| 0.3249                       | 0.1827                   | 4.29E-09             | 2.153 | (1.661, 2.79)   |
| rs4263838 | 1    | IL-23R | 0.2635                       | 0.4189                   | 9.29E-09             | 0.6613| (0.5293, 0.8263) |
| rs34035106| 1    | IL-23R | 0.2521                       | 0.3546                   | 9.29E-09             | 0.6613| (0.5293, 0.8263) |
| rs1154133  | 2    | IL-23R | 0.3831                       | 0.4692                   | 0.001275             | 0.7025| (0.5667, 0.871) |
| rs7329174  | 13   | ELF1   | 0.2785                       | 0.1988                   | 0.001077             | 1.535 | (1.186, 1.986) |
| rs422951   | 6    | NOTCH4 | 0.146                        | 0.2115                   | 0.001275             | 0.7348| (0.4843, 0.8391) |
| rs5171847  | 1    | IL-23R | 0.3831                       | 0.4692                   | 0.001275             | 0.7025| (0.5667, 0.871) |
| rs13361189 | 5    | IRGM   | 0.5031                       | 0.4205                   | 0.0002632            | 0.6613| (0.5293, 0.8263) |
| rs10065172 | 5    | IRGM   | 0.5                          | 0.4186                   | 0.0002632            | 0.6613| (0.5293, 0.8263) |
| rs11235604 | 11   | ATG16L2| 0.1374                       | 0.08687                  | 0.004163             | 0.674 | (0.1173, 2.388) |
| rs888208   | 10   | NKK2-3 | 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)  |
| rs4958487  | 5    | IRGM   | 0.3459                       | 0.4187                   | 0.006374             | 0.7342| (0.5879, 0.917) |
| rs9637876  | 5    | IRGM   | 0.4981                       | 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                      | 0.000879             | 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                   | 0.001347             | 1.31  | (1.057, 1.623) |
| rs3745603  | 19   | FUT3   | 0.1701                       | 0.1236                   | 0.01765              | 1.454 | (1.046, 1.983) |
| 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   | NKK2-3 | 0.4765                       | 0.5388                   | 0.02207              | 0.7792| (0.6292, 0.9649) |
| rs10947261 | 6    | BTN2L2| 0.335                        | 0.281                    | 0.03279              | 1.289 | (1.021, 1.628) |
| rs3749172  | 2    | GPR35  | 0.364                       | 0.3105                   | 0.04015              | 1.271 | (1.011, 1.599) |
| rs15310989 | 16   | IL7    | 0.2024                      | 0.1222                   | 0.04123              | 1.217 | (1.009, 1.573) |
| 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) |
| 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    | CD81 | 0.0155                       | 0.1185                   | 0.0887               | 0.7811 | (0.5827, 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) |
| rs6908417  | 2    | PUS10| 0.05260                      | 0.03846                  | 0.22                 | 1.389  | (0.82, 2.353) |
| rs2273650  | 14   | NFKBIA| 0.2684                      | 0.2934                   | 0.1302               | 0.8536 | (0.6635, 1.099) |
| 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                   | 0.3958               | 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) |
| rs134986   | 5    | 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| 0.2777                      | 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) |
| rs3765334  | 13   | ABCC4| 0.05183                      | 0.05385                  | 0.8676               | 0.9605 | (0.5981, 1.543) |

SNPs are ordered according to P values. Chr – chromosome.
## 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 |
|-----------------------|----------|---------------|-------------------|-------------|----------|-------------|-------------------|----------------|-----------------|
| rs7253867             | chr5     | IRGM          | C/A               | A           | 0.194    | 0.125       | 1.685 (1.188–2.390) | 0.003          | 0.002           |
| rs4958847             | chr5     | IRGM          | A/G               | A           | 0.688    | 0.617       | 1.365 (1.047–1.778) | 0.021          | 0.025           |
| rs4409764             | chr10    | NKK2-3        | G/T               | T           | 0.558    | 0.487       | 1.329 (1.033–1.709) | 0.027          | 0.007           |
| rs888208              | chr10    | NKK2-3        | A/G               | A           | 0.656    | 0.588       | 1.338 (1.032–1.735) | 0.028          | 0.004           |
| rs3731772             | chr2     | AOX1          | T/C               | T           | 0.681    | 0.615       | 1.335 (1.025–1.740) | 0.032          | 0.032           |
| rs1292053             | chr17    | TUBD1         | G/A               | A           | 0.482    | 0.417       | 1.300 (1.010–1.674) | 0.041          | 0.035           |
| rs3894326             | chr19    | FUT3          | A/T               | A           | 0.880    | 0.836       | 1.438 (1.001–2.064) | 0.049          | 0.045           |
| rs10883365            | chr15    | NKK2-3        | A/G               | G           | 0.548    | 0.496       | 1.234 (0.957–1.590) | 0.105          | 0.033           |
| rs3181374             | chr9     | TNFSF8        | A/G               | A           | 0.687    | 0.642       | 1.226 (0.939–1.600) | 0.135          | 0.069           |
| rs11235667            | chr11    | ATG16L2-FCHSD2| A/G               | A           | 0.877    | 0.844       | 1.314 (0.914–1.887) | 0.139          | 0.148           |
| rs3749172             | chr2     | GPR33         | C/A               | A           | 0.387    | 0.343       | 1.329 (1.033–1.709) | 0.027          | 0.007           |
| rs2241880             | chr2     | ATG16L1       | A/G               | G           | 0.394    | 0.355       | 1.234 (0.957–1.590) | 0.105          | 0.033           |
| rs153109              | chr16    | IL27          | T/C               | T           | 0.613    | 0.573       | 1.234 (0.957–1.590) | 0.105          | 0.033           |
| rs1800630             | chr6     | TNF           | C/A               | C           | 0.857    | 0.828       | 1.242 (0.981–1.672) | 0.126          | 0.168           |
| rs11235604            | chr11    | ATG16L2       | C/T               | C           | 0.875    | 0.848       | 1.256 (0.873–1.805) | 0.219          | 0.225           |
| rs7282490             | chr21    | ICOSLG        | G/A               | A           | 0.468    | 0.429       | 1.242 (0.981–1.672) | 0.126          | 0.168           |
| rs2107538             | chr17    | CCL5          | C/T               | T           | 0.360    | 0.325       | 1.242 (0.981–1.672) | 0.126          | 0.168           |
| rs10114470            | chr9     | TNFSF15       | C/T               | C           | 0.633    | 0.598       | 1.242 (0.981–1.672) | 0.126          | 0.168           |
| rs514000              | chr18    | PTPN2         | T/C               | T           | 0.600    | 0.564       | 1.242 (0.981–1.672) | 0.126          | 0.168           |
| rs11209032            | chr1     | IL23R-IL12RB2 | A/G               | G           | 0.464    | 0.429       | 1.242 (0.981–1.672) | 0.126          | 0.168           |

SNPs are ordered according to P values. *p value for the dominant model; **p value for the regressive model; ***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 |
| rs7253867*      | 1.874 (1.246–2.817) | 0.003 | 1.770 (1.151–2.723) | 0.009 |
| rs4958847       | 1.366 (1.045–1.786) | 0.023 | – | – |
| rs4409764*      | 1.780 (1.149–2.758) | 0.010 | 1.886 (1.181–3.012) | 0.008 |
| rs888208*       | 2.087 (1.205–3.616) | 0.009 | – | – |
| rs3731772*      | 1.941 (1.099–3.428) | 0.022 | 2.131 (1.150–3.949) | 0.016 |
| rs1292053*      | 1.487 (0.992–2.230) | 0.055 | – | – |
| rs3894326*      | 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 |

*Dominant model; **additive model.
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