PTPN2 rs1893217 single-nucleotide polymorphism is associated with risk of Behçet’s disease in a Chinese Han population


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Results. PTPN2 rs1893217 was associated with risk of developing BD (χ²=10.01, p=0.040), while the PTPN2 rs2542151 genotype had a weak association in basic genotype analysis (χ²=7.49, p=0.024), but it could not withstand the strongest Bonferroni correction (p=0.14). In contrast, PTPN2 rs2847297 and rs7234029 SNPs and ICOSLG rs2838519 and rs762421 SNPs using a Sequenom MassArray system.

Methods. A total of 407 BD patients and 679 ethnically matched healthy controls were recruited for genotyping of PTPN2 rs1893217, rs2542151, rs2847297 and rs7234029 SNPs and ICOSLG rs2838519 and rs762421 SNPs using a Sequenom MassArray system.

Introduction. Behçet’s disease (BD) is a rare, chronic, relapsing, systemic, immune-mediated vasculitis and the etiology remains to be defined. This study investigated single-nucleotide polymorphisms (SNP) of tyrosine-protein phosphatase non-receptor type 2 (PTPN2) and inducible T-cell co-stimulator-ligand gene (ICOSLG) in Chinese Han BD patients and healthy controls because SNPs of these two genes are associated with risk of developing other auto-inflammation diseases.

ABSTRACT  
Objective. Behçet’s disease (BD) is a rare, chronic, relapsing, systemic, immune-mediated vasculitis and the etiology remains to be defined. This study investigated single-nucleotide polymorphisms (SNP) of tyrosine-protein phosphatase non-receptor type 2 (PTPN2) and inducible T-cell co-stimulator-ligand gene (ICOSLG) in Chinese Han BD patients and healthy controls because SNPs of these two genes are associated with risk of developing other auto-inflammation diseases.

Methods. A total of 407 BD patients and 679 ethnically matched healthy controls were recruited for genotyping of PTPN2 rs1893217, rs2542151, rs2847297 and rs7234029 SNPs and ICOSLG rs2838519 and rs762421 SNPs using a Sequenom MassArray system.

Results. PTPN2 rs1893217 was associated with risk of developing BD (χ²=10.01, p=0.040), while the PTPN2 rs2542151 genotype had a weak association in basic genotype analysis (χ²=7.49, p=0.024), but it could not withstand the strongest Bonferroni correction (p=0.14). In contrast, PTPN2 rs2847297 and rs7234029 SNPs and ICOSLG rs2838519 and rs762421 SNPs did not correlate with BD risk. Moreover, logistic analysis with the additive, dominant and recessive genetic models did not reveal any statistical difference between BD cases and controls (p>0.05). In addition, associations were observed between the two SNPs (rs1893217, rs2542151) and the patients with gastrointestinal involvement (p=0.027, p=0.032, respectively).

Conclusion. PTPN2 variant rs1893217 was associated with risk of BD development in a Han Chinese population. Further study will confirm this finding and investigate the role of PTPN2 in development of BD.

Key words. Behçet’s disease, PTPN2, ICOSLG, single nucleotide polymorphisms, Han Chinese

Competing interests: none declared.
courses and symptoms (e.g., abdominal tenderness, bloating, and generic abdominal discomfort that closely mimics irritable bowel syndrome) similar to IBD including Crohn’s disease (CD) and ulcerative colitis (UC). Intestinal BD usually affects the ileocecal region and the colon (37), which makes it difficult for differentiation of diagnosis between BD and CD (18). In addition, both BD and CD are immune-mediated diseases and they may share certain genetic susceptibility. A previous GWAS identified SNPs of tyrosine-protein phosphatase non-receptor type 2 (PTPN2) and inducible T-cell co-stimulator-ligand gene (ICOSLG) as the common gene predisposing variants for CD (19, 20); thus, we investigated SNPs of these two genes in BD patients and compared them to healthy controls to evaluate the risks of BD development.

PTPN2 protein is a member of the protein tyrosine phosphatase (PTP) family and regulates a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation (21). PTPN2 SNPs are associated with risks of developing CD, UC, rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA) and type 1 diabetes mellitus (T1D) (19, 22-31), all of which are autoimmune and inflammatory diseases (21). Moreover, ICOSLG, a ligand for inducible T-cell co-stimulator (ICOS), is expressed on antigen presenting cells, like B-lymphocytes and dendritic cells (32). Binding of ICOSLG to ICOS activates T memory lymphocytes and promotes differentiation of B-lymphocytes and formation of memory B-lymphocytes and production of Th2 cytokine, especially IL-10 (32). Blockage of the ICOS/ICOSLG pathway ameliorates collagen type II-induced arthritis, and decreases cytokine secretion and IgG production (33). Alteration of ICOS expression is involved in formation of memory B-lymphocytes and plasma cells in systemic lupus erythematosus (SLE) patients (34). Thus, we analysed SNPs of PTPN2 (rs1893217, rs2542151, rs2847297 and rs7234029) and ICOSLG (rs2838519 and rs762421) for their association with risk of BD development.

**Materials and methods**

**Study population**

This study was approved by the Ethics Committee of Peking Union Medical College Hospital. A total of 407 BD patients and 679 healthy controls were recruited from Peking Union Medical College Hospital (Beijing, China) between May 2010 and April 2012. All participants were self-reported as Han Chinese and unrelated. The BD patients were diagnosed according to criteria of the International BD Study Group (35), i.e., presence of oral ulceration plus any two of genital ulceration, typical defined eye lesions, typical defined skin lesions, or a positive pathergy test. The healthy controls were enrolled during their physical examinations from the same hospital; these participants were healthy and did not have any autoimmune disorder or family history of BD. All participants signed a written informed consent form to participate in this study.

**DNA extraction and genotyping**

A 2-mL blood sample was collected in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube from each participant. Genomic DNA was extracted from the peripheral blood cells using a kit from Bioteke (Beijing, China) according to the manufacturer’s instructions. DNA concentration was determined by using a Nanodrop machine (Thermo, Wilmington, DE) with the 260/280 nm ratio and diluted to a working concentration of 15–20 ng/μL. The SNPs of PTPN2 and ICOSLG genes were genotyped using a Sequenom MassArray system (Sequenom iPLEX assay, San Diego, CA) following the manufacturer’s instructions. Briefly, both polymerase chain reaction (PCR) and single-base extension primers for PTPN2 (rs1893217, rs2542151, rs2847297 and rs7234029) and ICOSLG (rs2838519 and rs762421) were designed by the MassArray Assay Design 3.0 (Sequenom). After multiplex PCR amplifications, the products were used for locus-specific single-base extension reactions and the final products were desalted and transferred on to a 384-element SpectroCHIP array. Allele detection was conducted by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). The mass spectrometry data were analysed using the MassArray Typer 4.0 software (Sequenom). Duplicate samples, negative controls, and blank controls were included on the plates to ensure the accuracy of genotyping.

**Selection of SNPs**

Considering the vital effects of PTPN2 and ICOSLG in immune-mediated diseases, 6 SNPs of them, which had shown in a positive association with CD or other autoimmune disease based on GWAS and candidate gene association studies, were selected for further analysis (Table II).

**Statistical analysis**

Statistical analyses were performed using PLINK 1.07 software (Shaun Purcell, Boston, USA). The Hardy-Weinberg equilibrium (HWE) in healthy controls was assessed using a chi-squared test.

**Table I. Characteristics of BD patients and healthy controls.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BD patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Numbers</td>
<td>407</td>
<td>679</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>1.24</td>
<td>1.16</td>
</tr>
<tr>
<td>Average age</td>
<td>38.0±12.44</td>
<td>38.81±10.45</td>
</tr>
<tr>
<td>Clinical symptom [no./total (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral aphthous ulcer</td>
<td>401/407 (98.5)</td>
<td>0</td>
</tr>
<tr>
<td>Genital ulcers</td>
<td>310/407 (76.2)</td>
<td>0</td>
</tr>
<tr>
<td>Skin manifestations</td>
<td>239/407 (58.7)</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal involvement*</td>
<td>116/407 (28.5)</td>
<td>0</td>
</tr>
<tr>
<td>HLA-B5 positive**</td>
<td>62/384 (16.1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>68/128 (53.1)</td>
<td>--</td>
</tr>
</tbody>
</table>

*only 384 cases were available; **only 128 cases were available.*
PTPN2 SNP associated with Behçet’s disease / Z. Wu et al.

Table II. SNPs analysed in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNPs</th>
<th>Position(hg19)</th>
<th>Associated diseases</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTPN2</td>
<td>rs2542151</td>
<td>chr18:12779947</td>
<td>CD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>UC</td>
<td>19.28,29</td>
</tr>
<tr>
<td></td>
<td>rs1893217</td>
<td>chr18:12809340</td>
<td>JIA</td>
<td>22.28</td>
</tr>
<tr>
<td></td>
<td>rs7234029</td>
<td>chr18:12877060</td>
<td>TID</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>rs2847297</td>
<td>chr18:12797694</td>
<td>CD</td>
<td>15.29</td>
</tr>
<tr>
<td></td>
<td>rs2838519</td>
<td>chr21:45615023</td>
<td>JIA</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>rs762421</td>
<td>chr21:45615561</td>
<td>TID</td>
<td>31</td>
</tr>
</tbody>
</table>

Table III. Allele and genotype distributions of PTPN2 and ICOSLG in cases and controls.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNPs</th>
<th>Groups</th>
<th>Allele (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>Genotype (%)</th>
<th>( \chi^2 )</th>
<th>p</th>
<th>p&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTPN2</td>
<td>rs2542151</td>
<td>cases</td>
<td>135 (16.6)</td>
<td>679 (83.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>192 (14.1)</td>
<td>1166 (85.9)</td>
<td>1.21</td>
<td>(0.95-1.53)</td>
<td>0.12</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs1893217</td>
<td>cases</td>
<td>139 (17.1)</td>
<td>675 (82.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>190 (14.0)</td>
<td>1168 (86.0)</td>
<td>1.27</td>
<td>(1.00-1.67)</td>
<td>0.05</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2847297</td>
<td>cases</td>
<td>268 (32.9)</td>
<td>546 (67.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>403 (29.7)</td>
<td>955 (70.3)</td>
<td>1.16</td>
<td>(0.96-1.40)</td>
<td>0.11</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs7234029</td>
<td>cases</td>
<td>244 (30.0)</td>
<td>570 (70.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>378 (27.8)</td>
<td>980 (72.2)</td>
<td>1.11</td>
<td>(0.92-1.34)</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICOSLG</td>
<td>rs2838519</td>
<td>cases</td>
<td>362 (44.5)</td>
<td>452 (55.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>628 (46.2)</td>
<td>730 (53.8)</td>
<td>0.93</td>
<td>(0.78-1.11)</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs762421</td>
<td>cases</td>
<td>411 (50.5)</td>
<td>403 (49.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>653 (48.1)</td>
<td>705 (51.9)</td>
<td>1.10</td>
<td>(0.93-1.31)</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test for these six SNPs. A SNP would be excluded from subsequent analyses if there was significant deviation from the HWE (i.e. \( p<0.05 \)) in the control population. Allele and genotype distributions between cases and controls were analysed using a chi-squared test with a two-tailed significance threshold of 0.05. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated and \( p \)-values were corrected for multiple comparisons by the Bonferroni method (\( p_c \)) (36).

**Results**

**Characteristics of study subjects**

In this study, we successfully genotyped 407 BD patients and 679 healthy controls with an average call rate of more than 99%. The cases and controls were well matched in age and sex ratio (Table I). Briefly, the average age and male/female ratio of BD patients were 38.02±12.44 years and 1.24, respectively, and those of the controls were 38.81±10.45 years and 1.16, respectively. Moreover, these six SNPs of PTPN2 and ICOSLG did not show any deviation from HWE (\( p>0.05 \)) in controls; thus, the data were suitable for further comparisons. The power analysis revealed more than 80% power (\( \alpha=0.05 \)) for detecting association with an OR of 1.00–1.56 for both heterozygotes and homozygotes, based on an assumption of 0.25% BD prevalence in a Chinese population and 0.51 for the risk allele frequency, similar to the allele frequencies of the tested SNPs in studies of Asian population (37).

PTPN2 rs1893217 SNP associated with developing BD

We analysed PTPN2 SNPs (rs1893217, rs2542151, rs2847297 and rs7234029) in both cases and controls and then associated these SNPs with risk of BD development. We found that the PTPN2 rs1893217 genotype was associated with BD development (\( \chi^2=10.01, p_c=0.040 \)). Allele frequency analysis failed to reach the statistical significant threshold, though the frequency of C allele of PTPN2 rs1893217 was higher in the cases than in controls (17.0% vs. 14.1%). Moreover, the GT genotype of PTPN2 rs2542151 was more frequent in BD than that of controls (29.7% vs. 22.7%), but the trend was not statistically significant after Bonferroni correction (\( p_c=0.14 \)). In addition, PTPN2 rs2847297 and rs7234029 were not associated with BD (\( p_c>0.05 \); Table III). Logistic analysis with three genetic models (i.e. additive, dominant and recessive) also did not reveal any statistically significant difference in the genotypic distribution of PTPN2 in this Chinese Han population (\( p_c>0.05 \); Table IV).

**Table II.** SNPs analysed in this study.

**Table III.** Allele and genotype distributions of PTPN2 and ICOSLG in cases and controls.

\( p_c \): \( p \)-value corrected by the Bonferroni method (36); NS: not significant.
ICOSLG SNPs not associated with risk of BD development

We then detected ICOSLG SNPs (rs2838519 and rs762421) in this study population. Our data showed that neither genotype nor allele frequencies of these two ICOSLG SNPs were statistically different between BD and healthy controls ($p>0.05$; Table III). Moreover, logistic analysis with three genetic models (i.e., additive, dominant and recessive) also did not reveal any statistical difference between BD patients and the controls ($p>0.05$; Table IV).

**Correlation of PTPN2, ICOSLG SNPs and the sub-phenotypes of BD**

It had also been performed that the association analysis between PTPN2, ICOSLG SNPs and clinical sub-phenotypes of BD. The BD patients were classified by clinical characteristics, like oral aphthous ulcer, genital ulcers, skin manifestations, ocular manifestations and gastrointestinal involvement. ICOSLG SNPs (rs2838519, rs762421) were not associated with any clinical sub-phenotypes of BD in our study. And the same trend was also found for PTPN2 (rs2847297, rs7234029). While PTPN2 (rs1893217, rs2542151) were associated with BD patients with gastrointestinal involvement ($p_{add}=0.027$, $p_{dom}=0.032$, respectively; Table V).

**Discussion**

Due to unknown etiology, it has long been controversial to correctly classify BD. For example, BD has been considered a seronegative spontaneous arthritis, autoimmune disease or auto-inflammatory disorder (38). To date, knowledge shows that BD is an immune-mediated disease since BD sometimes is difficult to distinguish from CD due to similarities in clinical manifestations and treatment options (17), indicating BD may share certain genetic alterations with CD. Indeed, our current study investigated SNPs of PTPN2 and ICOSLG in BD patients and healthy controls and found that PTPN2 rs1893217 was associated with BD and PTPN2 rs1893217, rs2542151 were also associated with BD patients with gastrointestinal involvement, whereas other PTPN2 SNPs and two ICOSLG SNPs did not share a linkage with BD risk or any subgroups of BD. Future studies will further verify this finding and investigate the role of PTPN2 in development and severity of BD.

BD affects small-vessels to induce vasculitis; produces mucous membrane ulceration in the oral cavity, genitals, eyes, skin, gastrointestinal tract; and causes brain and ocular problems. In contrast, CD is an inflammatory bowel disease that may affect any part of the gastrointestinal tract from the mouth to anus, but may also cause complications outside the gastrointestinal tract, such as skin rashes, arthritis, inflammation of the eye and tiredness (39). In general, extra-intestinal manifestations, like oral and genital ulcers, are more frequent in intestinal BD than those in CD. Intestinal BD most frequently affects the ileocecal region, transverse colon and ascending colon could also be involved, and so is the oesophagus. And it rarely affects the anus. However, perianal region and small intestine are more common affected in CD. Intestinal ulcers in BD are round/oval or geographic in shape, and well-demarcated (focal distribution), whereas those in CD tend to be longitudinal lesions with a cobblestone appearance (segmental and diffuse distribution). Also, granuloma (in mucosal biopsy) could be used to distinguish intestinal BD from CD (40). A recently published research had found that there were no significant differences in the long-term clinical outcomes of CD and intesti-

| Table IV. Logistic regression analyses based in three genetic models. |
|------------------|------------------|------------------|------------------|
| Gene  | SNPs | Additive model | Dominant model | Recessive model |
|       |      | $p$   | OR (95% CI) | $p$   | OR (95% CI) | $p$   | OR (95% CI) |
| PTPN2 | rs2542151 | 0.75 | 1.21 (0.95-1.53) | 0.20 | 1.34 (1.02-1.76) | NS | 1.04 (0.87-1.26) |
|       | rs1893217 | 0.32 | 1.26 (1.00-1.60) | 0.06 | 1.43 (1.09-1.87) | NS | 1.12 (0.74-1.71) |
|       | rs2847297 | 0.66 | 1.17 (0.97-1.41) | 0.47 | 1.25 (0.97-1.60) | NS | 1.34 (0.85-2.10) |
|       | rs7234029 | NS  | 1.11 (0.92-1.35) | NS  | 1.09 (0.85-1.40) | NS  | 1.15 (0.86-1.53) |
| ICOSLG | rs2838519 | NS  | 0.93 (0.78-1.11) | NS  | 0.96 (0.73-1.26) | NS  | 0.84 (0.61-1.15) |
|       | rs762421 | NS  | 1.10 (0.93-1.31) | NS  | 1.13 (0.85-1.50) | NS  | 1.15 (0.86-1.53) |

$p_{add}$: $p$-value corrected by the Bonferroni method (36); NS: not significant.

| Table V. Association of the six SNPs with the sub-phenotypes of BD. |
|------------------|------------------|------------------|------------------|
| Genes  | SNPs | GI+ vs. GI- (n=62 vs. 322) | GI+ vs. Control (n=62 vs. 679) | GI- vs. Control (n=322 vs. 679) |
|       |      | $p$   | OR (95% CI) | $p$   | OR (95% CI) | $p$   | OR (95% CI) |
| PTPN2 | rs2542151 | 0.17 | 1.68(1.05-2.68) | 0.032 | 1.86(1.20-2.90) | NS | 1.11(0.85-1.44) |
|       | rs1893217 | 0.38 | 1.55(0.97-2.47) | 0.027 | 1.89(1.21-2.94) | NS | 1.22(0.94-1.58) |
|       | rs2847297 | 0.37 | 1.46(0.98-2.16) | 0.08 | 1.60(1.10-2.34) | NS | 1.10(0.90-1.35) |
|       | rs7234029 | 0.60 | 1.40(0.94-2.10) | 0.27 | 1.48(1.00-2.17) | NS | 1.06(0.86-1.30) |
| ICOSLG | rs2838519 | NS  | 0.82(0.55-1.21) | NS  | 0.79(0.54-1.15) | NS  | 0.96(0.80-1.16) |
|       | rs762421 | 0.84 | 1.34(0.91-1.97) | 0.46 | 1.40(0.91-2.02) | NS  | 1.04(0.87-1.26) |

$p_{add}$: $p$-value corrected by the Bonferroni method (36); NS: not significant.
nal BD, though CD patients tended to take more corticosteroid or immunosuppressant therapy than BD patients (41). At the molecular level, CD is associated with immune system disorder. Thus, *PTPN2* and *ICOSLG* SNPs are associated with CD risk (42). However, it is true that genetic factors do have indispensable risks in the initiation and improvement of BD and CD (4, 43). For example, previous studies confirmed that *IL10* and *IL23R* are associated with risk of these two diseases (6, 7, 12-15). SNP rs1800871 in the *IL10* promoter region and SNP rs1518111 in the *IL10* intron are associated with BD (6, 7, 12), while SNP rs3024505 of this gene is associated with susceptibility to CD and UC (13, 15). Moreover, *IL23R* SNP rs11209026 is correlated with CD and UC (14, 15), whereas *IL23R-IL12RB2* SNPs rs1495965 and rs924080 and *IL23R* SNP rs11209032 are associated with BD (6, 7, 12). Since *IL10* spans 4,892 bp and consists of 5 exons and 4 introns, it contains 913 SNPs, while *IL23R* consists of 11 exons and 10 introns spanning 93,481 bp with a total of 9,018 SNPs (45). Different SNPs in these two genes may have similar effects, altering expression and activity of the genes; but further study is needed to clarify. However, our current study on six SNPs of *PTPN2* and *ICOSLG* showed that just two SNPs of *PTPN2* were associated with risk of BD or intestinal BD, indicating that pathogenesis of BD may be different from CD. To support this notion, a previous study from Turkey strived to confirm a CD’s risk gene (*CARD15/NOD2*) in a cohort of 85 BD patients and 100 healthy controls, but there were no associations found (46).

*PTPN2* rs1893217 was mapped to *PTPN2* intron 7, and shared a linkage with CD and other immune-mediated diseases, such as UC, JIA and T1D (15, 25, 27). In this study, we found that *PTPN2* rs1893217 was associated with a risk of BD and intestinal BD. *PTPN2* rs2542151 was localised at 5.5 kb upstream of the *PTPN2* transcriptional start site and had a strong linkage disequilibrium (LD) with rs1893217 (r²=1), (45), and it was a risk factor for UC, CD, and T1D (19, 22, 26). A study based in Chinese Han had confirmed that *PTPN2* rs2542151 was associated with UC, but not CD (47); other SNPs of *PTPN2* and *ICOSLG* had not been confirmed in Chinese IBD so far. Moreover, our current study showed that the GT genotype of *PTPN2* rs2542151 was more frequent in BD than that of controls (29.7% vs. 22.7%), but the trend was not statistically significant after Bonferroni correction (p_{adj}=0.14). The Bonferroni correction, the most stringent one, was conducted to avoid false positive loci. It was commonly used in GWAS, while the SNPs were in local LD, it may be too conservative to have some nominal association polymorphisms be overcorrected (48), which may explain why rs1893217 was associated with BD in the genotype frequency analysis; while rs2542151, shared strong LD (r²=1) with rs1893217, was only associated with intestinal BD in the subgroup analysis. The other possible reason for this could be attributed to the relative small sample size of our study making it underpowered to find out those marginally associated SNPs. *PTPN2* (rs1893217 and rs2542151) was associated with BD or intestinal BD, which implied that, the possible overlaps in the susceptibility loci for CD and intestinal BD. The other two *PTPN2* SNPs (rs7234029 and rs2847297) were identified with RA, JIA and CD (23, 24, 28) – did not show any associations with BD in our current study. Rs2847297 was not in strong LD with rs1893217 and rs2542151 (r²=0.60, r²=0.60, respectively), so was rs7234029 (r²=0.60, r²=0.45, respectively) (45). Furthermore, our current study did not find any association of the two SNPs of *ICOSLG* with risk of BD, although previous GWAS studies did show the association with risk of CD and UC (19, 20, 22). These data further suggested that BD pathogenesis was different from other autoimmune diseases, like CD or UC. However, in the current study, we observed a correlation between risk of BD and the CD-associated SNPs of *PTPN2*, but we did not analyse other gene SNPs that were related to immune reactions or diseases. Until now, no studies about *PTPN2* in BD have been conducted. Only one study also came from China, strived to search out the genetic links between *PTPN22* and BD (49). Indeed, T cell protein tyrosine phosphatase (TCP-PTP), the product of *PTPN2*, is a classic non-receptor protein tyrosine phosphatase, whose substrates are epidermal growth factor receptor (EGFR), signal transducers and activators of transcription (STATs), and insulin receptors. TCP-PTP can negatively regulate proinflammatory cytokine (interferon-gamma [IFN-γ]) and has indispensable roles in regulation of immune responses (50). Also, TCP-PTP is a negative regulator of TCR, mediated by SFK, which supports the association of *PTPN2* with several autoimmune and inflammatory diseases (21). As TC-PTP is a tyrosine phosphatase, it could regulate TNF-induced MAPK signaling, but not the NF-κB pathway. Interacting with the adaptor protein – TRAF2 – it could inactivate the Src tyrosine kinases to suppress downstream kinases and the production of IL-6 (51). In animal experiments, TC-PTP−/− mice developed progressive systemic inflammatory diseases and the TC-PTP deficiency resulted in synovitis with several inducers of inflammatory arthritis (52, 53). In addition, TCP-PTP was overexpressed in the intestinal biopsy specimens of CD patients, indicating that TC-PTP may help to maintain the intestinal epithelial barrier. TC-PTP can also regulate autophagosome formation in the intestinal cells and trigger the chronic intestinal inflammation (50). Although rs1893217 is localised at the intron of *PTPN2*, it was identified that the C allele could decrease IL-2R signaling in CD4+ T cells, mediated by the phosphorylation of STAT5 (54). Also, it could activate MAPK, resulting in elevated mRNA expression of the T-bet transcription factor and increased IFN-γ secretion (54). The rs1893217 SNP may cause activation of inflammasome and the aberrant autophagosome assembly in human monocytic THP-1 cells and human colonic crypt Tαb epithelial cells (55). The two SNPs of *ICOSLG* were not in association with BD in our study, though previous GWAS had identified them as risk loci for CD and UC (19, 20, 22). The disparity between the results may be due to the differences in...
distinct genetic backgrounds for BD and IBD, or the limitation of the smaller sample size. When interpreting our results, it should be mentioned that we only tested CD-associated SNPs of PTPN2 and ICOSSLG in our work, while other gene variants still remained unknown. Furthermore, gene function studies will discover the roles of different genes in BD development and progression. In addition, studies with a large sample size from different populations are needed to extend and verify our current data.

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