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Original Article

Protein tyrosine phosphatase nonreceptor 2 (PTPN2) polymorphisms associated with inflammatory bowel disease in Guangxi Zhuang population

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Abstract: We aimed to investigate the association of protein tyrosine phosphatase nonreceptor 2 (PTPN2) genetic variants with inflammatory bowel disease (IBD) in Guangxi Zhuang population in China. Methods: Intestinal tissue samples were obtained from unrelated patients with IBD including 93 Zhuang [50 ulcerative colitis (UC) and 43 Crohn's disease (CD)] and 95 Han [50 ulcerative colitis (UC) and 45 Crohn's disease (CD)]. The control subjects included 76 Zhuang and 78 Han patients from Guangxi Zhuang population in the Guangxi Zhuang Autonomous Region from January 2009 to February 2013. Polymorphisms of PTPN2 gene rs2542151 and rs7234029 were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotype and allele frequencies of rs2542151 and rs7234029 polymorphisms of UC were significantly different from the controls ($P < 0.05$). No specific associations were detected between the two single nucleotide polymorphisms (SNPs) and gender, disease stage, age of onset, ethnic groups and site of origin of UC after further genotype-phenotype analysis ($P > 0.05$). No statistically significant differences were found in the genotype and allele frequency of PTPN2 gene rs2542151 as well as rs7234029 among CD and control subjects ($P > 0.05$). Gene polymorphisms of PTPN2 rs2542151 and rs7234029 may be associated with susceptibility to UC in Zhuang populations of Guangxi, China. No obvious correlations between the two SNPs and the disease phenotype of UC were found.

Keywords: PTPN2 gene, inflammatory bowel disease, ulcerative colitis, Crohn's disease, gene polymorphism

Introduction

Inflammatory bowel disease (IBD) is characterized by inflammation of the gastrointestinal tract, including ulcerative colitis (UC) and Crohn's disease (CD). The etiology and the pathogenesis of IBD remain unknown. Epithelial barrier defects coupled with a dysfunctional immune response to commensal microbiota, resulting in either excessive upregulation or impaired downregulation of inflammatory events drive the development of chronic intestinal inflammation [1]. Limited evidence suggests that protein tyrosine phosphatase nonreceptor 2 (PTPN2) maintains epithelial barrier function by restricting the capacity of IFN-γ to increase epithelial permeability and prevent expression of the pore-forming protein, claudin-2 [2]. Interferon γ (IFN-γ) is known not only to play an important role in IBD pathogenesis, but also increases permeability of the intestinal epithelial barrier [3, 4]. In addition to the discovery of NOD2 mutation in 2001 [5, 6], several genetic mutations associated with IBD have been found. The total number of confirmed CD risk loci is more than 71 and up to 47 for UC, with 28 shared loci [7, 8]. NOD2/CARD15 gene is the first identified human CD susceptibility gene, with three SNPs (Arg702Trp, Gly908Arg and Leu1007fsinsC) significantly correlated with CD in European Caucasians, but not with CD in Japanese [9] as well as Han population of Zhejiang [10], Han and Zhuang population of Guangxi [11] and Hong Kong in China [12]. The

Table 1. PCR reaction primers and restriction enzymes for each single nucleotide polymorphism

SNP	Primer sequences (5'→3')	DNA fragments (bp)	Enzymes	Digest TM (°C)
rs2542151	F-TGCTGTGCTGCGTGAGTT	291	Bsp1286I	37
	R-CACCATTGAGCGAAGTCC			
rs7234029	F-GGCAGTGCTGAAACGAGA	237	Hpy188I	37
	R-TCCCACCACCTACCTACGG			

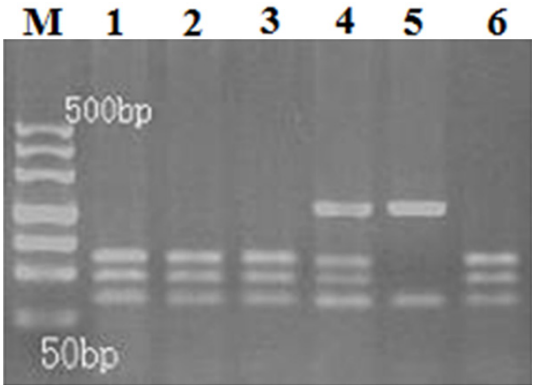

Figure 1. Electrophoresis of rs2542151 digestion products. M: Marker; 1-3, 6: TT homozygote; 4: GT heterozygote; 5: GG homozygote.

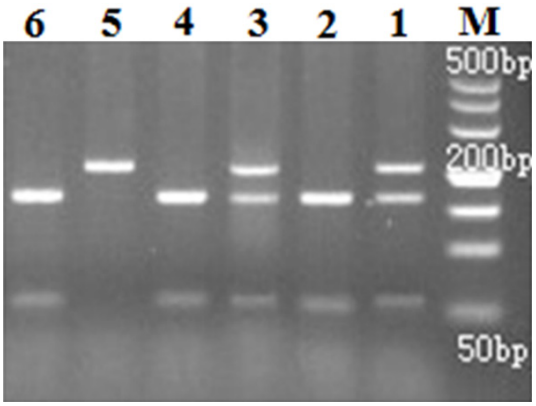

Figure 2. Electrophoresis of rs7234029 digestion products. M: Marker; 1, 3: A/G heterozygote; 2, 4, 6: AA homozygote; 5: GG homozygote.

gene loci correlating with IBD in Europeans include ATG16L1, TLR4 and TLR2, OCTN1 and OCTN2, P268S, JW1 and N852S. However, in the Guangxi Zhuang population, only the P268S was associated with CD [13, 14], link Germany [15] and Indian [16]. PTPN2 gene SNP rs2542151 has been repeatedly linked to susceptibility for CD in many Western countries, including Germany, Holland, Switzerland, Italy and New Zealand [17-21]. Recently, PTPN2 SNPrs-2542151 has been found significantly associated with increased risk for UC, and rs2542151 is a possible susceptibility marker for IBD in Han population of Guangdong, China [22]. We investigated the association of PTPN2 genetic variants (rs254215-15, rs7234029) with IBD in Guangxi Zhuang population.

Materials and methods

Subjects

Intestinal mucosa samples were obtained from unrelated patients with IBD including 93 Zhuang [50 ulcerative colitis (UC) and 43 Crohn's disease (CD)] and 95 Han [50 ulcerative colitis (UC) and 45 Crohn's disease (CD)]. The control subjects included 76 Zhuang and 78 Han from Guangxi Zhuang population. All participants were enrolled in the Department of Gastroenterology, the First Affiliated Hospital of Guangxi Medical University, from January 2009 to February 2013. All patients had a well-established diagnosis of UC or CD based on the modified criteria established by the World Gastroenterology Organization in 2010 [23]. The control group of individuals showed no evidence of intestinal infectious diseases, ischemic bowel disease or autoimmune diseases such as IBD or diabetes. There was no significant difference in gender or age between the patient group and the control group. All subjects provided written informed consent and the study protocol was approved by the Institutional Ethics Committee.

DNA extraction and genotyping of the PTPN2 polymorphisms

Intestinal tissue samples weighing about 30 to 50 mg were obtained from the participants. Phenol-chloroform method was used for genomic DNA extraction. The required DNA fragments were amplified by polymerase chain reaction (PCR) using specific primers (**Table 1**). The two PTPN2 SNP rs2542151 and rs7234029 were genotyped by PCR-RFLP. Primers were synthesized in ABGENT (Jiangsu, China) Biotechnology Company. The sequences of prim-

Table 2. Genotype and allele frequency distribution of the two SNPs

	Genotype	Allele	Control	UC	CD
				P value	*P* value
rs2542151				*[1]P=0.016*	*P=0.091*
	TT		51 (67.1)	25 (50.0)	22 (51.2)
	TG		25 (32.9)	21 (42.0)	20 (46.5)
	GG		0 (0.0)	4 (8.0)	1 (2.3)
		G	25 (16.4)	29 (29.0)	22 (25.6)
				[2]P=0.019	*P=0.093*
rs7234029				*[3]P=0.017*	*P=0.108*
	AA		45 (59.2)	21 (42.0)	19 (44.2)
	AG		30 (39.5)	23 (46.0)	21 (48.8)
	GG		1 (1.3)	6 (12.0)	3 (7.0)
		G	32 (21.1)	35 (35.0)	27 (31.4)
				[4]P=0.019	*P=0.087*

[1/3]comparison of genotype frequency. [2/4]comparison of allele frequency. UC: Ulcerative colitis; CD: Crohn's disease; A *P* value > 0.05: No significance.

Table 3. Genotype and allele frequency distribution of the two SNPs between IBD and controls in the Guangxi Han population

	Genotype	Allele	Control	UC	CD
				P value	*P* value
rs2542151				*[a]P=0.022*	*P=0.103*
	TT		52 (66.7)	24 (48.0)	22 (48.9)
	TG		25 (32.1)	21 (42.0)	22 (48.9)
	GG		1 (1.3)	5 (10.0)	1 (2.2)
		G	27 (17.3)	31 (31.0)	24 (26.7)
				[b]P=0.011	*P=0.081*
rs7234029				*[c]P=0.027*	*P=0.115*
	AA		46 (59.0)	21 (42.0)	20 (44.4)
	AG		31 (39.7)	24 (48.0)	22 (48.9)
	GG		1 (1.3)	5 (10.0)	3 (6.7)
		G	33 (21.2)	34 (34.0)	28 (31.1)
				[d]P=0.023	*P=0.082*

[a/c]Comparison of genotype frequency; [c/d]Comparison of allele frequency. UC: Ulcerative colitis; CD: Crohn's disease; A *P* value > 0.05: No significance.

ers and restriction enzymes are listed in **Table 1**.

The PCR system comprised a total volume of 20 μl, containing 2 × PCR Master-Mix 10 μl, disinfection DDW 7 μl, 1 μl each of upstream and downstream primers, and 1 μl of DNA extract.

The PCR conditions were as follows, the rs2542151 was subjected to an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 58°C for 30 s, extension at 72°C for 35 s, and final extension at 72°C for 7 min. The rs7234029 was subjected to initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 55°C for 30 s, extension at 72°C for 28 s, and final extension at 72°C for 5 min. All of the PCR products were stored at 4°C. The PCR products were electrophoresed in a 2% agarose gel, with 1 × TBE buffer, V=90-100V for 45 min, visualized under Gel imaging system (Bio-Rad Gel Doc-2000, United States) and were confirmed with the reference DNA. The PCR products (5 μl) were digested by the appropriate restriction enzyme (**Table 1**) for about 10 hours, followed by electrophoresis in a 2.5% agarose gel.

Statistical analysis

The genotype frequencies were calculated by direct counting. The Hardy-Wein-berg equilibrium test was used to check the mutation genotype frequency distributions. Fisher's exact test and chi-square test were used to compare the genotype frequencies and allele frequency distribution in patients and controls by SPSS13.0 software (SPSS Inc., Chicago, IL, U.S.A.), $P < 0.05$ was considered statistically significant.

Results

PTPN2 gene rs2542151 polymorphisms

After complete digestion, the PCR product of rs2542151 of the PTPN2 gene yielded three bands (74, 95, and 291 bp) in TT homozygotes, 74 bp and 217 bp in GG homozygotes, and all four bands (74, 95, 122, and 295 bp) in GT heterozygotes (**Figure 1**).

PTPN2 gene rs7234029 polymorphisms

The PCR product of PTPN2 gene rs7234029 generated three bands (76, 161, and 237 bp) in A/G heterozygotes, two bands (76, and 161 bp)

Table 4. Clinical profile of UC of SNP rs2542151 and rs7234029 genotype

		rs2542151			*P value*	rs7234029			*P value*
Onset age	N	TT	TG	GG	*P=0.799*	AA	AG	GG	*P=0.152*
≤ 40	45	23 (51.1)	19 (42.2)	3 (6.7)		19 (42.2)	24 (53.3)	2 (4.4)	
> 40	55	26 (47.3)	23 (41.8)	6 (10.9)		23 (41.8)	23 (41.8)	9 (16.4)	
Location					*P=0.457*				*P=0.569*
E1	18	9 (50.0)	8 (44.4)	1 (5.6)		9 (42.9)	11 (52.4)	1 (4.8)	
E2		21 (42.9)	21 (42.9)	7 (14.3)		22 (44.9)	20 (40.8)	7 (14.3)	
E3		19 (57.6)	13 (39.4)	1 (3.0)		11 (33.3)	19 (57.6)	3 (9.1)	
Gender					*P=0.333*				*P=0.627*
Male	53	25 (47.2)	21 (39.6)	7 (13.2)		20 (37.3)	27 (50.9)	6 (11.3)	
Female	47	24 (51.1)	21 (44.7)	2 (4.3)		22 (46.8)	20 (42.6)	5 (10.6)	
Ethnic Groups					*P=1.0*				*P=1.0*
Zhuang	50	25 (50.0)	21 (42.0)	4 (8.0)		21 (42.0)	23 (46.0)	6 (12.0)	
Han	50	24 (48.0)	21 (42.0)	5 (10.0)		21 (42.0)	24 (48.0)	5 (10.0)	
Stages					*P=0.871*				*P=0.571*
Activity	59	28 (47.5)	25 (42.4)	6 (10.2)		23 (39.0)	28 (47.5)	8 (13.6)	
Remission	41	21 (51.2)	17 (41.5)	3 (7.3)		19 (46.3)	19 (46.3)	3 (7.3)	

E1, rectum; E2, left hemicolon; E3, extensive colon; A *P* value > 0.05*:* No significance.

in AA homozygotes, and the one band (237 bp) in GG homozygotes (**Figure 2**).

Analysis of PTPN2 gene polymorphisms in IBD

All the genotypic distributions were in accordance with the Hardy-Weinberg equilibrium ($P > 0.05$). The genotype and allele frequencies of rs2542151 were statistically significant in UC group compared with control group in Zhuang and Han populations ($P < 0.05$) (**Table 2**). The genotype and allele frequencies of PTPN2 rs7234029 were also statistically significant in UC compared with control group in Zhuang and Han populations ($P < 0.05$) (**Table 2**). However, no significant differences were detected in genotype frequencies or allele frequencies of the two SNPs between CD group and control groups including Zhuang and Han ($P > 0.05$) (**Tables 2**, **3**). Further phenotype-genotype analysis revealed no associations between the two SNPs and the gender, disease stage or site of origin of UC (**Table 4**). In addition, as shown in the **Table 4**, no correlation existed between UC and CD in the Han and Zhuang population.

Discussion

The identification of IBD susceptibility genes provides insight into the pathogenic mechanisms. As one of the susceptibility genes, PTPN2 gene is located on chromosome 18p11.2-11.3. It encodes a key negative regulatory factor of inflammation of T-cell protein phosphatase (TC-PTP), which is expressed in all the tissues and cells. Recent studies have demonstrated that [24] defective PTPN2 was associated with significant elevation in IFN-γ and TNF-α levels in mice serum as well as increased production of nitric oxide. Additionally, the secretion of IL-6 and IL-8 induced by TNF-α was increased [25]. As a result, PTPN2 loss aggravates the epithelial barrier defect caused by IFN-γ [26]. Therefore, PTPN2 deficiency may cause autophagosome formation and impaired autophagy, significantly increasing the permeability of intestinal epithelium and the intestinal epithelial barrier dysfunction. However, the mechanisms are known for Crohn's disease in Europeans. The PTPN2 rs2542151 and rs7234029 polymorphisms were shown to result in CD with a strong diversity among different ethnicities, which is similar to the NOD2/CARD15 [27]. In our study, the two SNPs were associated with UC, but not with CD, unlike in most Europeans [17, 18, 20, 28, 29]. Several recent studies also showed the association of SNP rs2542151 with advanced CD, manifesting inflammatory and penetrating behavior, requiring bowel resection [20]. CD associated with colon was significantly associated with healthy controls among Canadians [30]. In the Netherlands, PTPN2-rs2542151 was only asso-

ciated with smoking CD patients, and not with the other cohorts [31]. It is plausible that genetic and environmental factors such as smoking interact with each other, contributing to the genotype distribution. Genetic studies of IBD should be stratified for environmental factors, race, geography, populations, especially of CD.

In Asia, researchers in Japan and Guangdong in China showed that rs2542151 was not associated with susceptibility to CD, which is consistent with our findings [31, 32]. Accordingly, we speculated that the gene PTPN2 may have little relevance with CD in Asia. SNP rs7234029 was associated with CD in Germans [16]. Interestingly, rs2542151 also revealed an association with UC in German patients [16]. Our study is consistent with Chinese Han population of Guangdong. The results were not consistent with the studies on Chinese Han patients with CD from Guangdong or patients with CD from Europe or Italy. The diversity may be due to geographic, environmental and racial heterogeneity, and a relatively small sample size. PTPN2 gene may be a susceptible gene with UC patients in Zhuang population in Guangxi of China. Comparative analysis of the genotype-phenotype did not yield any specific associations between the two SNPs and the clinical features of UC patients. The presence of rs2542151 and rs7234029 polymorphisms did not influence disease phenotype.

In conclusion, for the first time we identified PTPN2 gene rs2542151 and rs7234029 polymorphisms associated with UC in Guangxi Zhuang population of China. In the present study, no correlations between gene polymorphisms and CD were observed. The genes may be candidates for conferring susceptibility to UC in Zhuang population. There was no obvious association between the two SNPs and the clinical phenotype of UC. It may be limited by the relatively small sample size. Our results play an important role in the understanding of the pathogenesis of UC. Additional susceptibility genes need to be isolated and the pathogenesis of inflammatory bowel disease elucidated in future studies.

Comments

Background

The precise etiology of inflammatory bowel disease is not clear, but the genetic factors play a critical role. An increasing number of studies suggested the association between IBD susceptibility and PTPN2 expression.

Research frontiers

PTPN2 gene polymorphisms have been identified in CD among Europeans. In Asia, research studies involving Japanese and the Han population of Guangdong China showed that rs2542151 was not associated with susceptibility to CD. Furthermore, several studies in Zhuang population of Guangxi found no susceptible gene polymorphisms. The study assessed the association of PTPN2 genetic variants (rs25421515, rs7234029) with IBD in Guangxi Zhuang population.

Innovations and breakthroughs

To the best of our knowledge, it is the first time that SNPs were associated with UC in Zhuang population from the Guangxi Zhuang Autonomous Region of China.

Applications

The gene may be a susceptibility marker in UC among Zhuang population. However, there was no obvious association between the two SNPs and the clinical phenotype of UC.

Terminology

PTPN2 gene located on chromosome 18p11.2-11.3, encodes a key inflammatory negative regulatory factor of T-cell protein phosphatase (TC-PTP), which is expressed in all the tissues and cells.

Peer review

The study demonstrates the associations of PTPN2 polymorphisms with UC in a restricted Chinese cohort. The genetic variation in UC susceptibility among various ethnic and regional groups is meaningful.

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Disclosure of conflict of interest

None.

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References

[1] Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-429.

[2] McCole DF. Regulation of epithelial barrier function by the inflammatory bowel disease candidate gene, PTPN2. Ann N Y Acad Sci 2012; 1257: 108-114.

[3] Madara JL and Stafford J. Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. J Clin Invest 1989; 83: 724-727.

[4] Beaurepaire C, Smyth D and McKay DM. Interferon-gamma regulation of intestinal epithelial permeability. J Interferon Cytokine Res 2009; 29: 133-144.

[5] Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M and Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411: 599-603.

[6] Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G and Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411: 603-606.

[7] Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ and Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 2010; 42: 1118-1125.

[8] Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel JF, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RS, Floyd JA, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot JP, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra HJ, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK and Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 2011; 43: 246-252.

[9] Yamazaki K, Takazoe M, Tanaka T, Kazumori T and Nakamura Y. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. J Hum Genet 2002; 47: 469-472.

[10] Gao M, Cao Q, Luo LH, Wu ML, Hu WL and Si JM. NOD2/CARD15 gene polymorphisms and susceptibility to Crohn's disease in Chinese Han population. Zhonghua Nei Ke Za Zhi 2005; 44: 210-212.

[11] Lin MJ, Lv XP and Chen L. Correlation of R702W, G908R and L1007fs polymorphisms

of the NOD2/CARD15 gene with susceptibility in Zhuang population in Guangxi, China. World J Gastroenterol 2012; 20: 1210-1215.
[12] Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP and Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther 2003; 17: 1465-1470.
[13] Long WY, Chen L, Zhang CL, Nong RM, Lin MJ, Zhan LL and Lv XP. Association between NOD2/CARD15 gene polymorphisms and Crohn's disease in Chinese Zhuang patients. World J Gastroenterol 2014; 20: 4737-4744.
[14] Chen L, Lin MJ, Zhan LL and Lv XP. Analysis of TLR4 and TLR2 polymorphisms in inflammatory bowel disease in a Guangxi Zhuang population. World J Gastroenterol 2012; 18: 6856-6860.
[15] Wex T, Ebert MP, Kropf S, Dierkes J, Schüttler K, Röcken C, Höcker M and Malfertheiner P. Gene polymorphisms of the NOD-2/CARD-15 gene and the risk of gastric cancer in Germany. Anticancer Res 2008; 28: 757-762.
[16] Ng SC, Tsoi KK, Kamm MA, Xia B, Wu J, Chan FK and Sung JJ. Genetics of inflammatory bowel disease in Asia: systematic review and meta-analysis. Inflamm Bowel Dis 2012; 18: 1164-1176.
[17] Glas J, Wagner J, Seiderer J, Olszak T, Wetzke M, Beigel F, Tillack C, Stallhofer J, Friedrich M, Steib C, Göke B, Ochsenkühn T, Karbalai N, Diegelmann J, Czamara D and Brand S. PTPN2 gene variants are associated with susceptibility to both Crohn's disease and ulcerative colitis supporting a common genetic disease background. PLoS One 2012; 7: e33682.
[18] Weersma RK, Stokkers PC, Cleynen I, Wolfkamp SC, Henckaerts L, Schreiber S, Dijkstra G, Franke A, Nolte IM, Rutgeerts P, Wijmenga C and Vermeire S. Confirmation of multiple Crohn's disease susceptibility loci in a large Dutch-Belgian cohort. Am J Gastroenterol 2009; 104: 630-638.
[19] Scharl M, Mwinyi J, Fischbeck A, Leucht K, Eloranta JJ, Arikkat J, Pesch T, Kellermeier S, Mair A, Kullak-Ublick GA, Truninger K, Noreen F, Regula J, Gaj P, Pittet V, Mueller C, Hofmann C, Fried M, McCole DF and Rogler G. Crohn's disease-associated polymorphism within the PTPN2 gene affects muramyl-dipeptide-induced cytokine secretion and autophagy. Inflamm Bowel Dis 2012; 18: 900-912.
[20] Latiano A, Palmieri O, Latiano T, Corritore G, Bossa F, Martino G, Biscaglia G, Scimeca D, Valvano MR, Pastore M, Marseglia A, D'Incà R, Andriulli A and Annese V. Investigation of multiple susceptibility loci for inflammatory bowel disease in an Italian cohort of patients. PLoS One 2011; 6: e22688.
[21] Morgan AR, Han DY, Huebner C, Lam WJ, Fraser AG and Ferguson LR. PTPN2 but not PTPN22 is associated with Crohn's disease in a New Zealand population. Tissue Antigens 2010; 76: 119-125.
[22] Lv C, Yang X, Zhang Y, Zhao X, Chen Z, Long J, Zhang Y, Zhong C, Zhi J, Yao G, Jiang B and Zhi F. Confirmation of three inflammatory bowel disease susceptibility loci in a Chinese cohort. Int J Colorectal Dis 2012; 27: 1465-1472.
[23] Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Gearry R, Goh KL, Hamid S, Khan AG, LeMair AW, Malfertheiner, Ouyang Q, Rey JF, Sood A, Steinwurz F, Thomsen OO, Thomson A and Watermeyer G. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. Inflamm Bowel Dis 2010; 16: 112-124.
[24] Scharl M and Rogler G. The role for protein tyrosine phosphatase nonreceptor type 2 in regulating autophagosome formation. Ann N Y Acad Sci 2012; 1257: 93-102.
[25] Scharl M, McCole DF, Weber A, Vavricka SR, Frei P, Kellermeier S, Pesch T, Fried M and Rogler G. Protein tyrosine phosphatase N2 regulates TNF{alpha}-induced signaling and cytokine secretion in human intestinal epithelial cells. Gut 2011; 60: 189-197.
[26] Scharl M, Paul G, Weber A, Jung BC, Docherty MJ, Hausmann M, Rogler G, Barrett KE and McCole DF. Protection of epithelial barrier function by the Crohn's disease associated gene protein tyrosine phosphatase n2. Gastroenterology 2009; 137: 2030-2040.
[27] Peng H, Li J, Chen X, Zhou X, Zhu W and Li F. Genetic Variants of PTPN2 Gene in Chinese Children with Type 1 Diabetes Mellitus. Med Sci Monit 2015; 21: 2653-2658.
[28] Marcil V, Mack DR, Kumar V, Faure C, Carlson CS, Beaulieu P, Israel D, Krupoves A, Costea I, Lambrette P, Grimard G, Dong J, Seidman EG, Amre DK and Levy E. Association between the PTPN2 gene and Crohn's disease: dissection of potential causal variants. Inflamm Bowel Dis 2013; 19: 1149-1155.
[29] Fisher SA, Tremelling M, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ, Nimmo ER, Massey D, Berzuini C, Johnson C, Barrett JC, Cummings FR, Drummond H, Lees CW, Onnie CM, Hanson CE, Blaszczyk K, Inouye M, Ewels P, Ravindrarajah R, Keniry A, Hunt S, Carter M, Watkins N, Ouwehand W, Lewis CM, Cardon L; Wellcome Trust Case Control Consortium, Lobo A, Forbes A, Sanderson J, Jewell DP, Mansfield JC, Deloukas P, Mathew CG, Parkes M and Satsangi J. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet 2008; 40: 710-712.

[30] Waterman M, Xu W, Stempak JM, Milgrom R, Bernstein CN, Griffiths AM, Greenberg GR, Steinhart AH and Silverberg MS. Distinct and overlapping genetic loci in Crohn's disease and ulcerative colitis: correlations with pathogenesis. Inflamm Bowel Dis 2011; 17: 1936-1942.

[31] van der Heide F, Nolte IM, Kleibeuker JH, Wijmenga C, Dijkstra G and Weersma RK. Differences in genetic background between active smokers, passive smokers, and non-smokers with Crohn's disease. Am J Gastroenterol 2010; 105: 1165-1172.

[32] Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y and Hata A. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. J Hum Genet 2007; 52: 575-583.