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Original Article

Neuropeptide S receptor 1 gene polymorphisms are associated with susceptibility to inflammatory bowel disease in Chinese han population

Xiaobing Wang[1,2], Xiaomin Zhang[1,2], Shi Liu[1,2], Feng Zhu[1,2], Ge Wang[1,2], Feng Zhou[1,2*], Liping Chen[1,2*]

[1]Department of Gastroenterology/Hepatology, Zhongnan Hospital of Wuhan University, Wuhan City, Hubei Province, P.R. of China; [2]The Hubei Clinical Center & Key Laboratory of Intestinal & Colorectal Diseases, Wuhan City, Hubei Province, P.R. of China. [*]Equal contributors.

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Abstract: Background and objectives: Neuropeptide receptor 1 (*NPSR1*) has been identified as a susceptibility gene for inflammatory bowel disease (IBD) in Caucasians. However, whether there is an association between polymorphisms of *NPSR1* and IBD susceptibility in Asian populations, including the Chinese, is unknown. Clarifying this association will improve our understanding of the genetics of IBD in Eastern populations. Methods: The study included 107 patients with Crohn's disease (CD), 710 patients with ulcerative colitis (UC), and 605 healthy controls (HC). Peripheral blood was obtained from each study subject for DNA extraction. Six SNPs in *NPSR1* (rs323917, rs323922, rs324377, rs324384, rs324396, and rs740347) were genotyped, and haplotype blocks were constructed. Results: The genotype and allelotype distributions of *NPSR1* rs324377 in CD were significantly different from those in HC (P = 0.003 and 0.019, respectively). The differences in the genotype frequencies of all six SNPs between UC and HC didn't reach statistical significance. However, the differences in the allelotype frequencies of rs324384 and rs740347 between UC and HC were significantly different (P = 0.026 and 0.047, respectively). The haplotype frequencies between CD or UC and HC were significantly different. Analysis of the genotypes and clinical phenotypes revealed that polymorphisms in rs324377 for CD as well as rs324384 and rs740347 for UC were statistically associated with gender, age at diagnosis, disease location (extent), and disease behaviour. Conclusions: *NPSR1* gene is a susceptibility locus for IBD in Chinese Han population. *NPSR1* polymorphisms are associated with IBD clinical phenotype, and these results should be verified in a different population.

Keywords: NPSR1, polymorphisms, inflammatory bowel disease

Introduction

Inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic, debilitating disorders characterized by recurrent episodes of relapsing and remitting inflammation of the gastrointestinal tract [1]. Although the precise aetiology of IBD has not yet been elucidated, a complex interaction between predisposing genes, environmental factors, and dysregulated mucosal immune responses to the gut microbiome is thought to contribute to pathogenesis [2, 3]. The importance of genetic susceptibility to the pathogenesis of IBD is well known. Genetic studies have opened a window to understanding the nature of IBD, and pointed the way to subsequent functional studies as well as immunological, microbial, signalling pathway, epigenetic, and pharmacogenetic studies.

Thus far, more than 163 genes have been confirmed as susceptibility genes for IBD by Genome wide association study (GWAS), of which, 110 are common to UC and CD, whereas 23 and 38 are specific for UC and CD, respectively [4-7]. However, GWASs have many limitations, for example, there have been very few GWASs on non-white populations, including a limited number of recent studies in Japan and South Korea [8-10]. In addition, GWAS data can only account for a small fraction of the total heritability of IBD. Therefore, other genetic studies, such as candidate gene association studies,

Table 1. The inclusion and exclusion criteria for patients and healthy controls

Inclusion and exclusion criteria for patients	IBD (CD or UC) was diagnosed according to established clinical criteria [20], including endoscopic, radiologic, and histopathologic examinations.
	Patients with other autoimmune diseases, such as psoriasis, SLE, rheumatoid arthritis, or multiple sclerosis, were excluded from the study.
	Patients with other diseases that may impair immune function, such as viral hepatitis or HIV infection, were excluded.
	Patients who received a bone marrow transplant were excluded.
	None of the included patients were blood relatives.
Inclusion and exclusion criteria for control cohort	All healthy controls were assessed with a general health evaluation and routine blood, liver, and kidney function tests.
	Persons treated with glucocorticoid or immunosuppressant within the prior 3 months were excluded.
	Persons with obvious abdominal symptoms were excluded.
	Persons with a family history of autoimmune disease, including IBD, were excluded.

IBD, inflammatory bowel disease; CD, Cohn's disease; UC, ulcerative colitis; HC, healthy controls.

are still a useful supplement to GWASs. Neuropeptide receptor 1 (NPSR1), previously called G-protein-coupled receptor, GPR154, or GPRA, is a member of the G protein-coupled receptor family [11-13]. Very few studies on NPSR1 have been published; thus, very little is known about NPSR1. A few studies reported that *NPSR1* polymorphisms are associated with various diseases, such as asthma [14], rheumatoid arthritis [15], anxiety neurosis [16], and irritable bowel syndrome [13]. Only two studies had been focused on the association of NPSR1 polymorphisms with IBD, and the target populations in both studies were Caucasian [17, 18]. Thus, whether there are definite associations between the *NPSR1* polymorphisms and IBD susceptibility in Asian populations, including Chinese, is unknown. Clarifying this association will help expand our known of IBD genetics in Eastern populations, especially Chinese populations.

Methods

Study subjects

All the IBD patients and the healthy controls were recruited during the study period (2004-2013) at IBD clinics and the inpatient department of Zhongnan Hospital of Wuhan University. The study was approved by the ethics committee of Zhongnan Hospital of Wuhan University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants prior to their participation. For all patients, a diagnosis was made by incorporating clinical, endoscopic, radiologic, and histopathologic information, according to established clinical criteria [19], and those with indeterminate colitis or complicated by other immune-related diseases were excluded from the study. The detailed reasons for inclusion and exclusion for both patients and controls are shown in **Table 1**. Patients with CD were assessed according to the Montreal classification [19] based on age at diagnosis (A), location (L), and behaviour (B) of the disease. In addition, for patients with UC, anatomic location was assessed in accordance with the Montreal classification [19], as follows: ulcerative proctitis (E1), left-sided UC (distal UC; E2), or extensive UC (pancolitis; E3). Demographic data (name, gender, ethnicity, age at diagnosis, age at sample collection) and clinical characteristics (UC or CD, disease course, disease activity: active or remission, anatomic location of IBD, and disease behaviour for CD) for all participants are summarized. We obtained 2 mL of peripheral blood from all patients and controls and stored the samples at -80°C until DNA extraction.

DNA extraction, PCR, and sequencing

Blood samples were collected from all participants, and genomic DNA was isolated from

Table 2. The information for primers of the six SNPs within NPSR1

SNPs		Primer sequence (5'→3')	Length of PCR amplified product (bp)	Optimal annealing temperature (°C)
Rs323917	Forward	TGGAATTAGATTCAGCGCACAT	222	60
	Reverse	TCTTGAACCAACCGCCATC		
Rs323922	Forward	TCCACCATCTTGCCTGTGAG	217	60
	Reverse	CAGGACCAAGTTGTGGTAGCTGTATT		
Rs324377	Forward	GCTTGGACCGTGAGATGACTATAAC	261	60
	Reverse	AGAACGAGGATTTGAACCAGAAACT		
Rs324384	Forward	ATTTTTTAGTTTCATCATCTCACAA	422	60
	Reverse	ATACATCATGGGATACTATGCAATC		
Rs324396	Forward	GTGTTCGGATGCCGATGGGTAGT	177	60
	Reverse	TGCCAGAGGTTTGGATGGGATTG		
Rs740347	Forward	ACAATAATGAAGACTCAATTTCAGC	297	60
	Reverse	AGCAGGCAAGAAGAGGAGT		

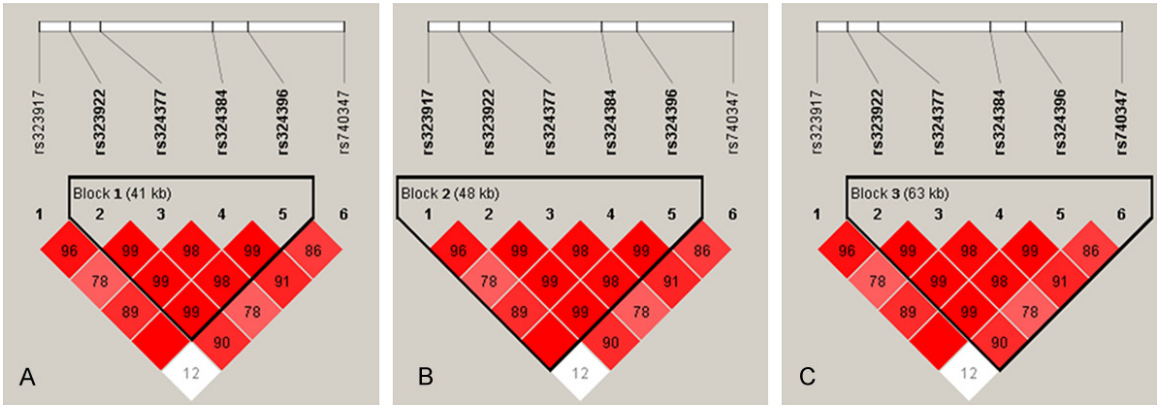

Figure 1. Linkage disequilibrium (LD) analysis of six *NPSR1* SNPs. LD was calculated using Haploview (v. 3.32) based on demographic and clinical characteristics and the genotypes of UC patients, CD patients, and healthy controls, and haplotype blocks 1, 2, and 3 were generated from original data. The numbers in each box correspond to the LD coefficient D' between the respective SNPs. Values greater than 80 usually indicate strong LD.

peripheral blood leukocytes using the DNA Blood Mini Kit (TIANGEN, Beijing) according to the manufacturer's instructions. The DNA extracted from each blood sample was stored at -80°C until polymerase chain reaction (PCR). All the primers used in our study were designed with Primer Premier 5.0 software based on sequences of six SNPs within *NPSR1* (rs323917, rs323922, rs324377, rs324384, rs324396, and rs740347) and synthesized by Wuhan Tsingke BioTech, Ltd. Then, we performed PCR with the following cycling parameters: an initial denaturation step (95°C for 5 min), followed by 30 cycles of denaturation (95°C for 30 sec), primer annealing (60°C for 30 sec), and extension (72°C for 1 min), and a final extension step (72°C for 10 min). Reactions were performed in triplicate for each polymorphism. The PCR-amplified products were sequenced by Wuhan Tsingke BioTech. Primer information is shown in **Table 2**.

Statistical analysis

Hardy-Weinberg calculations were performed to ensure that each SNP was in population equilibrium, and no significant deviation was observed for any of the six SNPs tested. Sequence mapping (from Wuhan Tsingke BioTech) was analysed with Chromas to identify genotypes. Then, genotype frequencies, allele frequencies, and correlations between genotypes and phenotypes were calculated by Fisher's exact test or chi-squared tests using SPSS 17.0 based on the observed genotypes

Table 3. Demographics and clinic data of patients and healthy controlS

	CD (n = 107)	UC (N = 710)	HC (N = 605)
Gender			
Female	39	317	264
Male	68	393	341
Age			
Mean ± standard deviation	33±12.0	44.1±14.4	40.0±11.49
Range	13-62	15-79	22-87
Age at diagnosis*			
A_1 (≤16)	10	29	-
A_2 (17-40)	71	319	-
A_3 (≥40)	20	362	-
Disease course			
Mean ± standard deviation	3.5±3.8	3.3±4.7	-
Range	0.1-20	0-30	-
Disease activity			
Active	87	428	-
Remission	20	282	-
Anatomic location of disease			
L_1 (ileum)	39	-	-
L_2 (colon)	38	-	-
L_3 (ileocolon)	22	-	-
L_4 (isolated upper gastrointestinal tract)	2	-	-
E_1 (rectum)	-	138	-
E_2 (descendent colon)	-	328	-
E_3 (ascendant colon)	-	244	-
Disease behavior			
B_1 (stricture type)	61	-	-
B_2 (inflammatory type)	21	-	-
B_3 (penetrating type)	11	-	
P (perianal fistula)	8	-	

IBD, inflammatory bowel disease; CD, Cohn's disease; UC, ulcerative colitis; HC, healthy controls, *Certain clinical information for 6 CD patients was incomplete.

Table 4. Hardy-Weinberg test for six SNPs of NPSR1

SNPs	χ^2	Degree of Freedom	*P* values
Rs323917	3.49	1	0.06
Rs323922	0.15	1	0.70
Rs324377	0.04	1	0.84
Rs324384	0.02	1	0.89
Rs324396	0.08	1	0.78
Rs740347	1.99	1	0.16

and the phenotype characteristics, including demographic data and clinical parameters. In addition, we applied a false discovery rate (FDR) to correct *P* values.

Haplotype analysis

H*aplot*ype frequencies and linkage disequilibrium (LD) were estimated and visualized using Haploview 3.32, and three NPSR1 haplotype blocks were generated using the algorithm proposed by Gabriel. The haplotype blocks in our study are presented in **Figure 1**. The Haploview tagger function was used to identify redundant SNPs, and SNPs were considered redundant if the pairwise LD (r^2) was > 0.8. Polymorphism loci rs323922, rs324377, rs324384, and rs324396 were retained in the first haplotype block (block 1); rs323917, rs323922, rs324377, rs324384, and rs324396 were retained in the second haplotype block (block 2); and rs323922, rs324377, rs324384, rs324396, and rs740347 were retained in the third block (block 3).

Results

Demographic and clinic characteristics of patients and controls

A consecutive series of 817 IBD patients (107 with CD and 710 with UC) and 605 age-, gender-, and ethnicity-matched, unrelated healthy controls were included in our study. The demographic and clinic characteristics of all participants are summarized in **Table 3**.

Hardy-Weinberg test of six NPSR1 SNPs in IBD patients and controls

Hardy-Weinberg equilibrium (HWE) analyses were assessed using Pearson's chi-square test, and no significant deviation was observed for any of the six SNPs tested (*P* > 0.05, **Table 4**). The analysis revealed that the population in our study was in genetic equilibrium at all six tested loci.

Table 5. Allele frequencies and genotyping analysis for six SNPs between controls and CD

SNP	HC (n = 605)	CD (n = 107)	χ^2	*P*	P_{FDR}	OR	95% CI
Rs323917							
CC	511 (508.8)	88 (90.2)				Reference	
CG	80 (83.2)	18 (14.8)	2.143	0.342	0.513	1.432	(0.815, 2.515)
GG	7 (5.9)	0 (1.1)				-	-
C carrier	1102 (1100.9)	194 (195.1)				Reference	
G carrier	94 (95.1)	18 (16.9)	0.098	0.754	0.953	1.088	(0.642, 1.842)
Rs323922							
CC	164 (170.2)	35 (18.8)				Reference	
CG	306 (298.6)	43 (50.4)	2.935	0.230	0.690	0.658	(0.405, 1.069)
GG	134 (135.2)	24 (22.8)				0.839	(0.476, 1.480)
C carrier	634 (639.1)	113 (107.9)				Reference	
G carrier	574 (568.9)	91 (96.1)	0.593	0.441	0.882	0.889	(0.660, 1.199)
Rs324377							
CC	148 (146.4)	24 (25.6)				Reference	
AC	300 (287.6)	38 (60.4)	11.428	0.003	0.018	0.781	(0.452, 1.351)
AA	157 (171.0)	44 (30.0)				1.728	(1.001, 2.983)
C carrier	596 (580.3)	86 (101.7)				Reference	
A carrier	614 (629.7)	126 (110.3)	5.458	0.019	0.114	1.422	(1.057, 1.913)
Rs324384							
TT	205 (206.6)	37 (35.4)				Reference	
CT	294 (290.3)	46 (49.7)	0.676	0.713	0.713	0.867	(0.543, 1.384)
CC	103 (105.0)	20 (18.0)				1.076	(0.594, 1.947)
T carrier	704 (703.6)	120 (120.4)				Reference	
C carrier	500 (500.4)	86 (85.6)	0.003	0.953	0.953	1.009	(0.748, 1.362)
Rs324396							
TT	100 (103.1)	21 (17.9)				Reference	
CT	287 (280.2)	42 (48.8)	2.150	0.341	0.682	0.697	(0.394, 1.234)
CC	216 (219.7)	42 (36.3)				0.926	(0.521, 1.645)
T carrier	487 (486.3)	84 (84.7)				Reference	
C carrier	719 (719.7)	126 (125.3)	0.011	0.917	0.953	1.016	(0.753, 1.370)
Rs740347							
GG	500 (497.3)	79 (81.7)				Reference	
CG	95 (96.2)	17 (15.8)	1.783	0.410	0.492	1.133	(0.642, 1.999)
CC	8 (9.4)	3 (1.6)				2.373	(0.617, 9.137)
G carrier	1095 (1090.9)	175 (179.1)				Reference	
C carrier	111 (115.1)	23 (18.9)	1.146	0.284	0.852	1.297	(0.805, 2.088)

CD, Cohn's disease; HC, healthy controls.

Allele frequencies and genotype analysis of six SNPs in the healthy controls and CD and UC patients

Allele frequencies and genotype frequencies of the six SNPs in CD patients and healthy controls are presented in **Table 5**. For the rs324377 polymorphisms, the A allele was a risk allele, and it was associated with an increased risk for CD (OR = 1.422, 95% CI: 1.05, 1.913). In addition, The AA homozygote (OR = 1.728, 95% CI: 1.001, 2.983) instead of the AC heterozygote (OR = 0.781, 95% CI: 0.452, 1.351) was significantly associated with a risk of CD, and such statistical significance remained even after FDR correction (*P* = 0.018). However, the AC heterozygote did not increase the risk of CD. For the other five SNPs (rs323917, rs323922,

Table 6. Allele frequencies and genotyping analysis for six SNPs between controls and UC

SNP	HC (n = 605)	UC (n = 710)	χ^2	*P*	P_{FDR}	OR	95% CI
Rs323917							
CC	511 (513.3)	604 (601.7)				Reference	
CG	80 (79.6)	93 (93.4)	1.394	0.498	0.672	0.984	(0.713, 1.356)
GG	7 (5.1)	4 (5.9)				0.483	(0.141, 1.661)
C carrier	1102 (1106.2)	1301 (1296.8)				Reference	
G carrier	94 (89.8)	101 (105.2)	0.400	0.527	0.632	0.910	(0.680, 1.219)
Rs323922							
CC	164 (160.3)	181 (184.7)				Reference	
CG	306 (301.5)	343 (347.5)	1.161	0.560	0.672	1.016	(0.782, 1.319)
GG	134 (142.2)	172 (163.8)				1.163	(0.854, 1.585)
C carrier	634 (622.1)	705 (716.9)				Reference	
G carrier	574 (585.9)	687 (675.1)	0.874	0.350	0.525	1.076	(0.922, 1.256)
Rs324377							
CC	148 (149.4)	175 (173.6)				Reference	
AC	300 (294.2)	336 (341.8)	0.465	0.793	0.793	0.947	(0.724, 1.239)
AA	157 (161.4)	912 (187.6)				1.034	(0.763, 1.402)
C carrier	596 (593.0)	686 (689.0)				Reference	
A carrier	614 (617.0)	720 (717.0)	0.056	0.812	0.812	1.019	(0.874, 1.188)
Rs324384							
CC	103 (118.2)	153 (137.8)				Reference	
CT	294 (291.8)	338 (340.2)	5.278	0.071	0.405	0.774	(0.576, 1.039)
TT	205 (192.0)	211 (224.0)				0.693	(0.506, 0.950)
C carrier	500 (528.1)	644 (615.9)				Reference	
T carrier	704 (675.9)	760 (788.1)	4.960	0.026	0.141	0.838	(0.717, 0.979)
Rs324396							
CC	216 (206.4)	232 (241.6)				Reference	
CT	287 (285.1)	332 (333.9)	3.046	0.218	0.436	1.077	(0.844, 1.374)
TT	100 (111.5)	142 (130.5)				1.322	(0.964, 1.813)
C carrier	719 (697.9)	796 (817.1)				Reference	
T carrier	487 (508.1)	616 (594.9)	2.809	0.094	0.188	1.143	(0.978, 1.335)
Rs740347							
GG	500 (485.7)	552 (566.3)				Reference	
CG	95 (108.0)	139 (126.0)	4.010	0.135	0.405	1.325	(0.994, 1.767)
CC	8 (9.2)	12 (10.8)				1.359	(0.551, 3.351)
G carrier	1095 (1079.5)	1243 (1258.5)				Reference	
C carrier	111 (126.5)	163 (147.5)	3.947	0.047	0.141	1.294	(1.003, 1.669)

UC, ulcerative colitis; HC, healthy controls.

rs324384, rs324396, and rs740347), no statistical difference in the frequencies of any allele or genotype was found between CD patients and healthy controls.

Comparison of UC patients and healthy controls (**Table 6**) showed that none of the genotype frequencies at any of the six tested SNPs reached statistical difference. Only the allele frequencies at rs324384 and rs740347 varied significantly between UC patients and healthy controls (*P* = 0.026, 0.047, respectively). Our results demonstrated that a T allele at rs324384 was a protective factor against UC (OR = 0.083, 95% CI: 0.717, 0.979), but only the TT homozygote was associated with a decreased risk of UC (OR = 0.693, 95% CI: 0.506, 0.950). For the rs740347 polymor-

Table 7. Haplotype analysis for comparison of CD patients, UC patients, and healthy controls

Block	HC (n = 605)	CD (n = 107)				UC (n = 710)			
	Freq	Freq	OR (95% CI)	*P*	P_{FDR}	Freq	OR (95% CI)	*P*	P_{FDR}
Block 1									
CATC	0.505	0.541	1.176 (0.876, 1.578)	0.289	0.385	0.486	0.942 (0.808, 1.098)	0.433	0.433
GCCT	0.400	0.367	0.860 (0.635, 1.165)	0.323	0.323	0.419	1.087 (0.929, 1.270)	0.310	0.414
GCTC	0.062	0.025	0.441 (0.190, 1.027)	0.036	0.071	0.048	0.732 (0.521, 1.027)	0.080	0.160
CCTC	0.022	0.000	-	0.032	0.128	0.000	0.097 (0.029, 0.321)	1.87E-0.6	7.47E-0.6
Block 2									
CCATC	0.435	0.456	1.108 (0.825, 1.488)	0.495	0.660	0.418	0.955 (0.818, 1.116)	0.554	0.692
CGCCT	0.400	0.367	0.861 (0.636, 1.166)	0.327	0.655	0.417	1.082 (0.926, 1.266)	0.316	0.527
GCATC	0.069	0.085	1.187 (0.699, 2.016)	0.518	0.518	0.069	0.953 (0.707, 1.285)	0.785	0.785
CGCTC	0.062	0.025	0.440 (0.189, 1.024)	0.035	0.140	0.048	0.745 (0.531, 1.044)	0.086	0.428
CGCCC	0.000	0.000	-	-	-	0.013	1.553 (0.714, 0.978)	0.241	0.603
Block 3									
CATCG	0.420	0.430	1.041 (0.774, 1.400)	0.751	0.751	0.375	0.836 (0.714, 0.978)	0.024	0.097
GCCTG	0.399	0.360	0.837 (0.617, 1.136)	0.274	0.343	0.419	1.078 (0.922, 1.260)	0.355	0.355
CATCC	0.085	0.112	1.397 (0.872, 2.237)	0.1878	0.313	0.112	1.339 (1.030, 1.740)	0.029	0.059
GCTCG	0.061	0.025	0.373 (0.149, 0.934)	0.040	0.200	0.048	0.752 (0.535, 1.058)	0.112	0.150
CCTCG	0.018	0.000	-	0.049	0.122	-	-	-	-

CD, Cohn's disease; UC, ulcerative colitis; HC, healthy controls.

phisms, a C allele was a risk factor for UC (OR = 1.294, 95% CI: 1.003, 1.669). However, neither the CC homozygote (OR = 1.359, 95% CI: 0.551, 3.351) nor the CG heterozygote (OR = 1.325, 95% CI: 0.994, 1.767) was found to be significantly associated with UC risk. None of the differences in allele and genotype frequencies remained statistically significant after FDR correction.

Haplotype analysis of IBD patients and healthy controls

LD was calculated using Haploview software based on demographic and clinical characteristics and the genotypes of UC patients, CD patients, and healthy controls. Haplotype blocks 1, 2, and 3, which were generated from original data, are shown in **Figure 1**. The haplotype frequencies within subgroups (UC & controls, CD & controls) are shown in **Table 7**.

Haplotype analysis for comparison of CD patients and healthy controls

In block 1, there was a significant difference between CD patients and healthy controls for the haplotype GCTCII (0.025 vs. 0.062, *P* = 0.036); however, this difference was not associated with a risk of CD (OR = 0.441, 95% CI: 0.190, 1.027). For another haplotype in block 1, CCTCII, the haplotype frequency in CD patients was obviously lower than that in healthy controls (0.000 vs. 0.022, *P* = 0.032).

In block 2, the haplotype frequency of CGCTCII was significantly lower in CD patients than in healthy controls (0.025 vs. 0.062, *P* = 0.035); however, this difference was not associated with disease risk (OR = 0.440, 95% CI: 0.189, 1.024).

However, in block 3, we found that the haplotype frequencies of GCTCGII and CCTCGII in CD patients were considerably lower than in the healthy controls (0.025 vs. 0.061, *P* = 0.040 and 0.000 vs. 0.018, *P* = 0.049, respectively), and haplotype GCTCGII was a protective factor for CD (OR = 0.373, 95% CI: 0.149, 0.934). The haplotype frequencies of the other haplotypes are shown in **Table 7**.

Haplotype analysis for comparison of UC patients and healthy controls

We observed quite different results in the haplotype analysis of UC patients and healthy controls. In block 1, the haplotype CCTCII occurred more frequently in the healthy controls than in the UC patients (0.022 vs. 0.000, $P = 1.87 \times 10^{-6}$, $P_{FDR} = 7.47 \times 10^{-6}$), which indicated that CCTCII was a strong protective factor against

Table 8. The association between NPSR1 rs324377 polymorphisms and clinical phenotypes of Crohn's disease

		Genotype			AC vs. CC			AA vs. CC			Allele				
		AA	AC	CC	*P*	OR	95% CI	*P*	OR	95% CI	A	C	*P*	OR	95% CI
Male	Controls	86 (25.4)	176 (51.9)	77 (22.7)	-	1.000	-	-	1.000	-	348 (51.3)	330 (48.7)	-	1.000	-
	CD Patients	31 (46.3)	19 (28.4)	17 (25.4)	0.044	0.489	0.241-0.992	0.147	1.633	0.838-3.180	81 (60.4)	53 (39.6)	0.053	1.449	0.993-2.114
Female	Control	71 (26.7)	124 (46.6)	71 (26.7)	-	1.000	-	-	1.000	-	266 (50.0)	266 (50.0)	-	1.000	-
	CD Patients	13 (33.3)	19 (48.7)	7 (17.9)	0.342	1.554	0.623-3.878	0.290	1.857	0.700-4.928	45 (57.7)	33 (42.3)	0.204	1.364	0.844-2.204
Control (Reference)		157 (26.0)	300 (49.6)	148 (24.5)	-	1.000	-	-	1.000	-	614 (50.7)	596 (49.3)	-	1.000	-
Age at diagnosis	A1 (≤16y)	4 (40.0)	3 (30.0)	3 (30.0)	0.404	0.493	0.098-2.474	1.000	1.257	0.277-5.711	11 (55.0)	9 (45.0)	0.706	1.186	0.488-2.884
	A2 (17-40y)	31 (43.7)	25 (35.2)	15 (21.1)	0.566	0.822	0.421-1.606	0.044	1.948	1.011-3.755	87 (61.3)	55 (38.7)	0.018	1.535	1.076-2.192
	A3 (≥40y)	6 (30.0)	10 (50.0)	4 (20.0)	0.951	1.233	0.380-3.998	0.834	1.414	0.391-5.111	22 (55.0)	18 (45.0)	0.596	1.186	0.630-2.234
Location at diagnosis	L1 (Ileal)	15 (38.5)	10 (25.6)	14 (35.9)	0.011	0.352	0.153-0.812	0.980	1.010	0.471-2.164	40 (51.3)	38 (48.7)	0.927	1.022	0.646-1.615
	L2 (Colonic)	17 (44.7)	15 (39.5)	6 (15.8)	0.670	1.233	0.469-3.244	0.038	2.671	1.025-6.958	49 (64.5)	27 (35.5)	0.020	1.762	1.087-2.856
	L3 (Ileocolonic)	9 (40.9)	11 (50.0)	2 (9.1)	0.299	2.713	0.594-12.399	0.048	4.242	0.902-19.957	29 (65.9)	15 (34.1)	0.048	1.877	0.996-3.536
	L4 (Upper GI)	0 (0)	2 (100.0)	0 (0)	1.000	-	-	-	-	-	2 (50.0)	2 (50.0)	1.000	0.971	0.136-6.913
Behaviour at diagnosis	B1 (Non-stricturing, non-penetrating)	23 (37.7)	24 (39.3)	14 (23.0)	0.633	0.846	0.425-1.683	0.219	1.549	0.768-3.123	70 (57.4)	52 (42.6)	0.162	1.307	0.897-1.903
	B2 (Stricturing)	10 (47.6)	5 (23.8)	6 (28.6)	0.242	0.411	0.123-1.369	0.390	1.571	0.557-4.431	25 (59.5)	17 (40.5)	0.263	1.427	0.763-2.671
	B3 (Penetrating)	5 (45.5)	6 (54.5)	0 (0)	0.184	-	-	0.062	-	-	16 (72.7)	6 (27.3)	0.041	2.588	1.006-6.660
	P (Perianal fistula)	3 (37.5)	3 (37.5)	2 (25)	0.668	0.740	0.122-4.477	1.000	1.414	0.233-8.582	9 (56.3)	7 (43.8)	0.662	1.248	0.462-3.373

CD, Cohn's disease.

Table 9. The association between NPSR1 rs324384 polymorphisms and clinical phenotypes of ulcerative colitis

		Genotype			TC vs. CC			TT vs. CC			Allele				
		TT	TC	CC	*P*	OR	95% CI	*P*	OR	95% CI	T	C	*P*	OR	95% CI
Male	Controls	114 (33.3)	172 (50.3)	56 (16.4)	-	1.000	-	-	1.000	-	400 (58.5)	284 (41.5)	-	1.000	-
	UC Patients	119 (30.7)	185 (47.7)	84 (21.6)	0.100	0.717	0.482-1.066	0.094	0.696	0.455-1.064	423 (54.5)	353 (45.5)	0.127	0.851	0.691-1.047
Female	Control	91 (25.3)	122 (33.9)	147 (40.8)	-	1.000	-	-	1.000	-	304 (42.2)	416 (57.8)	-	1.000	-
	UC Patients	92 (29.3)	153 (48.7)	69 (22.0)	0.000	2.672	1.842-3.876	0.000	2.154	1.433-3.236	337 (53.7)	291 (46.3)	0.000	1.585	1.277-1.966
Control (Reference)		205 (29.2)	294 (41.9)	203 (28.9)	-	1.000	-	-	1.000	-	704 (50.1)	700 (49.9)	-	1.000	-
Age at diagnosis	A1 (≤16 y)	4 (13.8)	19 (65.5)	6 (20.7)	0.093	2.187	0.858-5.570	0.522	0.660	0.184-2.374	27 (46.6)	31 (53.4)	0.592	0.866	0.512-1.466
	A2 (17-40 y)	108 (34.4)	135 (43.0)	71 (22.6)	0.114	1.313	0.936-1.842	0.024	1.506	1.054-2.152	351 (55.9)	277 (44.1)	0.017	1.260	1.043-1.522
	A3 (≥40 y)	99 (27.6)	184 (51.3)	76 (21.2)	0.002	1.672	1.212-2.306	0.161	1.290	0.903-1.842	382 (53.2)	336 (46.8)	0.182	1.130	0.944-1.354
Extent at diagnosis	E1 (Proctitis)	44 (32.4)	68 (50.0)	24 (17.6)	0.008	1.956	1.188-3.221	0.027	1.815	1.064-3.096	156 (57.4)	116 (42.6)	0.029	1.337	1.029-1.738
	E2 (Left Sided colitis)	96 (29.7)	147 (45.5)	80 (24.8)	0.152	1.269	0.916-1.757	0.340	1.188	0.834-1.394	339 (52.5)	307 (47.5)	0.326	1.098	0.911-1.323
	E3 (Extensive colitis)	71 (29.2)	123 (50.6)	29 (20.2)	0.004	1.733	1.190-2.525	0.085	1.435	0.950-2.167	265 (54.5)	221 (45.5)	0.096	1.192	0.969-1.466

UC, ulcerative colitis.

Table 10. The association between NPSR1 rs740347 polymorphisms and clinical phenotypes of ulcerative colitis

		Genotype			TC vs. CC			TT vs. CC			Allele				
		CC	CG	GG	*P*	OR	95% CI	*P*	OR	95% CI	C	G	*P*	OR	95% CI
Male	Controls	5 (1.5)	54 (15.9)	281 (82.6)	-	1.000	-	-	1.000	-	64 (9.4)	616 (90.6)	-	1.000	-
	UC Patients	5 (1.3)	78 (20.1)	305 (78.6)	0.143	1.331	0.907-1.952	1	0.921	0.264-3.216	88 (11.3)	688 (88.7)	0.230	1.231	0.876-1.729
Female	Control	3 (1.1)	41 (15.6)	219 (83.3)	-	1.000	-	-	1.000	-	47 (8.9)	479 (91.1)	-	1.000	-
	UC Patients	7 (2.2)	61 (19.4)	247 (78.4)	0.212	1.319	0.853-2.039	0.458	2.069	0.529-8.098	75 (11.9)	555 (88.1)	0.102	1.377	0.938-2.023
Control (Reference)		8 (1.3)	95 (15.8)	500 (82.9)	-	1.000	-	-	1.000	-	111 (9.2)	1095 (90.8)	-	1.000	-
Age at diagnosis	A1 (≤16 y)	2 (0.9)	6 (2.7)	212 (96.4)	0.000	0.149	0.064-0.345	0.746	0.590	0.124-2.800	10 (2.3)	430 (97.7)	0.000	0.229	0.119-0.442
	A2 (17-40 y)	7 (2.2)	72 (22.9)	235 (74.8)	0.006	1.613	1.144-2.273	0.354	1.862	0.677-5.195	86 (13.7)	542 (86.3)	0.003	1.565	1.160-2.112
	A3 (≥40 y)	3 (0.8)	61 (16.9)	296 (82.2)	0.651	1.085	0.763-1.543	0.717	0.633	0.167-2.406	67 (9.3)	653 (90.7)	0.941	1.012	0.736-1.392
Extent at diagnosis	E1 (Proctitis)	4 (3.0)	29 (21.5)	102 (75.6)	0.089	1.496	0.938-2.387	0.271	2.451	0.724-8.293	37 (13.7)	233 (86.3)	0.026	1.567	1.052-2.332
	E2 (Left Sided colitis)	5 (1.5)	64 (19.7)	256 (78.8)	0.125	1.316	0.926-1.869	0.959	1.221	0.395-3.769	74 (11.4)	576 (88.6)	0.135	1.267	0.929-1.730
	E3 (Extensive colitis)	3 (1.2)	46 (18.9)	194 (79.8)	0.264	1.248	0.846-1.842	1.000	0.966	0.254-3.688	52 (10.7)	434 (89.3)	0.345	1.182	0.835-1.673

UC, ulcerative colitis.

UC (OR = 0.097, 95% CI: 0.029, 0.321). No significant differences were found between the UC patients and healthy controls for the genotypes in block 2. In block 3, the frequency of the CATCGII haplotype in UC patients was lower than that in the healthy controls (0.375 vs. 0.420, *P* = 0.024), and haplotype CATCGII was considered to be a protective factor for UC (OR = 0.836, 95% CI: 0.714, 0.978). However, haplotype CATCCII was found to be a risk factor for UC, as there was a higher frequency in UC patients than in the healthy controls (0.112 vs. 0.085, *P* = 0.029; OR = 1.339, 95% CI: 1.030, 1.740). The haplotype frequencies for the other haplotypes are shown in **Table 7**.

Associations between NPSR1 genotypes and IBD phenotypes

Three polymorphic loci (rs324377, rs324384, and rs740347) associated with IBD susceptibility were analysed for their associations with various phenotypes.

Association of rs324377 polymorphisms with CD phenotypes

The association of the rs324377 polymorphisms with CD phenotypes was explored within stratified subgroups (gender, age at diagnosis, anatomic location, and disease behaviour). The rs324377 AC heterozygote was found to be a protective factor for males with CD (*P* = 0.044, OR = 0.489, 95% CI: 0.241, 0.992) and for ileum involvement in CD (*P* = 0.011, OR = 0.352, 95% CI: 0.153, 0.812). The rs324377 AA homozygote was a risk factor for CD in the 17-40 age group (*P* = 0.044, OR = 1.948, 95% CI: 1.011, 3.755) and for colon involvement (*P* = 0.038, OR = 2.671, 95% CI: 1.025, 6.958). Our results also revealed that carriage of an A allele was a risk factor for CD in the 17-40 age group (*P* = 0.018, OR = 1.535, 95% CI: 1.076, 2.192), colon involvement (*P* = 0.020, OR = 1.762, 95% CI: 1.087, 2.856), and penetrating behaviour (*P* = 0.041, OR = 2.588, 95% CI: 1.006, 6.660). Additional details of the analysis are shown in **Table 8**.

Association of rs324384 polymorphisms with UC phenotypes

Analysis of UC (for both rs324384 and rs740347) was conducted within stratified subgroups (gender, age at diagnosis, and anatomic area involved in the disease). At rs324384, the TC heterozygote and TT homozygote were associated with an increased risk of UC for females (for TC: *P* = 0.000, OR = 2.672, 95% CI: 1.842, 3.876; for TT: *P* = 0.000, OR = 2.154, 95% CI: 1.433, 3.236) and for rectum involvement (for TC: *P* = 0.008, OR = 1.956, 95% CI: 1.188, 3.221; for TT: P = 0.027, OR = 1.815, 95% CI: 1.064, 3.096). The main difference between TC and TT was the age at diagnosis; specifically, TC was a risk factor for the > 40 age group (*P* = 0.002, OR = 1.672, 95% CI: 1.212, 2.306), whereas TT was a risk factor for the 17-40 age group (*P* = 0.024, OR = 1.506, 95% CI: 1.054, 2.152). In the analysis of allele distribution, carriage of a T allele at rs324384 was associated with a higher risk of UC in females (*P* = 0.000, OR = 1.585, 95% CI: 1.277, 1.966), in 17-40-year olds (*P* = 0.017, OR = 1.260, 95% CI: 1.043, 1.522), and in the group with rectum involvement (*P* = 0.029, OR = 1.337, 95% CI: 1.029, 1.738; **Table 9**).

Association of rs740347 polymorphisms with UC phenotypes

For rs740347, our results indicated that the CG heterozygote was a protective factor for those under 16 years old with UC (*P* = 0.000, OR = 0.149, 95% CI: 0.064, 0.345), whereas it was a risk factor for UC in 17-40-years olds (*P* = 0.006, OR = 1.613, 95% CI: 1.144, 2.273). However, the CC homozygote was not associated with any UC phenotype. In the analysis for associations between allele frequencies and UC phenotypes, we found that a C allele was protective for patients <16 years old with UC (*P* = 0.000, OR = 0.229, 95% CI: 0.119, 0.442) and was a risk factor for UC in 17-40-year olds (*P* = 0.003, OR = 1.565, 95% CI: 1.160, 2.112) and for rectum involvement (*P* = 0.026, OR = 1.567, 95% CI: 1.052, 2.332; **Table 10**).

Discussion

Since *NOD2* was identified as the first susceptibility gene for IBD, genetic studies have promoted our understanding of the pathogenesis of IBD. To the best of our knowledge, this study included the largest number of participants (IBD patients and healthy controls) of all studies on IBD genetics in China, and it is also the first study to report an association between *NPSR1* polymorphisms and IBD susceptibility in an eastern population.

The NPSR1 protein is found to be expressed in the epithelial cells of some organs and tissues, including the lung, large intestine, small intestine, and skin [13, 17, 20-22]. It is also worth mentioning that under inflammatory conditions, for example, in some immune-related diseases such as rheumatoid arthritis, asthma, and IBD, the expression of NPSR1 in these organs or tissues is markedly up-regulated [13, 17, 20-22]. The NPSR1 protein has several isoforms which differ in the sequences of their C-termini [23]. The ligand of NPSR1, neuropeptide S (NPS), is a 20-amino acid peptide (SFRNGVGTGMKKTSFQRAKS) that is highly conserved among species [11]. Previous studies showed that NPS could be selectively coupled to and activated by NPSR1 to induce elevation of intracellular Ca^{2+} levels and cAMP production [13, 18]; thus, it may play a key role in cell proliferation, gene transcription, morphogenesis, immune responses, chemotaxis, intercellular contact, and the response to invasion by pathogenic microorganisms [24]. Another study reported higher expression of both NPSR1-A mRNA and NPSR1-B mRNA in activated T cells, especially CD4+ T cells, than in quiescent T cells [17]. Based on the above, we speculated that NPSR1 might be closely associated with inflammation or immune responses. This is why we chose *NPSR1* as the candidate gene for our study.

We used a candidate gene association study to explore the associations between *NPSR1* polymorphisms and IBD susceptibility in a Chinese Han population, and found that rs324377 polymorphisms were associated with CD susceptibility, and rs324384 and rs740347 polymorphisms were associated with UC susceptibility. Carriage of an A allele, especially an AA homozygote, at rs324377 was a risk factor for CD. The distribution of genotype frequencies remained significant, even after correction by FDR, indicating that NPSR1 rs324377 was a susceptibility locus for CD in this Chinese Han population. Our study also revealed that carrying a T allele at rs324384 and a C allele at rs740347 was a protective factor and a risk factor for UC susceptibility, respectively. However, the differences failed to remain statistically significant after FDR correction. Then, we generated three haplotype blocks to identify their associations with CD and UC susceptibility. However, after FDR correction, only the CCTCII haplotype was found to be a strong protective factor for UC. Relatively speaking, our results were very different from those reported in Western populations, and ethnic variation might account for this difference. Numerous studies have confirmed genetic heterogeneity across different races and ethnicities [25, 26], emphasizing the importance of performing studies in different populations, ethnicities, and regions to improve our understanding of genetic diversity and provide effective criteria for individual treatment.

Analysis of the association between genotypes and phenotypes was expected to more or less predict IBD phenotypes such as age onset, extent of disease, disease behaviour, extraintestinal manifestations, and the need for surgery, which would make a difference in individualized prevention and treatment plans. Ours was the first analysis of the association of *NPSR1* genotypes with IBD phenotypes. Our study revealed that the rs324377 AC heterozygote was a strong protective factor for males with CD and ilea involvement in CD. Conversely, the rs324377 AA homozygote was considered to be a risk factor for colonic CD and CD onset in 17-40 year olds. Thus, harbouring an allele A at rs324377 might increase the risk for penetrating behaviour in CD. For the UC phenotypes, the rs324384 TC heterozygote was a risk factor for females with UC, UC onset over 40 years old, and extensive colonic UC. The TT homozygote was a risk factor for females with UC, UC onset over 40 years old, and rectum involvement in UC. For rs740347, the CG heterozygote was a strong protective factor against UC onset <16 years old, but was a risk factor for UC onset in 17-40-year olds. However, the biggest problem for these types of studies is that the results usually cannot be replicated in different populations, which might be caused by unrecognized confounding factors in the current model. Thus, a better model is urgently needed to promote the application of genotype and phenotype association studies in clinical practice.

In conclusion, our study confirmed *NPSR1* as an IBD susceptibility gene in a Chinese Han population, and some *NPSR1* genotypes were associated with some IBD phenotypes. However, the exact role of NPSR1 in IBD pathogenesis is still unknown, and further study is needed to clarify it.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Liping Chen and Feng Zhou, Department of Gastroenterology/Hepatology, Zhongnan Hospital of Wuhan University, Donghu Road 169, Wuhan 430071, Hubei Province, P.R. of China. Tel: +86 027-67813061; Fax: +86 027-67812892; E-mail: chenlipingzhongnan@163.com (LPC); zhouf04@163.com (FZ)

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