World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i17.2112

World J Gastroenterol 2012 May 7; 18(17): 2112-2120
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Association of NOD1 and NOD2 genes polymorphisms with *Helicobacter pylori* related gastric cancer in a Chinese population

Peng Wang, Li Zhang, Jian-Ming Jiang, Dan Ma, Hao-Xia Tao, Sheng-Ling Yuan, Yan-Chun Wang, Ling-Chun Wang, Hao Liang, Zhao-Shan Zhang, Chun-Jie Liu

Peng Wang, Li Zhang, Hao-Xia Tao, Sheng-Ling Yuan, Yan-Chun Wang, Ling-Chun Wang, Zhao-Shan Zhang, Chun-Jie Liu, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, Beijing 100071, China
Jian-Ming Jiang, Dan Ma, Hao Liang, Department of Gastroenterology and Hepatology, China People's Liberation Army General Hospital, Beijing 100853, China
Author contributions: Wang P and Zhang L contributed equally to this work; Wang P, Zhang L, Liang H and Liu CJ designed the research; Wang P, Zhang L, Jiang JM, Ma D, Tao HX, Yuan SL, Wang YC and Wang LC performed the research; Wang P and Zhang L analyzed the data; Wang P, Zhang ZS and Liu CJ wrote the paper.
Supported by The Major Foundation of Vaccines and Antibody Program during the Eleventh Five-Year Plan Period (863 Program), No. 2006AA02A219; the National Specialized Research Fund for Control of Major Infectious Diseases during the Eleventh Five-Year Plan Period, No. 2008ZX10004-015; the National Major Science and Technology Project of China (Innovation and Development of New Drugs), No. 2009ZX09301-002
Correspondence to: Dr. Chun-Jie Liu, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, 20 Feng Tai Dong Da Jie Street, Beijing 100071, China. liucj@nic.bmi.ac.cn
Telephone: +86-10-66948834 Fax: +86-10-63833521
Received: May 11, 2011 Revised: December 6, 2011
Accepted: March 10, 2012
Published online: May 7, 2012

Abstract

AIM: To investigate the association between the tag single nucleotide polymorphisms (TagSNPs) of NOD1 and NOD2 and the risk of developing gastric cancer.

METHODS: We conducted a hospital-based case-control study including 296 incident gastric cancer patients and 160 gastritis controls. Eight TagSNPs in the NOD1 and NOD2 genes were selected from the Hapmap database using the haploview software and genotyped by the Sequenom MassArray system. The serum levels of anti-*Helicobacter pylori* (*H. pylori*) IgG were measured by enzyme-linked immunosorbent assay to indicate *H. pylori* infection. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression, including sex and age as confounding factors.

RESULTS: The NOD1 rs2907749 GG genotype showed a decreased risk for gastric cancer (OR 0.50, 95% CI: 0.26-0.95, P = 0.04) while the rs7789045 TT genotype showed an increased risk (OR 2.14, 95% CI: 1.20-3.82, P = 0.01). An elevated susceptibility to gastric cancer was observed in the subjects with *H. pylori* infection and the NaOD1 rs7789045 TT genotype (OR 2.05, 95% CI: 1.07-3.94, P = 0.03) or the NOD2 rs7205423 GC genotype (OR 2.52, 95% CI: 1.05-6.04, P = 0.04). Haplotype analysis suggested that the distribution of AGT (rs2907749, rs2075820 and rs7789045) in NOD1 between the cases and control groups was significantly different (P corrected: 0.04), and the diplotype AGT/AGT was associated with an elevated gastric cancer risk (OR 1.98, 95% CI: 1.04-3.79, P = 0.04). The association of the NOD1 rs7789045 TT genotype and the diplotype AGT/AGT was significant with *H. pylori*-related diffuse-type gastric cancer (OR 3.00, 95% CI: 1.38-6.53, P = 0.01; OR 4.02, 95% CI: 1.61-10.05, P < 0.01, respectively).

CONCLUSION: Genetic polymorphisms in NOD1 and NOD2 may interact with *H. pylori* infection and may play important roles in promoting the development of gastric cancer in the Chinese population.

Key words: Gastric cancer; NOD1; NOD2; Gene polymorphisms; *Helicobacter pylori* infection

Peer reviewer: Dr. Jianyuan Chai, PhD, MS, BS, Assistant Professor, Research (09-151), VA Long Beach Healthcare System, 5901 E. 7th St, Long Beach, CA 90822, United States

Wang P, Zhang L, Jiang JM, Ma D, Tao HX, Yuan SL, Wang YC, Wang LC, Liang H, Zhang ZS, Liu CJ. Association of NOD1 and NOD2 genes polymorphisms with *Helicobacter pylori* related gastric cancer in a Chinese population. *World J Gastroenterol* 2012; 18(17): 2112-2120 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i17/2112.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i17.2112

INTRODUCTION

The average prevalence of *Helicobacter pylori* (*H. pylori*) infection in people worldwide is approximately 50%. An epidemiology meta analysis has indicated that the *H. pylori* prevalence ranges from 35% to 81% in different districts within China, and the average infection rate is 58%[1]. *H. pylori* was estimated to be responsible for approximately 65% of all stomach cancers worldwide[2]. It has been reported that gastric cancer-associated mortality rates accounted for nearly one-quarter of the total malignant tumor-related mortalities in China[3]. Together with *H. pylori* infection, host genetic susceptibility, diet, a high salt intake and smoking have all been proposed to be risk factors for gastric cancer.

Clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation, and cancer[4-6]. Gastric cancer develops very rarely in the normal gastric mucosa. Most of the *H. pylori*-infected individuals showed gastritis, but very few people develop gastric cancer. The genetic variations between the gastritis and gastric cancer patients may play important roles in the *H. pylori*-related clinical outcomes[7]. The host immune response has a strong role in determining the outcome of *H. pylori* infection, and the polymorphisms in genes that control this immune response have been shown to affect the risk of gastric cancer[8-11]. *H. pylori* trigger inflammation through activation of the receptors that recognize pathogen-associated molecular patterns (PAMPs), and these PAMPs are recognized through a set of germline-encoded pattern recognition receptors (PRRs). The activation of PRRs leads to rapid production of a range of pro-inflammatory cytokines with a profound impact on both the innate and adaptive immune responses.

Among the cytosolic PRRs is the nucleotide-binding oligomerization domain (NOD)-like receptor family. Two members of this family, known as NOD1 and NOD2, have been recently identified[12]. NOD1 and NOD2 are characterized by a central NOD, an N-terminal effector-binding domain (CARD) and a C-terminal ligand recognition domain that is comprised of leucine-rich repeats (LRR)[13]. NOD1 senses a muropeptide found mostly in Gram-negative bacterial peptidoglycans, whereas NOD2 senses bacterial molecules produced during peptidoglycan synthesis or degradation[14]. NOD1 and NOD2 are becoming known as key regulators of chronic inflammatory conditions[15]. The NODs ultimately activate transcription factors such as nuclear factor (NF)-κB, STAT1 and so on, which play important roles in inflammation-linked tumor development. It is important to understand how the NOD family proteins work together in coordinating the host response to a given pathogen. Direct evidence for NOD family-mediated host defense derived mostly from an *in vivo* study in which NOD1-deficient mice were reported to be more susceptible to infection by *H. pylori* strains with functional type Ⅳ secretion systems[16]. Additionally, NOD2 was reported to regulate antimicrobial peptide synthesis as part of the host defense strategies against *L. monocytogenes* infection *in vivo*[17].

There are several reports that demonstrated that the polymorphisms of the NOD1 and NOD2 genes in different populations were related to variant clinical outcomes of *H. pylori* infection. Although two studies have shown that the NOD1 E266K (rs2075820) mutation increased the risk of peptic ulceration, antral atrophy and intestinal metaplasia[18,19], there is little research related to the association between NOD1 polymorphisms and gastric cancer. NOD2 polymorphisms have been proven to significantly correlate with the incidence of gastric cancer in European populations[20-22], whereas all of the SNPs studied proved to be monomorphic sites in the Chinese population. To verify that the polymorphisms of the *H. pylori*-recognized NOD1 and NOD2 contribute to gastric cancer carcinogenesis through gene-gene and gene-environment interactions, we performed a hospital-based case-control study with 296 incident gastric cancer patients (hospital case subjects) and 160 gastritis patients (hospital control subjects).

MATERIALS AND METHODS

Study population

The hospital-based case-control study consisted of 466 hospitalized patients recruited sequentially in the China People's Liberation Army General Hospital from January 2009 to June 2010. The 296 case subjects were histopathologically verified gastric cancer patients (GC group) and the 160 control subjects were gastritis patients (GA group) who had undergone gastroscopy. All subjects were unrelated Han Chinese. The exclusion criteria for the hospital control subjects included previous cancer and previous chemotherapy or radiotherapy. Upon recruitment, informed consent was obtained from each subject or their relatives, and this study was approved by the Institutional Review Board of the Institute of Biotechnology.

Genotyping and tag single nucleotide polymorphisms selection

Eight TagSNPs for the NOD1 and NOD2 genes were chosen from the designable set of common SNPs [minor allele frequency (MAF) ⩾ 0.05] genotyped in the Han Chinese (CHB) population samples of the HapMap Project (Data Release 24/Phase Ⅱ, NCBI B36 assembly, dbSNP b126). The TagSNPs selection was done using

Table 1 Primer details for genotyping of single nucleotide polymorphisms from NOD1 and NOD2 genes

SNP ID	PCR 1st primer	PCR 2nd primer	Amplication length (bp)	Extension sequence primer
rs17159048	ACGTTGGATGTCAAGAGGAGGGTATTAGGC	ACGTTGGATGCTGTGTGCTTGGGCAGTAAC	93	TTTGGCAGTAACAGTGACAAG
rs2907749	ACGTTGGATGGCTGTGAAGAACAGCAAATC	ACGTTGGATGCACACAGCAGGTTGTACCAC	99	GTAGGTTGTACCACATACATCC
rs2075820	ACGTTGGATGAAGCGCAGCAGGAAGGCAAA	ACGTTGGATGACCTGCTCTTCAAGCACTAC	90	CACTCCTGCTACCCAGAGCGGGACCCC
rs7789045	ACGTTGGATGAGCAGACACAGACAGGGTTC	ACGTTGGATGTTGAGATTGCTGACTGGTGG	95	GTGGTCTCTTCCAGC
rs2067085	ACGTTGGATGATCAGGTTGCCGATCTTCAC	ACGTTGGATGCCTTTCCTGAGAACTCTGTG	95	GTGCCTCACCCTCTG
rs1861759	ACGTTGGATGTGACATTTCTCTTGGCTTCC	ACGTTGGATGTGATGTGAAGGAATTCCAGG	100	AAGACACGACACCTTTGGC
rs3135500	ACGTTGGATGGGCCATGTTGTCTATAAGAG	ACGTTGGATGGATGTGTGAAAACTGGTTAA	99	ATTTGTGAAAACTGGTTAATATTTATAG
rs7205423	ACGTTGGATGCTTGGCTCCAGCCCATTTTG	ACGTTGGATGTATGGCTGCTGCAGGAAATG	99	CACAAATTATCCCCTTATAGTC

SNP: Single nucleotide polymorphism; PCR: Polymerase chain reaction.

the software Haploview version 4.0 with pairwise tagging mode. For the NOD1 gene, four TagSNPs were selected (rs17159048, rs2907749, rs2075820 and rs7789045), which captured 36 out of 45 (80%) of the SNPs covering the whole gene. For the NOD2 gene, four TagSNPs were selected (rs2067085, rs1861759, rs3135500 and rs72054230), which captured 17 out of 21 (80%) of the SNPs covering the whole gene, and the 3'-flanking 2 kb regions; TagSNPs were selected with pairwise $r^2 \geqslant 0.80$.

The genotypes of all the SNPs were determined by the MassArray system (Sequenom iPLEXassay, San Diego, United States). The polymerase chain reaction (PCR) primers (MassExtend; Sequenom) used in this study were listed in Table 1. Briefly, approximately 15 ng of genomic DNAs isolated from the peripheral blood lymphocytes of the study subjects were used to genotype each sample. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom, San Diego, United States). The sample DNA was amplified by a multiplex PCR reaction, and the PCR products were then used for locus-specific single-base extension reaction. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. The alleles were discriminated by mass spectrometry. (Sequenom, San Diego, United States). Genotyping was performed without knowledge of the case or control status. Twenty random samples were tested in duplicate by different persons, and the reproducibility was 100%.

Helicobacter pylori detection

The *H. pylori* infection status was evaluated by the detection of serum-specific IgG antibodies against *H. pylori* in duplicate with enzyme-linked immunosorbent assay procedures. The sonicated *H. pylori* strain SS1 antigen was used to coat 96-well microplates at a concentration of 2 μg/mL. The sera of the samples were diluted 1:100 when measured. Twenty serum samples, which were verified by *H. pylori* histology culture, rapid urease test and Carbon-14-Urea Breath Test, were considered the candidate positive controls, whereas twenty serum samples were considered candidate negative controls by the same three tests. Six serum samples were ultimately confirmed as the negative control criteria, the mean absorbance of which was identical to that of the 20 candidate negative controls. Finally, the samples with a mean absorbance 2.1-fold or greater than the mean absorbance of the six negative reference samples were considered to be positive reactions. The sensitivity of the *H. pylori* detection system was 100% (20 of 20) in the control groups.

Haplotype construction and statistical analysis

The Pearson's χ^2 test was used to examine the differences between the case and the control groups in sex, *H. pylori* infection and age groups. The genotype frequencies in the cases and controls were compared and both the OR and 95% CI: of each genotype were estimated by applying unconditional logistic regression adjusting for age, sex and *H. pylori* infection when it was appropriate. The homozygotes of the most frequent allele in controls were used as the reference group. The Hardy-Weinberg equilibrium was performed using PLINK version1.07[23]. The haplotypes were inferred using Haploview 4.0[24]. The pairwise linkage disequilibrium (LD) among the SNPs was assessed using Haploview 4.0. The case-control comparisons of the haplotype distributions were carried out by applying the inbuilt permutation test based on 10 000 permutations. SPSS, version 15.0 (Chicago, IL, United States) was used for all the statistical analyses.

RESULTS

Characteristics of the study population

A total of 296 incident patients with gastric cancer and 160 incident patients with gastritis were enrolled in this case-control study. Table 2 shows that the distributions of sex between the two groups were not significantly dif-

Table 2 Baseline clinical characteristics of cases and controls *n* (%)

	GC group (*n* = 296)	GA group (*n* = 160)	*P*[1]
Sex			0.455
Male	222 (75.0)	125 (78.1)	
Female	74 (25.0)	35 (21.9)	
Helicobacter pylori infection			0.946
Positive	221 (74.7)	119 (74.4)	
Negative	75 (25.3)	41 (25.6)	
Age (yr)			0.193
≤ 55	131 (43.9)	81 (50.6)	
> 55	165 (56.1)	79 (49.4)	
Histological type			
Intestinal	129 (43.6)		
Diffuse	125 (42.2)		
Unknown	42 (14.2)		

[1]Two-sided χ^2 test. GC: Gastric cancer group; GA: Gastritis group.

ferent. The age groups distribution between the gastric cancer patients and gastritis controls was also similar. The percentage of patients having *H. pylori* infection was almost the same in both the cases and controls. Among the gastric cancer cases, 129 (44%) were intestinal-type cancer, 125 (42%) were diffuse-type cancer, and 42 (14%) were unknown histology-type cases.

Genetic association of the polymorphisms in NOD1 and NOD2 with gastric cancer

The distribution of each of the eight SNPs genotyped in the gastric cancer and gastritis group fitted the Hardy-Weinberg equilibrium law except for NOD1 rs7789045. For rs7789045, this Hardy-Weinberg equilibrium option is available for the gastritis subjects (χ^2 = 0.735, *P* = 0.391) but not for the gastric cancer subjects (χ^2 = 5.221, *P* = 0.022). The major allele homozygotes in all the SNPs were used as the reference genotypes. There were no significant differences between the gastric cancer case and gastritis control in the genotype frequency of the 4 polymorphisms of NOD2 gene. For NOD1 gene, the rs2907749 GG homozygote genotype and the recessive model (genotype GG *vs* GA + AA) showed a reduced risk for gastric cancer (adjusted OR, 0.50, 95% CI: 0.26-0.95, *P* = 0.04 and adjusted OR, 0.52, 95% CI: 0.28-0.96, *P* = 0.04, respectively), whereas the rs7789045 TT homozygote genotype and both the dominant model (genotype TT + TA *vs* AA) and the recessive model (genotype TT *vs* TA + AA) showed an elevated risk for gastric cancer (adjusted OR, 2.14, 95% CI: 1.20-3.82, *P* = 0.01; adjusted OR, 1.50, 95% CI: 1.01-2.22, *P* = 0.04 and adjusted OR, 1.87, 95% CI: 1.09-3.20, *P* = 0.02, respectively) (Table 3).

We next examined the joint effects of NOD1, NOD2 polymorphisms and *H. pylori* infection. Because of the limited number in *H. pylori* seronegative subjects (with 75 and 41 subjects in gastric cancer and gastritis groups, respectively), only the *H. pylori* seropositive subjects were considered for analysis. Logistic regression analysis showed that the NOD1 rs7789045 TT homozygote and the recessive model (genotype TT *vs* TA+AA) carriers had an elevated risk for gastric cancer, with adjusted OR, 2.05, 95% CI: 1.07-3.94, *P* = 0.03 and adjusted OR, 2.06, 95% CI: 1.13-3.76, *P* = 0.02, respectively. For the NOD2 gene, the rs3135500 AG heterozygote genotype had an increased risk for gastric cancer in *H. pylori*-positive subjects (adjusted OR, 2.65, 95% CI: 1.02-6.89, *P* = 0.05). Both the GC heterozygote and the dominant model (genotype CC+GC *vs* GG) of rs7205423 showed an elevated risk for gastric cancer (adjusted OR, 2.52, 95% CI: 1.05-6.04, *P* = 0.04 and adjusted OR, 2.38, 95% CI: 1.03-5.48, *P* = 0.04, respectively). We examined the association of the gene variations in NOD1 and NOD2 with intestinal-type and diffuse-type gastric cancer as well. The results showed that the NOD1 rs2907749 AA homozygote and the dominant model (genotype AA+GA *vs* GG) carriers (adjusted OR, 2.66, 95% CI: 1.10-6.44, *P* = 0.03 and adjusted OR, 2.47, 95% CI: 1.05-5.81, *P* = 0.04, respectively) together with the rs7789045 TT homozygote and the recessive model (genotype TT *vs* TA+AA) carriers (adjusted OR 2.97, 95% CI: 1.49-5.95, *P* < 0.01 and adjusted OR 2.53, 95% CI: 1.35-4.74, *P* < 0.01, respectively) had a significantly elevated risk for developing diffuse-type gastric cancer. When *H. pylori* infection and the gastric cancer type were considered simultaneously, both the recessive model of rs2075820 (genotype GG *vs* GA+AA) and the rs7789045 TT homozygote or the recessive model (genotype TT *vs* TA+AA) in the NOD1 gene showed a significantly elevated risk for developing diffuse-type gastric cancer in *H. pylori*-positive subjects (adjusted OR 1.89, 95% CI: 1.07-3.32, *P* = 0.03; adjusted OR 3.00, 95% CI: 1.38-6.53, *P* < 0.01; and adjusted OR 2.91, 95% CI: 1.45-5.87, *P* < 0.01, respectively) (Table 4).

Haplotype and diplotype analysis of NOD1 tag single nucleotide polymorphisms selection

In a linkage disequilibrium analysis for all of the polymorphisms, we found suggestive evidence for the linkage of rs2907749, rs2075820 and rs7789045 polymorphisms (for rs2907749 and rs2075820, D':0.935, LOD:19.93, r2:0.168; for rs2075820 and rs7789045, D':1.0, LOD:49.28, r2:0.307; for rs2907749 and rs7789045, D':0.949, LOD:38.97, r2:0.251) in the NOD1 gene. The five common haplotypes (AGT, AAA, GGA, GAA and AGA) in the gastritis control group accounted for 99% of all haplotypes (Table 5). The most common haplotype was AGT, occurring in 42% and 33% of the case and control groups, respectively, and the distribution of AGT was significantly different between cases and controls (*P* = 0.01).

For the NOD1 gene, the diplotypes with frequencies > 5% include AGT/AAA, AAA/GGA, AGT/GGA, AGT/AGT, GGA/GGA and AAA/AAA, which accounted for 94% of all the diplotypes in the controls. Using the most common diplotype AGT/AAA as a reference group, our data showed that diplotype AGT/AGT was significantly associated with elevated gastric cancer risk, with OR, 1.98, 95% CI: 1.04-3.79, *P* = 0.04 (Table 6).

Table 3 Adjusted odds ratios for gastric cancer associated with NOD1 and NOD2 polymorphisms

Gene	rsID	Chr	1[1]	2[1]	GC group	GA group	AOR[2] (95% CI)			
							Heterozygote	Homozygote	Dominant	Recessive
					11/12/22	11/12/22				
NOD1	rs17159048	7	G	T	1/62/233	3/38/118[3]	0.82 (0.52-1.31)	/	0.78 (0.49-1.22)	/
	rs2907749	7	G	A	23/117/156	22/62/76	0.92 (0.61-1.39)	0.50 (0.26-0.95)[a]	0.81 (0.55-1.19)	0.52 (0.28-0.96)[a]
	rs2075820	7	A	G	32/118/145[3]	12/78/68[3]	0.70 (0.47-1.06)	1.27 (0.62-2.64)	0.78 (0.53-1.16)	1.51 (0.75-3.04)
	rs7789045	7	T	A	65/126/105	21/67/72	1.30 (0.85-1.99)	2.14 (1.20-3.82)[a]	1.50 (1.01-2.22)[a]	1.87 (1.09-3.20)[a]
NOD2	rs2067085	16	G	C	0/46/250	0/24/136	1.04 (0.61-1.78)	/	/	/
	rs1861759	16	C	A	8/69/219	3/43/114	0.79 (0.50-1.23)	1.29 (0.33-4.97)	0.82 (0.53-1.27)	/
	rs3135500	16	A	G	16/111/169	12/51/97	1.22 (0.80-1.85)	0.73 (0.33-1.61)	1.13 (0.76-1.67)	0.68 (0.31-1.47)
	rs7205423	16	G	C	20/135/141	14/61/85	1.31 (0.87-1.96)	0.82 (0.39-1.72)	1.22 (0.82-1.79)	0.72 (0.35-1.48)

[1]"1"designates the minor allele,"2"designates the major allele; [2]ORs were adjusted for the covariates (age, sex and Helicobacter pylori infection); [3]One or two subjects failed to be genotyped. GC: Gastric cancer group; GA: Gastritis group. [a]$P < 0.05$, GC *vs* GA.

Table 4 Association of the risk single nucleotide polymorphisms in NOD1and NOD2 with two major gastric cancer types and *Helicobacter pylori* infection status

Type	Genotype			Heterozygote		Homozygote		Dominant model		Recessive model	
	AA[1]	Aa	aa	AOR[2] (95% CI)	*P*	AOR (95% CI)	*P*	AOR (95% CI)	*P*	AOR (95% CI)	*P*
NOD1 rs2907749 risk allele:A											
Diffuse 125 cases	68	49	8	2.24 (0.91-5.53)	0.08	2.66 (1.10-6.44)	0.03[a]	2.47 (1.05-5.81)	0.04[a]	1.39 (0.86-2.24)	0.18
Intestinal 129 cases	66	52	11	1.61 (0.71-3.67)	0.26	1.58 (0.71-3.55)	0.27	1.59 (0.73-3.46)	0.24	1.09 (0.68-1.75)	0.72
160 controls	91	61	8	/	/	/	/	/	/	/	/
NOD1 rs2075820 risk allele:G											
Diffuse 125 cases	60	55	13	0.45 (0.19-1.07)	0.07	0.82 (0.36-1.90)	0.65	0.63 (0.28-1.41)	0.26	1.55 (0.96-2.51)	0.08
Intestinal 128 cases	68	41	16	0.54 (0.22-1.31)	0.17	0.77 (0.32-1.85)	0.56	0.65 (0.28-1.51)	0.32	1.29 (0.79-2.08)	0.31
158 controls	68	78	12	/	/	/	/	/	/	/	/
HP^+ 220 cases	119	78	23	0.39 (0.16-0.99)	0.05	0.674 (0.27-1.68)	0.40	0.53 (0.22-1.28)	0.16	1.47 (0.93-2.31)	0.10
HP^+ diffuse 93 cases	56	27	10	0.38 (0.13-1.13)	0.08	0.85 (0.29-2.46)	0.76	0.60 (0.21-1.70)	0.34	1.89 (1.07-3.32)	0.03[a]
HP^+ 117 controls	52	58	7	/	/	/	/	/	/	/	/
NOD1 rs7789045 risk allele:T											
Diffuse 125 cases	32	51	42	1.36 (0.79-2.33)	0.26	2.97 (1.49-5.95)	0.00[a,b]	1.719 (1.045-2.828)	0.03[a]	2.53 (1.35-4.74)	0.00[a,b]
Intestinal 129 cases	22	60	47	1.39 (0.83-2.32)	0.21	1.55 (0.76-3.16)	0.23	1.42 (0.88-2.31)	0.15	1.31 (0.67-2.53)	0.43
160 controls	20	85	55	/	/	/	/	/	/	/	/
HP^+ 221 cases	56	86	79	0.99 (0.60-1.62)	0.96	2.05 (1.07-3.94)	0.03[a]	1.24 (0.79-1.97)	0.35	2.06 (1.13-3.76)	0.02[a]
HP^+ diffuse 93 cases	28	34	31	1.06 (0.56-2.01)	0.87	3.00 (1.38-6.53)	0.01[a,b]	1.50 (0.84-2.69)	0.17	2.91 (1.45-5.87)	0.00[a,b]
HP^+ 119 controls	17	53	49	/	/	/	/	/	/	/	/
NOD2 rs3135500 risk allele:G											
HP^+ 221 cases	132	79	10	2.65 (1.02-6.89)	0.05[a]	2.20 (0.88-5.51)	0.091	2.35 (0.96-5.79)	0.06	0.98 (0.62-1.55)	0.92
HP^+ 119 controls	73	35	11	/	/	/	/	/	/	/	/
NOD2 rs7205423 risk allele:C											
HP^+ 221 cases	112	97	12	2.52 (1.05-6.04)	0.04[a]	2.26 (0.96-5.35)	0.06	2.38 (1.03-5.48)	0.04[a]	1.04 (0.66-1.63)	0.88
HP^+ 119 controls	61	45	13	/	/	/	/	/	/	/	/

[1]Genotypes are shown as AA for risk allele homozygotes, Aa for heterozygotes and aa for the nonrisk allele homozygotes; [2]ORs were adjusted for the covariates (age, sex and/or *Helicobacter pylori* infection). [a]$P < 0.05$, [b]$P < 0.01$, cases *vs* controls.

The distribution of the NOD1 diplotypes between gastric cancer and gastritis subjects infected with *H. pylori* was significantly different from when the *H. pylori* infection status was not considered. The results showed that the diplotypes AGT/GGA, AGT/AGT and AAA/AAA were significantly associated with elevated gastric cancer risk when compared with the diplotype AGT/AAA, with adjusted OR, 2.14, 95% CI: 1.01-4.51, $P = 0.05$, adjusted OR, 3.07, 95% CI: 1.47-6.41, $P < 0.01$, adjusted OR, 2.96, 95% CI: 1.10-7.92, $P = 0.03$, respectively. The risks of intestinal-type and diffuse-type gastric cancer associated with diplotypes in the NOD1 and NOD2 genes were also estimated. The results showed that AGT/AGT was significantly associated with elevated diffuse-type gastric cancer risk compared with the diplotype AGT/AAA, with adjusted OR, 2.56, 95% CI: 1.17-5.58, $P = 0.02$. The risk of diffuse-type gastric cancer related to the NOD1 diplotype AGT/AGT was further examined with stratification by *H. pylori* infection. As expected, the OR value of diffuse-type gastric cancer with *H. pylori* infection for subjects carrying the AGT/AGT diplotype was 4.02, 95% CI: 1.61-10.05, $P < 0.01$, which was higher than that of the *H. pylori* infection group or diffuse-type group alone (Table 7). The NOD2 polymorphism was associated with neither the intestinal-type nor the diffuse-type gastric cancer in this study.

Table 5 Frequencies of haplotype of NOD1

Haplotype[1]	Frequency		*P*	*P* corrected[2]
	GC (*n* = 296)	GA (*n* = 160)		
AGT	0.42	0.33	0.01[a]	0.04
AAA	0.29	0.32	0.37	0.84
GGA	0.25	0.32	0.02[a]	0.08
GAA	0.02	< 0.01	0.05	0.23
AGA	0.01	0.01	0.55	0.96

[1]The order of the haplotype is rs2907749, rs2075820 and rs7789045; [2]Corrected by 10 000 times permutation test. GC: Gastric cancer group; GA: Gastritis group. [a]$P < 0.05$, GC *vs* GA.

Table 6 Associations of NOD1 diplotypes and gastric cancer risk *n* (%)

Diplotype	GC (*n* = 296)	GA (*n* = 160)	AOR[1] (95% CI)	*P*
AGT/AAA	58 (20)	38 (24)	1	
AAA/GGA	47 (16)	35 (22)	0.87 (0.48-1.59)	0.65
AGT/GGA	54 (18)	25 (16)	1.45 (0.77-2.74)	0.25
AGT/AGT	63 (21)	21 (13)	1.98 (1.04-3.80)	0.04[a]
GGA/GGA	22 (7)	20 (13)	0.72 (0.34-1.50)	0.38
AAA/AAA	30 (10)	12 (8)	1.69 (0.76-3.72)	0.20
Others	22 (7)	9 (6)	1.58 (0.65-3.81)	0.31

[1]ORs were adjusted for the covariates (age, sex and *Helicobacter pylori* infection). GC: Gastric cancer group; GA: Gastritis group. [a]$P < 0.05$, GC *vs* GA.

DISCUSSION

In the present study, we investigated the association between NOD1 and NOD2 gene polymorphisms and the risk of gastric cancer in a Chinese population. To clarify the impact of genetic variation in NOD1 and NOD2 on the difference of *H. pylori*-related clinical outcomes, gastritis patients and gastric cancer patients were selected as cases and controls. We found that subjects who carried the NOD1 rs7789045 TT genotype had an increased risk for gastric cancer. Furthermore, the risk was even more distinct when stratified by *H. pylori* infection and in the diffuse-type gastric cancer group. Moreover, individuals with certain haplotypes and diplotypes derived from three TagSNPs of the NOD1 gene had a significantly elevated risk of gastric cancer, suggesting that the combined effects of several SNPs may be detected by haplotype-based analyses. To our best knowledge, this is the first study investigating the impact of the NOD1 and NOD2 polymorphisms on susceptibility to gastric cancer in a Chinese population.

NOD1 consists of a C-terminal LRR (Leucine-rich region), a central NOD, and an N-terminal CARD (caspase-activating domain) domain[14]. NOD1 has emerged as a crucial factor for maintaining a basal level of immune activation. Clarke *et al*[25] showed the important role for peptidoglycan in priming systemic innate immunity and for NOD1 as a homeostatic regulator. The majority of patients with *H. pylori*-associated gastritis have higher NOD1 expression in gastric epithelial cells as compared with controls or *H. pylori*-non-associated gastritis[21], which suggests the involvement of NOD1 signaling in the development of human gastric inflammation. Recently, it has been demonstrated that *H. pylori* virulence factors and the NOD1 receptor ubiquitin-activating enzyme E1 accumulated in human superficial-foveolar epithelium and its metaplastic or dysplastic foci in a discrete cytoplasmic structure named the particle-rich cytoplasmic structure (PaCS). PaCS modulates immune-inflammatory and proliferative responses of the gastric epithelium of potential pathologic relevance[26]. Therefore, the function alteration of NOD1 due to the gene polymorphisms may contribute to the development of *H. pylori*-related gastric cancer.

It has been suggested that the AA homozygote of the E266K (rs2075820) NOD1 gene polymorphism increases the risk of peptic ulceration in *H. pylori*-positive patients in the Hungarian population[18]. Another report indicated that E266K A allele carriers have an increased risk of occurrence of intestinal metaplasia and atrophy and eradication failure in the Turkish population[19]. The rs2075820 SNP was chosen in the coding sequence of the NOD1 gene in exon 3 as it was earlier reported to encode a changed protein (E266K) in the nucleotide-binding domain altering a glutamic acid residue, suggesting a potential functional effect of the mutation[27]. Our result indicated that the AG heterozygote of rs2075820 was protective against the risk of gastric cancer ($P = 0.026$) while the AA homozygote showed moderate risk of gastric cancer ($P = 0.397$) in the *H. pylori*-positive subjects. There are no exact data that demonstrate how the NOD1 polymorphism alters the function of NOD1, but our results suggest that the change of negatively-charged glutamine to positively-charged lysine may cause a drastic change in the structure or regulation of the NOD1 protein that alters the reactivity to *H. pylori* or the nature of downstream inflammatory pathways.

Two studies focusing on the association of several NOD1 polymorphisms with colorectal and endometrial cancer, which include SNP rs2907749, did not find any relationship between individual NOD1 genotypes and the susceptibility to these cancers[28,29]. However, an association of the NOD1 polymorphisms with atopic eczema in the German population has been reported in a study that examined the effects of 11 SNPs, which covering the complete NOD1 gene, on atopy phenotypes[30]. One NOD1 haplotype and three polymorphisms (rs2907748, rs2907749, and rs2075822) were significantly associated with atopic eczema in a population-based cohort, case-control population, and/or family-based association analysis. The results indicated that genetic variants within the NOD1 gene were important determinants of atopy susceptibility. Especially, it showed that the A allele at rs2907749 is significantly associated with elevated IgE levels. Similarly, our study found that the A allele at rs2907749 elevated the risk of gastric cancer; moreover, the risk association was strengthened in diffuse-type gastric cancer patients. Rs2907749 is located in intron 9 of the NOD1 gene where two putative transcription factor-

Table 7 Associations of NOD1 diplotypes and gastric cancer with two major types and *Helicobacter pylori* infection status

	Diplotype						
	AGT/AAA	AAA/GGA	AGT/GGA	AGT/AGT	GGA/GGA	AAA/AAA	Others
HP$^+$ GC (n = 221)	34 (15)	35 (16)	41 (19)	54 (24)	18 (8)	22 (10)	17 (8)
HP$^+$ GA (n = 119)	32 (27)	22 (18)	18 (15)	17 (14)	16 (13)	7 (6)	7 (6)
AOR[1] (95% CI)	1	1.49 (0.72-3.08)	2.14 (1.01-4.51)[a]	3.07 (1.47-6.41)[a]	1.09 (0.47-2.53)	2.96 (1.10-7.92)[a]	2.44 (0.89-6.71)
P		0.28	0.05[a]	0.00[2,a,b]	0.83	0.03[a]	0.08
Diffuse GC (n = 125)	21 (17)	17 (14)	26 (21)	30 (24)	8 (6)	16 (13)	7 (6)
GA (n = 160)	38 (24)	35 (22)	25 (16)	21 (13)	20 (13)	12 (8)	9 (6)
AOR (95% CI)	1	0.84 (0.38-1.88)	1.66 (0.76-3.61)	2.56 (1.17-5.58)[a]	0.66 (0.24-1.77)	2.01 (0.78-5.16)	1.33 (0.43-4.14)
P		0.68	0.20	0.02[a]	0.41	0.15	0.62
Intestinal GC (n = 129)	29 (23)	22 (17)	25 (19)	22 (17)	11 (9)	12 (9)	8 (6)
GA (n = 160)	38 (24)	35 (22)	25 (16)	21 (13)	20 (13)	12 (8)	9 (6)
AOR (95% CI)	1	0.83 (0.40-1.73)	1.64 (0.76-3.54)	1.43 (0.65-3.14)	0.84 (0.34-2.06)	1.62 (0.619-4.26)	1.13 (0.38-3.34)
P		0.62	0.20	0.38	0.69	0.32	0.83
HP$^+$ diffuse GC (n = 93)	12 (13)	13 (14)	19 (20)	26 (28)	7 (8)	10 (11)	6 (7)
HP$^+$ GA (n = 119)	32 (27)	22 (19)	18 (15)	17 (14)	16 (13)	7 (6)	7 (6)
AOR (95% CI)	1	1.42 (0.53-3.76)	2.36 (0.91-6.08)	4.02 (1.61-10.05)[a]	1.09 (0.35-3.38)	3.12 (0.94-10.40)	2.30 (0.63-8.44)
P		0.48	0.08	0.00[2,a,b]	0.88	0.06	0.21

[1]ORs were adjusted for the covariates (age, sex and/or *Helicobacter pylori* infection); [2]Remained significant after Bonferroni adjustment for multiple comparisons. GC: Gastric cancer group; GA: Gastritis group. [a]$P < 0.05$, [b]$P < 0.01$, GC *vs* GA.

binding sites for Pax1 are found. The alteration of the allele changes the computer-predicted Pax1-binding probability next to exon 9, which may influence the T-regulatory cell development[30].

The NOD1 rs7789045 TT genotype was at a significantly elevated risk for gastric cancer in this study. Few studies have addressed the relationship of the polymorphism in rs7789045 with clinical diseases before, whereas our result indicated that this polymorphism was worthy to be further studied because it may play important roles in the *H. pylori*-related gastric carcinogenesis. Rs7789045 is located in intron 5 of the NOD1 gene, which is in the splicing region. The possible significance of T/A alteration was predicted by NetGene2 and SpliceView computer programs[31-33]. Different splicing may lead to the alteration of NOD1 caspase activity in the CARD domain; therefore, this may imply a difference in the signal pathway regulation downstream.

Because haplotype analyses may be of higher informative value to draw associations between the phenotypes and genetic variation than SNPs[34], we also assessed the effects of haplotypes and diplotypes in our studies. Analyses revealed significant association between the NOD1 haplotype AGT (rs2907749, rs2075820, and rs7789045) and gastric cancer, and the difference remained significant after a 10 000-times permutation test. Our results also showed that the AGT/AGT diplotype was associated with an increased risk of diffuse-type gastric cancer, and the risk was more evident in *H. pylori*-positive subjects. Some studies have observed the inverse associations of *H. pylori* and atopic diseases such as asthma and atopic eczema[35,36]. Epidemiological observations are consistent with the hypothesis that *H. pylori*, which has been colonizing the human stomach for ⩾ 58 000 years and is usually acquired within the first few years of life, may play distinct roles in the maturation of the immune system[37]. Weidinger *et al*[30] demonstrated that the haplotype A-G-T-A-C-C-G-T-A-C-G, defined by the eleven polymorphic alleles of NOD1 including rs2907749 A allele and rs2075818 C allele, rs2235099 C allele, rs2075821 G allele, the last three of which are in a linkage with rs2075820 G allele in the AGT haplotype, is significantly protective against the development of atopic eczema, whereas haplotype AGT is associated with an increased risk of *H. pylori*-related gastric cancer in this study. The different relationships of the similar haplotype of NOD1 between two diseases may imply the distinct roles of NOD1 in the pathogenesis of atopy and gastric cancer.

Some studies have investigated the relationships among the three major mutations, R702W (rs2066844), G908R (rs2066845) and 3020insC (rs5743293), in the coding region of the NOD2 gene with colorectal cancer[38], gastric cancer[20] and gastrointestinal diseases[22] in the European population. The results showed that NOD2 polymorphisms increase the susceptibility to gastrointestinal cancer. These three polymorphisms were shown to be monomorphic sites in the Chinese population due to the ethnic difference (Hapmap and Genome Variation Server). In this study, the association between the other four NOD1 SNPs and gastric cancer was investigated in the Chinese population. Although no significant differences on genotype distribution were found between gastric cancer and gastritis patients, our results indicated that the AG heterozygote genotype of rs3135500 and CC genotype of rs7205423 were associated with an increased risk for gastric cancer in *H. pylori*-positive subjects. It is notable that rs3135500 is located in the 3'UTR of the NOD2 gene while rs7205423 is located in the intergenic region between the NOD2 gene and the CYLD gene. The latter is a de-ubiquitinating enzyme that inhibits the activation of the NF-κB, which has key roles in inflammation, immune responses, carcinogenesis, and protection against apoptosis[39]. And the G allele of rs7205423 may be at a splice site, which was predicted by NetGene2

and SpliceView computer programs[31-33]. Another article of Weidinger *et al*[40] showed that the presence of the A allele at rs3135500 was significantly associated with an increased risk of developing asthma. On the contrary, our results showed that A allele at rs3135500 was associated with a slightly reduced risk of developing gastric cancer. The results of the two association studies of the NOD2 polymorphisms were in accordance with those of the NOD1 polymorphisms we mentioned above. These results emphasized that polymorphisms of NOD1 and NOD2 may contribute differently to the development of atopic diseases and gastric cancer.

Our study has some limitations. The number of participants in this study was relatively small, and thus, future replication studies with large cohorts are needed. Further expression analysis and transcription factor-binding studies are needed to clarify the functional role of NOD1 and NOD2 polymorphisms. Finally, *H. pylori* is genetically a highly diverse bacteria, and the virulence of *H. pylori* is related to different subtypes that contribute differently to clinical outcomes. However, anti-CagA antibodies were not available in our study.

In conclusion, to our knowledge, this study is the first one to indicate that the NOD1 rs7789045 polymorphism increases the genetic susceptibility of gastric cancer in a Chinese population, and it is observed to be enhanced in *H. pylori*-positive and diffuse-type gastric cancer subjects. The other two polymorphisms, rs2907749 and rs2075820, showed an association with gastric cancer as well. In addition, *H. pylori*-positive subjects carrying the NOD2 rs7205423 C allele have an increased risk of gastric cancer. These findings suggest that the polymorphisms of the NOD1 and NOD2 genes may play a role between *H. pylori* infection and development of gastric cancer. The underlying mechanism needs further investigation.

COMMENTS

Background

The role of *Helicobacter pylori* (*H. pylori*) in the development of gastric cancer has been confirmed. It is known that *H. pylori* is an important factor in both the induction of gastritis and the histological progression to gastric cancer. The NOD (nucleotide-binding oligomerization domain) proteins NOD1 and NOD2 play distinct roles in innate immunity as sensors of *H. pylori* components derived from bacterial peptidoglycan. The *H. pylori* infection may interact with the polymorphisms of NOD1 and NOD2, which influence the development of gastric cancer. In this hospital-based case-control study, the author analyzed the associations between the polymorphisms of NOD1 and NOD2 and the risk for *H. pylori*-related gastric cancer in a Chinese population.

Research frontiers

It has been confirmed that the *H. pylori* peptidoglycan delivered by the type Ⅳ secretion system can be sensed *via* NOD1. The polymorphisms of NOD2 was associated with gastric lymphoma. The current study is the first to access the impact of the TagSNPs of NOD1 and NOD2 and disease susceptibility to gastric cancer in a Chinese population.

Innovations and breakthroughs

This study indicated that genetic polymorphisms of NOD1 and NOD2 may interaction with *H. pylori* infection and may play distinct roles in developing gastric cancer in the Chinese population.

Applications

This is an original report of the association between NOD1 and NOD2 polymorphisms and Chinese patients with gastric cancer. It is believed these findings will be valuable in clarifying the relationship between genetic variation within innate immune molecules and *H. pylori* infection-related gastric cancer.

Peer review

The study examined NOD1/NOD2 polymorphisms in association with *H. pylori* infection in the patients of gastric cancer (296) *vs* gastritis (160). The results indicate that *H. pylori*-induced gastric cancer is associated with the genetic background of the patients. The data are useful. The study is in focus but can be expanded to include more factors such as smoking status, body mass index, age, *etc*. The written English needs some improvement.

REFERENCES

1 **Wang KJ**, Wang RT. [Meta-analysis on the epidemiology of Helicobacter pylori infection in China]. *Zhonghua Liuxingbingxue Zazhi* 2003; **24**: 443-446
2 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044
3 **Sun XD**, Mu R, Zhou YS, Dai XD, Zhang SW, Huangfu XM, Sun J, Li LD, Lu FZ, Qiao YL. [Analysis of mortality rate of stomach cancer and its trend in twenty years in China]. *Zhonghua Zhongliu Zazhi* 2004; **26**: 4-9
4 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867
5 **Fox JG**, Wang TC. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 2007; **117**: 60-69
6 **Shacter E**, Weitzman SA. Chronic inflammation and cancer. *Oncology* (Williston Park) 2002; **16**: 217-226, 229; discussion 230-232
7 **Vieth M**, Stolte M. Elevated risk for gastric adenocarcinoma can be predicted from histomorphology. *World J Gastroenterol* 2006; **12**: 6109-6114
8 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. *Nature* 2001; **412**: 99
9 **El-Omar EM**, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003; **124**: 1193-1201
10 **Lochhead P**, El-Omar EM. Helicobacter pylori infection and gastric cancer. *Best Pract Res Clin Gastroenterol* 2007; **21**: 281-297
11 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371
12 **Inohara C**, Nuñez G. NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu Rev Biochem* 2005; **74**: 355-383
13 **Inohara N**, Nuñez G. NODs: intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol* 2003; **3**: 371-382
14 **Strober W**, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* 2006; **6**: 9-20
15 **Girardin SE**, Tournebize R, Mavris M, Page AL, Li X, Stark GR, Bertin J, DiStefano PS, Yaniv M, Sansonetti PJ, Philpott DJ. CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive Shigella flexneri. *EMBO Rep* 2001; **2**: 736-742
16 **Hirata Y**, Ohmae T, Shibata W, Maeda S, Ogura K, Yoshida H, Kawabe T, Omata M. MyD88 and TNF receptor-associated factor 6 are critical signal transducers in Helicobacter pylori-infected human epithelial cells. *J Immunol* 2006; **176**: 3796-3803
17 **Kobayashi KS**, Chamaillard M, Ogura Y, Henegariu O, Ino-

hara N, Nuñez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731-734
18 **Hofner P**, Gyulai Z, Kiss ZF, Tiszai A, Tiszlavicz L, Tóth G, Szõke D, Molnár B, Lonovics J, Tulassay Z, Mándi Y. Genetic polymorphisms of NOD1 and IL-8, but not polymorphisms of TLR4 genes, are associated with Helicobacter pylori-induced duodenal ulcer and gastritis. *Helicobacter* 2007; **12**: 124-131
19 **Kara B**, Akkiz H, Doran F, Bayram S, Erken E, Gumurdullu Y, Sandikci M. The significance of E266K polymorphism in the NOD1 gene on Helicobacter pylori infection: an effective force on pathogenesis? *Clin Exp Med* 2010; **10**: 107-112
20 **Angeletti S**, Galluzzo S, Santini D, Ruzzo A, Vincenzi B, Ferraro E, Spoto C, Lorino G, Graziano N, Calvieri A, Magnani M, Graziano F, Pantano F, Tonini G, Dicuonzo G. NOD2/CARD15 polymorphisms impair innate immunity and increase susceptibility to gastric cancer in an Italian population. *Hum Immunol* 2009; **70**: 729-732
21 **Rosenstiel P**, Hellmig S, Hampe J, Ott S, Till A, Fischbach W, Sahly H, Lucius R, Fölsch UR, Philpott D, Schreiber S. Influence of polymorphisms in the NOD1/CARD4 and NOD2/CARD15 genes on the clinical outcome of Helicobacter pylori infection. *Cell Microbiol* 2006; **8**: 1188-1198
22 **Yazdanyar S**, Nordestgaard BG. NOD2/CARD15 genotype and common gastrointestinal diseases in 43,600 individuals. *J Intern Med* 2010; **267**: 228-236
23 **Purcell S**, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559-575
24 **Barrett JC**, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263-265
25 **Clarke TB**, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat Med* 2010; **16**: 228-231
26 **Necchi V**, Sommi P, Ricci V, Solcia E. In vivo accumulation of Helicobacter pylori products, NOD1, ubiquitinated proteins and proteasome in a novel cytoplasmic structure. *PLoS One* 2010; **5**: e9716
27 **Zouali H**, Lesage S, Merlin F, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Christensen S, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Chamaillard M, Thomas G, Hugot JP. CARD4/NOD1 is not involved in inflammatory bowel disease. *Gut* 2003; **52**: 71-74
28 **Ashton KA**, Proietto A, Otton G, Symonds I, McEvoy M, Attia J, Scott RJ. Toll-like receptor (TLR) and nucleosome-binding oligomerization domain (NOD) gene polymorphisms and endometrial cancer risk. *BMC Cancer* 2010; **10**: 382
29 **Möckelmann N**, von Schönfels W, Buch S, von Kampen O, Sipos B, Egberts JH, Rosenstiel P, Franke A, Brosch M, Hinz S, Röder C, Kalthoff H, Fölsch UR, Krawczak M, Schreiber S, Bröring CD, Tepel J, Schafmayer C, Hampe J. Investigation of innate immunity genes CARD4, CARD8 and CARD15 as germline susceptibility factors for colorectal cancer. *BMC Gastroenterol* 2009; **9**: 79
30 **Weidinger S**, Klopp N, Rummler L, Wagenpfeil S, Novak N, Baurecht HJ, Groer W, Darsow U, Heinrich J, Gauger A, Schafer T, Jakob T, Behrendt H, Wichmann HE, Ring J, Illig T. Association of NOD1 polymorphisms with atopic eczema and related phenotypes. *J Allergy Clin Immunol* 2005; **116**: 177-184
31 **Brunak S**, Engelbrecht J, Knudsen S. Prediction of human mRNA donor and acceptor sites from the DNA sequence. *J Mol Biol* 1991; **220**: 49-65
32 **Hebsgaard SM**, Korning PG, Tolstrup N, Engelbrecht J, Rouzé P, Brunak S. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. *Nucleic Acids Res* 1996; **24**: 3439-3452
33 **Rogozin IB**, Milanesi L. Analysis of donor splice sites in different eukaryotic organisms. *J Mol Evol* 1997; **45**: 50-59
34 **Gabriel SB**, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science* 2002; **296**: 2225-2229
35 **Chen Y**, Blaser MJ. Helicobacter pylori colonization is inversely associated with childhood asthma. *J Infect Dis* 2008; **198**: 553-560
36 **Herbarth O**, Bauer M, Fritz GJ, Herbarth P, Rolle-Kampczyk U, Krumbiegel P, Richter M, Richter T. Helicobacter pylori colonisation and eczema. *J Epidemiol Community Health* 2007; **61**: 638-640
37 **Malaty HM**, El-Kasabany A, Graham DY, Miller CC, Reddy SG, Srinivasan SR, Yamaoka Y, Berenson GS. Age at acquisition of Helicobacter pylori infection: a follow-up study from infancy to adulthood. *Lancet* 2002; **359**: 931-935
38 **Papaconstantinou I**, Theodoropoulos G, Gazouli M, Panoussopoulos D, Mantzaris GJ, Felekouras E, Bramis J. Association between mutations in the CARD15/NOD2 gene and colorectal cancer in a Greek population. *Int J Cancer* 2005; **114**: 433-435
39 **Courtois G**. Tumor suppressor CYLD: negative regulation of NF-kappaB signaling and more. *Cell Mol Life Sci* 2008; **65**: 1123-1132
40 **Weidinger S**, Klopp N, Rümmler L, Wagenpfeil S, Baurecht HJ, Gauger A, Darsow U, Jakob T, Novak N, Schäfer T, Heinrich J, Behrendt H, Wichmann HE, Ring J, Illig T. Association of CARD15 polymorphisms with atopy-related traits in a population-based cohort of Caucasian adults. *Clin Exp Allergy* 2005; **35**: 866-872

S- Editor Shi ZF **L- Editor** A **E- Editor** Zheng XM