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BRIEF ARTICLE

Association of NOD1 (CARD4) insertion/deletion polymorphism with susceptibility to IBD: A meta-analysis

Wei-Guo Lu, Yan-Feng Zou, Xiao-Liang Feng, Feng-Lai Yuan, Yuan-Long Gu, Xia Li, Cheng-Wan Li, Cheng Jin, Jian-Ping Li

Wei-Guo Lu, Feng-Lai Yuan, Xia Li, Department of Clinical Pharmacology, The Third Hospital Affiliated to Nantong University, Wuxi 214041, Jiangsu Province, China
Yan-Feng Zou, Xiao-Liang Feng, Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei 230032, Anhui Province, China
Yuan-Long Gu, Cheng-Wan Li, Cheng Jin, Jian-Ping Li, Department of Hepatobiliary Pancreatic Center, The Third Hospital Affiliated to Nantong University, Wuxi 214041, Jiangsu Province, China
Author contributions: Lu WG, Li JP and Zou YF designed the study, did data interpretation, and wrote the manuscript; Feng XL, Yuan FL, Gu YL and Li X performed the majority of data analysis; Li CW and Jin C were involved in the study design.
Supported by The National Natural Science Foundation of China (30972530)
Correspondence to: Jian-Ping Li, MD, PhD, Department of Hepatobiliary Pancreatic Center, The Third Hospital Affiliated to Nantong University, Wuxi 214041, Jiangsu Province, China. wxsyljp@163.com
Telephone: +86-510-82607561 Fax: +86-510-82607561
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Abstract

AIM: To find evidences about whether NOD1/CARD4 insertion/deletion polymorphism is associated with inflammatory bowel disease by meta-analysis.

METHODS: We surveyed the studies on the association of NOD1/CARD4 insertion/deletion polymorphism with inflammatory bowel disease in PubMed. Meta-analysis was performed for genotypes GG/T *vs* T/T, GG/GG *vs* T/T, GG/T + GG/GG *vs* T/T, GG/GG *vs* T/T + GG/T, and GG allele *vs* T allele in a fixed/random effect model.

RESULTS: We identified 8 studies (6439 cases and 4798 controls) in Caucasian populations using PubMed search. We found no association between NOD1/CARD4 insertion/deletion polymorphism and inflammatory bowel disease, Crohn's disease, and ulcerative colitis. Stratification of cases by age showed that NOD1/CARD4 insertion/deletion polymorphism was associated with inflammatory bowel disease in younger age group at onset (< 40 years) (GG *vs* T: OR = 0.68, 95% CI: 0.50-0.93, P = 0.02; GG/T + GG/GG *vs* T/T: OR = 0.71, 95% CI: 0.59-0.85, P = 0.0003).

CONCLUSION: This meta-analysis demonstrates an association between NOD1/CARD4 insertion/deletion polymorphism and inflammatory bowel disease in the younger age group at onset (< 40 years) in Caucasian populations.

Key words: NOD1; CARD4; Genetic polymorphisms; Inflammatory bowel disease; Meta-analysis

Peer reviewers: AM El-Tawil, MSc, MRCS, PhD, Department of Surgery, University Hospital of Birmingham, East Corridor, Ground Floor, Birmingham, B15 2TH, United Kingdom; Francesco Manguso, MD, PhD, UOC di Gastroenterologia, AORN A. Cardarelli, Via A. Cardarelli 9, Napoli, 80122, Italy

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INTRODUCTION

The inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a com-

mon relapsing condition characterized by both gastrointestinal and systemic manifestations and is responsible for a significant morbidity in both adults and children. Clinical symptoms of IBD include weight loss, abdominal pain, as well as diarrhea accompanied by blood, and disease progression is often accompanied by an increase in granulomas and activated monocytes, which produce significant amounts of eicosanoids and cytokines[1,2]. The etiology of IBD most likely involves a complex interaction of genetic, environmental and immunoregulatory factors[3].

The normal gut consists of an epithelial barrier, the mucosal immune system, and a number of stromal/supportive cells. The external environment comprises native mucosal microbiota, potential pathogenic microorganisms, abundant food antigens and allergens, all of which are encountered mainly at the vast surface areas of mucosal membranes, and forms the most important source of stimulation of the entire immune system. The induction of preventive and protective immune responses to mucosal infectious agents and to inert food antigens and environmental allergens that would limit their absorption, is usually the most emphasized functional aspect of the mucosal immune system[4]. Dysfunctional innate immune response seems important in the pathogenesis of IBD[5]. By means of genome-wide scans, numerous IBD susceptibility loci have been identified[6]. Specific single gene defects have been discovered, including mutations in the leucine rich region (LRR) of the nucleotide-binding oligomerization domain 2 (NOD-2) gene, also known as CARD-15 (caspase activation and recruitment domain 15)[7,8]. The identification of the NOD2 is a breakthrough in IBD genetics, which heralded extensive analyses of signaling pathways of the innate immune system implicated in the pathogenesis of IBD[9,10].

Innate immunity depends on the specific recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs). The NOD protein is a family of intracellular PRRs. After the intracellular PRRs recognized PAMPs, the pro-inflammatory pathways would be activated[11-13]. NOD1 (also known as CARD-4) is a host cytosolic signaling PRR, and acts as a cytosolic receptor for the diaminopimelate (DAP)-containing GlcNAc-tripeptide muropeptide found mostly in Gram-negative bacterial peptidoglycans[14]. NOD1/CARD4 signaling leads to activation of NF-κB, and plays an important role in innate immunity[15]. In 1903, Sutton[16] explained that dominance and recessiveness were features of "chromatin entities" rather than morphological characters. In other words, dominance and recessiveness are properties of genetic information resulting in a certain function rather than the function itself. This means that certain polymorphisms and mutations in NOD1/CARD4 may result in dysfunctional innate immune response during bacterial recognition with direct implications for IBD pathogenesis. Genome-wide scans for IBD linkage demonstrated a susceptibility locus on chromosome 7p14, and the same locus where the NOD1/CARD4 gene is located[17]. Therefore, NOD1/CARD4 gene is a perfect candidate for predisposition to IBD.

NOD1/CARD4 insertion/deletion polymorphism (rs6958571) was identified by Hysi *et al*[18]. Since its discovery in 2005, this polymorphism has attracted widespread attention. A number of case-control studies were conducted to investigate the association of this polymorphism with human IBD[19-25]. However, these studies reported conflicting results. There are several possible explanations for this, such as small sample size, ethnic background, uncorrected multiple hypothesis test, and publication bias.

Meta-analysis is a means of increasing the effective sample size under investigation through the pooling of data from individual association studies, thus enhancing the statistical power of the analysis for the estimation of genetic effects[26]. The aim of the present study was to investigate the association of NOD1/CARD4 insertion/deletion polymorphism with human IBD, using a meta-analysis.

MATERIALS AND METHODS

Identification of eligible studies

Available articles were identified through a literature search using the keywords "nucleotide-binding oligomerization domain 1" or "NOD1" or "caspase activation and recruitment domain 4" or "CARD4" and "polymorphism" in the PubMed database. Additional literature was collected from cross-references within both original and review articles. We only recruited data from the wholly published paper, but not from meeting or conference abstracts. No language restrictions were applied. A study was included in the current meta-analysis if (1) it was published up to December 2009; and (2) it was a case-control study. We excluded the studies containing overlapping data and the family-based studies because our analysis was based on linkage considerations. When there were multiple publications from the same population, only the largest study was included. When a study reported the results on different subpopulations, we took it as a separate study.

Additionally, an independent PubMed search was done (by Lu WG and Feng XL) by the same method. The abstracts were reviewed independently by two investigators (Yuan FL and Gu YL) to determine if they met the eligibility criteria for inclusion. References in the studies were reviewed (by Jin C, Li X and Li CW) to identify additional studies. If discrepancies occurred, a third investigator (Li JP) did an additional assessment.

Data extraction

If original genotype frequency data was unavailable in relevant articles, a request for additional data was sent to the corresponding authors. In addition, two investigators (Feng XL and Li JP) independently extracted the data with the standard protocol and the result was reviewed by a third investigator (Zou YF). Discrepancies were resolved

Table 1 Characteristics of the studies included in the meta-analysis

ID	Study	Yr	Ethnic group	Diseases	Sample size (frequency of GG allele, %)		OR (95%CI) for GG *vs* T allele	Hardy-Weinberg equilibrium of genotype control
					Case	Control		
1	Hancock *et al*[19]	2008	Caucasian	CD	594 (27.1)	1024 (24.6)	1.130 (0.960-1.330)	0.010
2	Cantó *et al*[20]	2007	Caucasian	CD	97 (21.7)	50 (31.0)	0.615 (0.357-1.060)	0.147
3	Henckaerts *et al*[21]	2007	Caucasian	IBD	1052 (24.5)	280 (25.4)	0.952 (0.768-1.180)	0.751
				CD	809 (24.8)		0.973 (0.780-1.214)	
				UC	222 (22.9)		0.878 (0.656-1.175)	
4	Van Limbergen *et al*[22]	2007	Caucasian (Scottish)	IBD	1079 (26.1)	1233 (26.4)	0.984 (0.863-1.089)	0.261
				CD	515 (26.4)		1.003 (0.850-1.182)	
				UC	537 (26.0)		0.985 (0.837-1.160)	
5	Van Limbergen *et al*[22]	2007	Caucasian (Swedish)	IBD	632 (25.3)	277 (23.3)	1.112 (0.880-1.406)	0.741
				CD	244 (24.7)		1.086 (0.817-1.444)	
				UC	388 (25.5)		1.129 (0.875-1.456)	
6	Franke *et al*[23]	2006	Caucasian	IBD	961 (22.4)	841 (21.5)	1.055 (0.900-1.235)	0.958
				CD	633 (21.1)		0.983 (0.822-1.174)	
				UC	332 (24.4)		1.181 (0.955-1.460)	
7	Tremelling *et al*[24]	2006	Caucasian	IBD	1360 (25.2)	758 (25.7)	0.975 (0.844-1.126)	0.580
				CD	641 (24.9)		0.964 (0.812-1.144)	
				UC	665 (25.4)		0.991 (0.837-1.173)	
8	McGovern *et al*[25]	2005	Caucasian	IBD	664 (26.6)	335 (31.8)	0.777 (0.634-0.952)	0.195
				CD	358 (24.4)		0.694 (0.548-0.878)	
				UC	306 (29.1)		0.880 (0.693-1.117)	

OR: Odds ratio; IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

through discussion among our research team. From each study, we extracted the first author's name, year of publication, source of publication, racial ancestry, type of diseases, the number of cases and controls, and the available genotype and allele frequency information from the NOD1/CARD4 insertion/deletion polymorphism.

Meta-analysis methods

Allele frequencies at the NOD1/CARD4 insertion/deletion polymorphism from the respective studies were determined by the allele counting method. A χ^2 test was used to determine if the observed frequencies of genotypes conformed to Hardy-Weinberg equilibrium expectations.

We examined the relationship between the allele and susceptibility to IBD (GG *vs* T), and the genotypes. The following genotype contrasts were included: GG/T + GG/GG *vs* T/T, GG/GG *vs* T/T + GG/T, GG/GG *vs* T/T, and GG/T *vs* T/T. The contrast of GG/T + GG/GG *vs* T/T genotypes corresponds to a dominant genetics effect of the GG allele. The contrast of GG/GG *vs* T/T + GG/T genotypes corresponds to a recessive genetics effect of the GG allele. The odds ratio (OR) and its 95% confidence interval (95% CI) were estimated for each study. The heterogeneity between studies was assessed by the Chi-square test based Q-statistics[27]. A significant Q-statistics ($P < 0.10$) indicated the heterogeneity among studies, and then the result of the random effect model was selected. Otherwise, the result of fixed effect model was selected. We also measured the effect of heterogeneity using the formula: $I^2 = 100\% \times (Q\text{-}df)/Q$[28].

Finally, the pooled OR was obtained by Mantel-Haenszel method in the fixed effect model and by DerSimonian and Laird method in the random effect model[29,30]. The pooled OR was performed, weighting the individual OR by the inverse of their variance. The significance of the pooled OR was determined by the *Z* test.

Evaluation of publication bias

Publication bias was investigated with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test[26]. Analyses were performed using the software Review Manager 4.2 (Cochrane Collaboration, http://www.cc-ims.net/RevMan/relnotes.htm/) and Stata version 10 (StataCorp LP, College Station, Texas, USA). A *P* value less than 0.05 was considered statistically significant, and all the *P* values were two sided.

RESULTS

Characteristics of eligible studies

Characteristics of studies included in the current meta-analysis are presented in Table 1[19-25]. There were 46 papers relevant to the searching words. Through the screening of the abstract, 19 of these articles were excluded (5 were reviews; 4 were not conducted in humans; 10 did not explore NOD1/CARD4 gene polymorphisms), leaving 27 studies for full publication review. Of the 27 studies, 13 without focusing on IBD, were excluded, leaving 14 studies[19-25,31-37] for more detailed assessment. Seven of them were excluded (one was a family-based study; one was a duplicate report; and 5 did not study the NOD1/CARD4 insertion/deletion polymorphism)[31-37]. As a result, 7 studies were included in the current meta-analysis (Figure 1). One of the eligible studies contained data on two dif-

Table 2 Meta-analysis of association between NOD1/CARD4 insertion/deletion polymorphism and inflammatory bowel disease

Polymorphism	Disease	Sample size		*n*	Test of association				Test of heterogeneity		
		Case	Control		OR (95%CI)	*Z*	*P* value	Model	χ^2	*P* value	I^2 (%)
GG *vs* T	Overall	12878	9596	8	0.98 (0.90-1.07)	0.39	0.70	R	12.60	0.08	44.4
	CD	7782	9596	8	0.96 (0.86-1.07)	0.78	0.43	R	14.49	0.04	51.7
	UC	4900	7448	6	1.01 (0.92-1.09)	0.14	0.89	F	5.12	0.40	2.3
	IBD onset < 40	1486	4752	4	0.68 (0.50-0.93)	2.38	0.02	R	8.30	0.04	63.9
GG/T + GG/GG *vs* T/T	Overall	6439	4798	8	1.00 (0.98-1.08)	0.01	0.99	F	10.69	0.15	34.5
	CD	3891	4798	8	0.97 (0.86-1.10)	0.42	0.67	R	12.36	0.09	43.4
	UC	2450	3724	6	1.00 (0.90-1.11)	0.06	0.95	F	4.64	0.46	0.0
	IBD onset < 40	743	2376	4	0.71 (0.59-0.85)	3.65	0.0003	F	4.83	0.18	37.8
GG/GG *vs* T/T + GG/T	Overall	6439	4798	8	0.95 (0.81-1.11)	0.62	0.53	F	12.02	0.10	41.8
	CD	3891	4798	8	0.91 (0.76-1.09)	0.99	0.32	F	11.74	0.11	40.4
	UC	2450	3724	6	1.03 (0.83-1.27)	0.23	0.81	F	4.89	0.43	0.0
	IBD onset < 40	743	2376	4	0.61 (0.28-1.35)	1.22	0.22	R	7.98	0.05	62.3
GG/GG *vs* T/T	Overall	4033	3020	8	0.93 (0.74-1.17)	0.63	0.53	R	12.59	0.08	44.4
	CD	2442	3020	8	0.88 (0.68-1.14)	0.96	0.34	R	12.96	0.07	46.0
	UC	1623	2312	6	0.97 (0.78-1.20)	0.32	0.75	F	3.04	0.69	0.0
	IBD onset < 40	500	1435	4	0.52 (0.22-1.21)	1.52	0.13	R	8.84	0.03	66.0
GG/T *vs* T/T	Overall	2816	2098	8	0.95 (0.80-1.12)	0.64	0.53	F	10.85	0.15	35.5
	CD	1685	2098	8	0.92 (0.76-1.11)	0.92	0.36	F	9.94	0.19	29.6
	UC	1093	1648	6	1.03 (0.83-1.28)	0.27	0.79	F	4.67	0.46	0.0
	IBD onset < 40	290	1112	4	0.94 (0.64-1.37)	0.33	0.74	F	5.95	0.11	49.6

R: Random effect model; F: Fixed effect model; IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

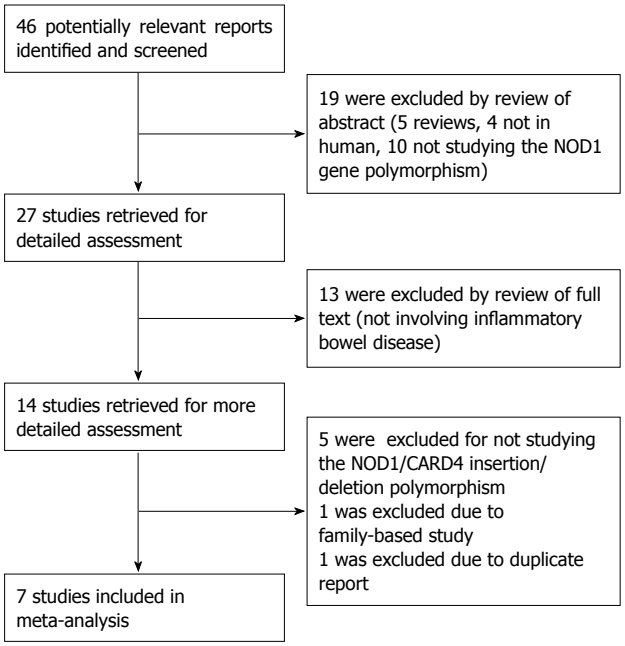

Figure 1 Study selection in Medline.

ferent subpopulations and we treated it independently[22]. Finally, a total of 8 separate studies were considered in the current meta-analysis (Table 1).

We got the data from the corresponding author of the study by Franke *et al*[23]. Thus, the allele and the genotype frequencies of the NOD1/CARD4 insertion/deletion polymorphism were extracted from all the eligible studies. The 8 separate studies were conducted in Caucasian populations. Of these, 8[19-25] were conducted in patients with CD and 6[21-25] were conducted in patients with UC. Meanwhile, 4 studies[20,22,24,25] showed stratified data of cases by age (IBD onset at < 40 years). The results of Hardy-Weinberg equilibrium test for the distribution of the genotype in control populations are shown in Table 1. Only one study belonged to Hardy-Weinberg equilibrium among the eligible studies[19]. The distribution of the genotype in the overall control population was consistent with Hardy-Weinberg equilibrium ($P = 0.240$).

Meta-analysis

The summary of the meta-analysis for the NOD1/CARD4 insertion/deletion polymorphism and IBD is shown in Table 2.

Overall effects

The *Q*-test of heterogeneity was not significant and we conducted analyses using fixed effect models except in the contrasts of GG *vs* T and GG/GG *vs* T/T. We found no association between NOD1/CARD4 insertion/deletion polymorphism and IBD in the overall population (GG *vs* T: OR = 0.98, 95% CI: 0.90-1.07, $P = 0.70$; GG/T + GG/GG *vs* T/T: OR = 1.00, 95% CI: 0.98-1.08, $P = 0.99$; GG/GG *vs* T/T + GG/T: OR = 0.95, 95% CI: 0.81-1.11, $P = 0.53$; GG/GG *vs* T/T: OR = 0.93, 95% CI: 0.74-1.17, $P = 0.53$; GG/T *vs* T/T: OR = 0.95, 95% CI: 0.80-1.12, $P = 0.53$).

Subgroup analyses

We performed group-specific meta-analysis of CD, UC and IBD onset in the populations aged < 40 years.

Analysis of CD population: The *Q*-test of heterogeneity was significant and we conducted analyses using random effect models except in the contrasts of GG/GG *vs* T/T + GG/T and GG/T *vs* T/T. No association of

Table 3 Egger's linear regression test to measure the funnel plot asymmetry[1]

Comparisons	Y axle intercept: a (95%CI)				
	GG *vs* T	GG/T + GG/GG *vs* T/T	GG/GG *vs* T/T + GG/T	GG/GG *vs* T/T	GG/T *vs* T/T
Overall	-1.92 (-5.57-1.72)	0.96 (-2.65-4.58)	2.38 (-0.87-5.64)	2.38 (-1.00-5.77)	2.32 (-0.71-5.36)
CD	-2.62 (-6.54-1.29)	1.60 (-2.38-5.59)	2.79 (-0.20-5.78)	2.89 (-0.31-6.09)	2.51 (-0.29-5.33)
UC	-0.39 (-6.54-5.75)	0.57 (-5.25-6.39)	-0.57 (-7.13-5.97)	-0.43 (-7.00-6.13)	-0.57 (-6.89-5.74)
IBD onset at < 40 yr of age	-2.16 (-8.80-4.47)	1.49 (-4.16-7.15)	1.86 (-4.04-7.77)	1.94 (-4.40-8.29)	1.63 (-3.48-6.75)

[1]All $P > 0.05$. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

NOD1/CARD4 insertion/deletion polymorphism was found with CD (GG *vs* T: OR = 0.96, 95% CI: 0.86-1.07, $P = 0.43$; GG/T + GG/GG *vs* T/T: OR = 0.97, 95% CI: 0.86-1.10, $P = 0.67$; GG/GG *vs* T/T + GG/T: OR = 0.91, 95% CI: 0.76-1.09, $P = 0.32$; GG/GG *vs* T/T: OR = 0.88, 95% CI: 0.68-1.14, $P = 0.34$; GG/T *vs* T/T: OR = 0.92, 95% CI: 0.76-1.11, $P = 0.36$).

Analysis of UC population: The *Q*-test of heterogeneity was not significant and we conducted analyses using fixed effect models in the UC population. No association of NOD1/CARD4 insertion/deletion polymorphism with UC was discovered (GG *vs* T: OR = 1.01, 95% CI: 0.92-1.09, $P = 0.89$; GG/T + GG/GG *vs* T/T: OR = 1.00, 95% CI: 0.90-1.11, $P = 0.95$; GG/GG *vs* T/T + GG/T: OR = 1.03, 95% CI: 0.83-1.27, $P = 0.81$; GG/GG *vs* T/T: OR = 0.97, 95% CI: 0.78-1.20, $P = 0.75$; GG/T *vs* T/T: OR = 1.03, 95% CI: 0.83-1.28, $P = 0.79$).

Analysis of IBD onset in a population aged < 40 years: The *Q*-test of heterogeneity was significant and we conducted analyses using random effect models except in the contrasts of GG/T + GG/GG *vs* T/T, and GG/T *vs* T/T. We found an association between NOD1/CARD4 insertion/deletion polymorphism and IBD in a younger age group at onset (< 40 years) when examining the contrasts of GG *vs* T, and GG/T + GG/GG *vs* T/T (GG *vs* T: OR = 0.68, 95% CI: 0.50-0.93, $P = 0.02$; GG/T + GG/GG *vs* T/T: OR = 0.71, 95% CI: 0.59-0.85, $P = 0.0003$), and the forest plots are shown in Figure 2. However, the association was not found when the contrasts of GG/GG *vs* T/T + GG/T, GG/GG *vs* T/T and GG/T *vs* T/T were examined (GG/GG *vs* T/T + GG/T: OR = 0.61, 95% CI: 0.28-1.35, $P = 0.22$; GG/GG *vs* T/T: OR = 0.52, 95% CI: 0.22-1.21, $P = 0.13$; GG/T *vs* T/T: OR = 0.94, 95% CI: 0.64-1.37, $P = 0.74$).

Evaluation of publication bias

Funnel plot asymmetry was assessed by the method of Egger's linear regression test. If there was asymmetry, the regression line would not run through the origin. The larger its deviation from zero, the more pronounced the asymmetry. The results of Egger's linear regression test are shown in Table 3. It was shown that there was no publication bias (all $P > 0.05$). For the association of NOD1/CARD4 insertion/deletion polymorphism with IBD in the group of younger age at onset (< 40 years), the Egger's linear regression test provided no evidence of publication bias (GG *vs* T: $t = -1.40$, $P = 0.296$; GG/T + GG/GG *vs* T/T: $t = 1.14$, $P = 0.373$) (Figure 3A). Figure 3B shows that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot.

DISCUSSION

The identification of NOD2/CARD15 as a CD susceptibility gene makes its homologous gene NOD1/CARD4 a potential candidate gene for predisposition to IBD[8,38,39]. NOD1/CARD4 is the founding member of the Nod-like receptors (NLRs) family, and is expressed in large and small bowel[18]. It plays an important role in colonic epithelial defenses against intracellular organisms, such as enteroinvasive *E. coli* and *Shigella flexneri*[40,41]. The presence of bacterial flora is essential for IBD to develop in animal models[42]. Antibiotics and fecal diversion are effective therapies for CD[43,44]. NOD1/CARD4 has been mapped to chromosome bands 7p14-p15 (UniGene Cluster Hs 19405), a region which was previously reported to contain an IBD susceptibility locus[17]. Thus, NOD1/CARD4 appeared to be a good candidate for IBD. Recently, many studies have been conducted to test the association of NOD1/CARD4 insertion/deletion polymorphism with IBD, but the association trends observed have been variable with several studies showing an association while others do not[19-25]. It is, therefore, necessary to perform a comprehensive meta-analysis to assess the importance of the NOD1/CARD4 insertion/deletion polymorphism for IBD pathogenesis.

In the present study, we retrieved 8 studies, including 6439 cases and 4798 controls, to evaluate the association of NOD1/CARD4 insertion/deletion polymorphism with IBD in Caucasian populations. Our meta-analysis did not detect the association of NOD1/CARD4 insertion/deletion polymorphism with IBD, CD, and UC in the overall population. However, we did find a significant genetic association between NOD1/CARD4 insertion/deletion polymorphism and IBD in the group of younger age at onset (< 40 years), and GG allele was a protective allele for IBD pathogenesis. As far as we know, this is the first meta-analysis carried out so far which aimed at investigating the association of NOD1/CARD4 insertion/deletion polymorphism with IBD.

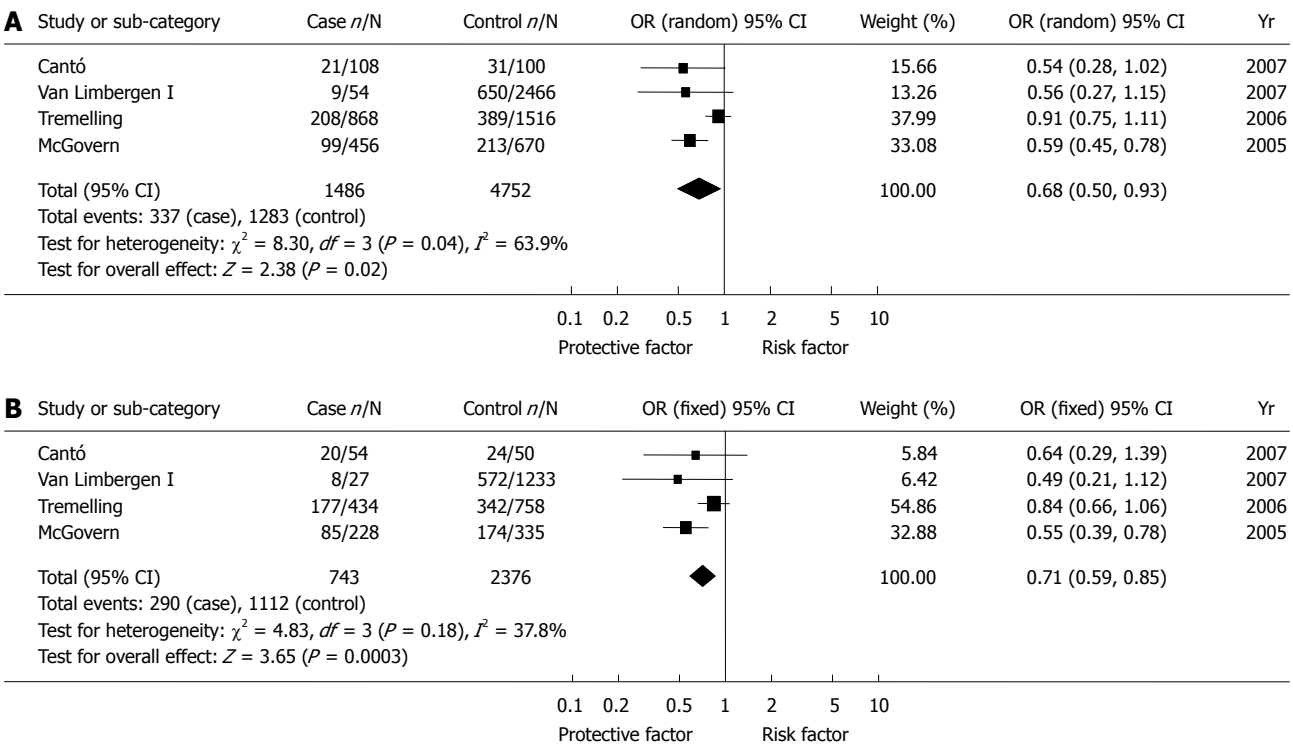

Figure 2 Forest plots for meta-analysis of positive results. Inflammatory bowel disease onset at < 40 years of age. A: GG *vs* T; B: GG/T + GG/GG *vs* T/T.

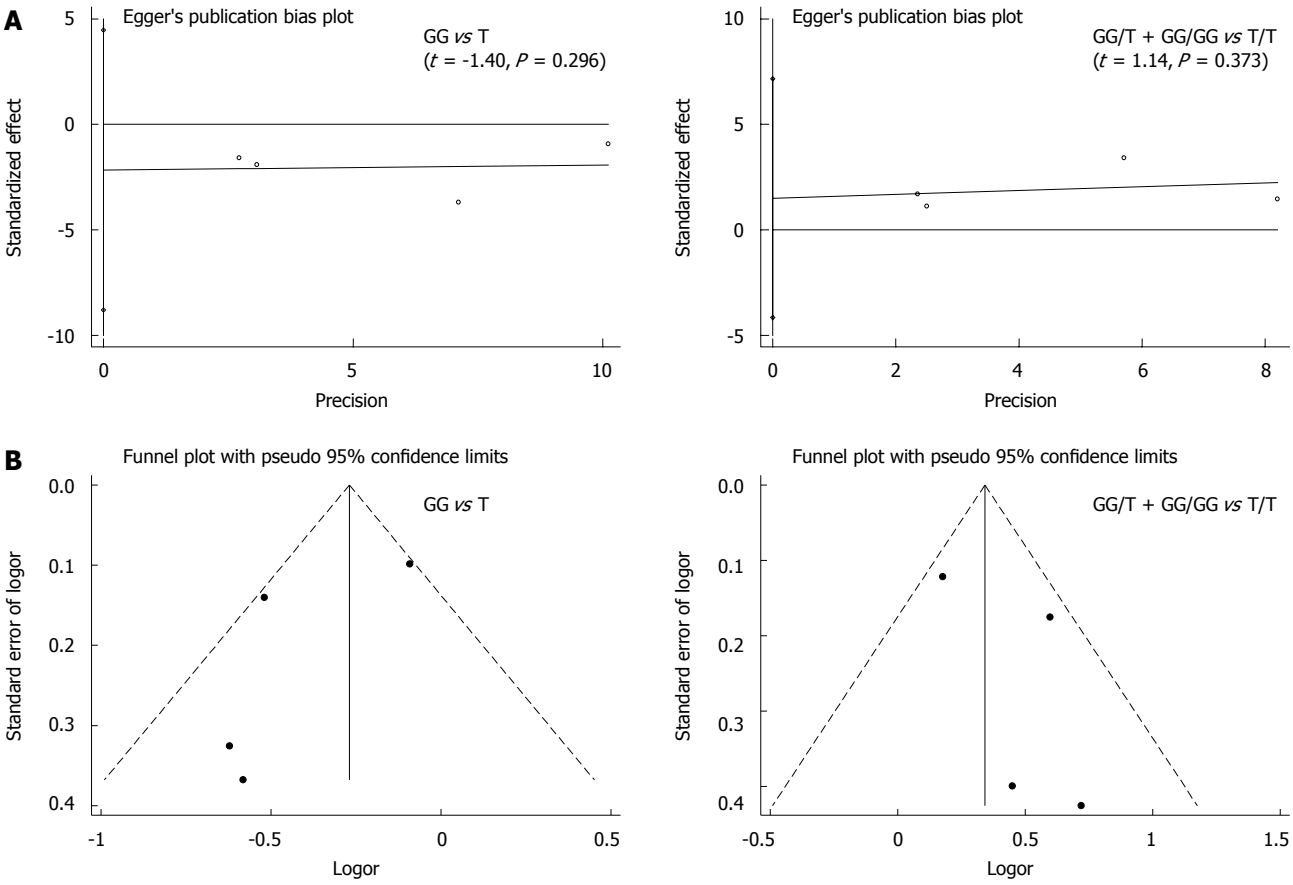

Figure 3 Egger's linear regression test for publication bias of positive results (A), funnel plots for meta-analysis of positive results; inflammatory bowel disease onset at < 40 yeas of age (B).

In our study, we found that the NOD1/CARD4 GG allele decreased the risk of IBD in the group of younger

age at onset (< 40 years), indicating that this locus is important in determining the susceptibility to IBD. The result is not surprising, since NOD1, similar to NOD2, is involved in the recognition of intracellular bacterial PAMPs[45]. The two molecules share structure and functional similarities. NOD1/CARD4 insertion/deletion polymorphism is located at the beginning of intron IX[18]. Hysi *et al*[18] firstly demonstrated an effect of this polymorphism on the binding of an unidentified nuclear protein. Hysi *et al*[18] demonstrated the presence of different isoforms of NOD1 transcripts. A recent study showed that some of these isoforms resulted in disruption of the LRR region critical for NOD1 mediated bacterial sensing[46]. Therefore, although noncoding, this polymorphism may affect immune response with direct implications for IBD pathogenesis either by altered binding of a cis/trans activating protein, resulting in abnormal gene expression, or by the generation of functionally significant splice variants. However, to date, the detailed functions of this polymorphism are still unclear. Further studies on the function of NOD1/CARD4 insertion/deletion polymorphism are required. Of course, the association may result from the direct effect of the polymorphism itself, or through linkage disequilibrium with another functional polymorphism in the structural part of the gene or in regulatory regions. Additionally, the association of NOD1/CARD4 insertion/deletion polymorphism with IBD was only detected in the contrasts of GG *vs* T and GG/T + GG/GG *vs* T/T, indicating that the GG allele of this polymorphism may have a dominant effect on risk for IBD.

Some limitations of this study should be discussed. Firstly, the current meta-analysis only included the wholly-published studies, not the meeting or conference abstracts. Thus, publication bias may have occurred, even though the use of a statistical test did not show it. Secondly, significant heterogeneity between studies was detected in the current meta-analysis, which may distort the analysis. However, it is not a major problem because IBD itself is heterogeneous, and different populations may contribute to the heterogeneity. Thirdly, these results should be interpreted with caution because the population from six countries was not uniform. Fourthly, the analysis in IBD onset in the population aged < 40 years only included four studies (743 cases and 2376 controls), and more studies based on a larger sample size, case-control design and stratification by age are still needed in the future research. Finally, meta-analysis remains retrospective that is subject to the methodological deficiencies of the included studies. Therefore, we minimized the likelihood of bias by developing a detailed protocol before initiating the study by performing a meticulous search for published studies and by using explicit methods for study selection, data extraction and data analysis.

In conclusion, our study demonstrates the association of NOD1/CARD4 insertion/deletion polymorphism with inflammatory bowel disease in the younger age group at onset (< 40 years) in Caucasian populations.

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COMMENTS

Background

Recently, many studies have been conducted to prove the association of NOD1/CARD4 insertion/deletion polymorphism with inflammatory bowel disease (IBD), but the association trends observed have been variable with several studies showing an association while others do not. It is, therefore, necessary to perform a comprehensive meta-analysis to assess the importance of the NOD1/CARD4 insertion/deletion polymorphism for IBD pathogenesis.

Research frontiers

The etiology of IBD most likely involves a complex interaction of genetic, environmental and immunoregulatory factors. The identification of the NOD2 is a breakthrough in IBD genetics, which heralded extensive analyses of signaling pathways of the innate immune system implicated in the pathogenesis of IBD. NOD1/CARD4 signaling leads to activation of nuclear factor-κB, and plays an important role in innate immunity. Certain polymorphisms and mutations in NOD1/CARD4 may result in dysfunctional innate immune response during bacterial recognition with direct implications for IBD pathogenesis.

Innovations and breakthroughs

The authors collected 8 studies (6439 cases and 4798 controls) in Caucasian populations to evaluate whether NOD1/CARD4 insertion/deletion polymorphism is associated with IBD by meta-analysis. They found the association of NOD1/CARD4 insertion/deletion polymorphism with IBD in the younger age group at onset (< 40 years) in Caucasian populations.

Applications

The authors found that the NOD1/CARD4 GG allele decreased the risk of IBD in the younger age group at onset (< 40 years), indicating that this locus is important in determining the susceptibility to IBD. The association of NOD1/CARD4 insertion/deletion polymorphism with IBD was only detected in the contrasts of GG *vs* T and GG/T + GG/GG *vs* T/T, indicating that the GG allele of this polymorphism may have a dominant effect on risk for IBD.

Terminology

Meta-analysis is a means of increasing the effective sample size under investigation through the pooling of data from individual association studies, thus enhancing the statistical power of the analysis.

Peer review

This is a very interesting meta-analytic study dealing with an important topic in IBD.

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