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

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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.

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

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. The etiology of IBD most likely involves a complex interaction of genetic, environmental and immunoregulatory factors.

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. Dysfunctional innate immune response seems important in the pathogenesis of IBD. By means of genome-wide scans, numerous IBD susceptibility loci have been identified. 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). 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.

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. 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. NOD1/CARD4 signaling leads to activation of NF-κB, and plays an important role in innate immunity. In 1903, Sutton 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. Therefore, NOD1/CARD4 gene is a perfect candidate for predisposition to IBD.

NOD1/CARD4 insertion/deletion polymorphism (rs6958571) was identified by Hysi et al. 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. 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. 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
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 $\chi^2$ 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-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.$^{20,30}$ 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.$^{29}$ 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.$^{19-26,31-37}$ 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$^{19-22,31-37}$ 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 disparate populations (Caucasian vs. Asian). We included both studies in the meta-analysis as the same polymorphism was investigated.

#### 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 |
|-----|---------------------|----|--------------|----------|----------------------------------------|-------------------------------|-----------------------------------------------|
| 1   | Hancock et al$^{[20]}$ | 2008 | Caucasian    | CD       | 961 (22.4)                                      | 0.010                         |                                               |
| 2   | Canto et al$^{[21]}$  | 2007 | Caucasian    | CD       | 335 (31.8)                                      | 0.147                         |                                               |
| 3   | Henckaerts et al$^{[22]}$ | 2007 | Caucasian    | IBD      | 641 (24.9)                                      | 0.741                         |                                               |
| 4   | Van Limbergen et al$^{[23]}$ | 2007 | Caucasian (Scottish) | IBD     | 841 (26.6)                                      | 0.985                         | 0.873-1.160                                   |
| 5   | Van Limbergen et al$^{[24]}$ | 2007 | Caucasian (Swedish) | IBD     | 1024 (24.6)                                     | 0.995                         | 0.870-1.160                                   |
| 6   | Franke et al$^{[25]}$  | 2006 | Caucasian    | CD       | 332 (24.4)                                      | 0.958                         |                                               |
| 7   | Tremelling et al$^{[26]}$ | 2006 | Caucasian    | IBD      | 388 (25.5)                                      | 0.958                         |                                               |
| 8   | McGovern et al$^{[27]}$ | 2005 | Caucasian    | IBD      | 332 (25.2)                                      | 0.958                         |                                               |

OR: Odds ratio; IBD: Inflammatory bowel disease; CD: Crohn’s disease; UC: Ulcerative colitis.
different subpopulations and we treated it independently\cite{23}. 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\cite{23,24,25}. 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, 4 studies\cite{20,22,24,25} were conducted in patients with CD and 4 were conducted in patients with UC. Meanwhile, 4 studies\cite{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\cite{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.

### 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 |                      |                       |
| GG vs T      | Overall | 12878       | 9596   | 8                   | OR (95% CI) Z $P$ value Model $\chi^2$ $P$ (%) |
| CD           | 7782    | 9596        | 8      | 0.98 (0.90-1.07) 0.39 0.70 R | 12.60 0.08 44.4 |
| 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/GG vs T/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 | 12.29 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   | 6      | 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 (0.64-1.57) 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.

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 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.
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/GG 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).

Dissection 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. NOD1/CARD4 is the founding member of the Nod-like receptors (NLRs) family, and is expressed in large intestinal epithelial defenses against intracellular organisms, such as enteroinvasive Escherichia coli and Shigella flexneri. Antibiotics and fecal diversion are effective therapies for CD. 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. 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. There 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. The 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. 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.

Table 3  Egger’s linear regression test to measure the funnel plot asymmetry

| Comparisons             | Y axis intercept: a (95%CI) |
|-------------------------|-----------------------------|
| GG vs T                 | GG/T + GG/GG vs T/T         |
| Overall                 | -1.92 (-5.57-1.72)          |
| CD                      | 2.62 (-6.54-1.49)           |
| UC                      | -0.39 (-6.54-5.75)          |
| IBD onset at < 40 yr of age | -2.16 (-8.80-4.47)         |

1All P > 0.05. IBD: Inflammatory bowel disease; CD: Crohn’s disease; UC: Ulcerative colitis.
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.[46] 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.[18] 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.

REFERENCES

1 Podolsky DK. The current future understanding of inflammatory bowel disease. Best Pract Res Clin Gastroenterol 2002; 16: 933-943
2 Kakazu T, Haraj, Matsumoto T, Nakamura S, Oshitani N, Arakawa T, Kitano A, Nakatani K, Kinjo F, Kuroki T. Type 1 T-helper cell predominance in granulomas of Crohn’s disease. Am J Gastroenterol 1999; 94: 2149-2155
3 Hugot JP, Zouali H, Lesage S, Thomas G. Etiology of the inflammatory bowel diseases. Int J Colorectal Dis 1999; 14: 2-9
4 Mestecky J, Moldoveanu Z, Elson CO. Immune response versus mucosal tolerance to mucosally administered antigens. Vaccine 2005; 23: 1800-1803
5 Neuman MG. Immune dysfunction in inflammatory bowel disease. Transit Res 2007; 149: 173-186
6 Satsangi J, Morecroft J, Shah NB, Nimmo E. Genetics of inflammatory bowel disease: scientific and clinical implications. Best Pract Res Clin Gastroenterol 2003; 17: 3-18
7 Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to
monocytes and activates NF-kappaB. J Biol Chem 2001; 276: 4812-4818

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

9 Martinon F, Tschopp J. NLRs join TLRs as innate sensors of pathogens. Trends Immunol 2005; 26: 447-454

10 Strober W, Murray PJ, Kilani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. Nat Rev Immunol 2006; 6: 9-20

11 Inohara N, Núñez G. NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 2003; 3: 371-382

12 Chamaillard M, Girardin SE, Vila J, Philpott DJ. Nod, Naips and Naip: intracellular regulators of bacterial-induced inflammation. Cell Microbiol 2005; 3: 381-392

13 Kufer TA, Fritz JH, Philpott DJ. NACHT-LRR proteins (NLRs) in bacterial infection and immunity. Trends Microbiol 2005; 13: 381-388

14 Girardin SE, Boneca IG, Carneiro LA, Antignac A, Jehanno M, Vila J, Tedin K, Taha MK, Labigne A, Zähringer U, Cote AJ, DiStefano PS, Bertin J, Sansonetti PJ, Philpott DJ. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 2003; 300: 1584-1587

15 Manon F, Favier A, Núñez G, Simorre JP, Cusack S. Solution structure of NOD1 CARD and mutational analysis of its interaction with the CARD of downstream kinase RICK. J Mol Biol 2007; 365: 160-174

16 Sutton WS. The chromosomes in heredity. Biol Bull 1903; 4: 231-251

17 Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3,7 and 12. Nat Genet 1996; 14: 199-202

18 Hysy P, Kabesch M, Moffatt MF, Schleder M, Carr D, Zhang Y, Boardman b, von Mutius E, Weiland SK, Leupold W, Fritzsche C, Klopp N, Musk AW, James A, Núñez G, Inohara N, Cookson WO. NOD1 variation, immunoglobulin E and asthma. Hum Mol Genet 2005; 14: 935-941

19 Hancock L, Beckly J, Geremia A, Cooney R, Cummings F, Pathan S, San C, Warren BF, Mortensen N, Ahmad T, Jewell D. Clinical and molecular characteristics of isolated colonic Crohn’s disease. Inflamm Bowel Dis 2008; 14: 1667-1677

20 Centó E, Ricart E, Busquets D, Monfort D, García-Planella E, González D, Balanzó J, Rodríguez-Sanchez JL, Vidal S. Influence of a nucleotide oligomerization domain 1 (NOD1) polymorphism and NOD2 mutant alleles on Crohn’s disease phenotype. World J Gastroenterol 2007; 13: 5464-5465

21 Henckaerts L, Pierik M, Joosens M, Ferrante R, Rutgeerts P, Vermeire S. Mutations in pattern recognition receptor genes modulate seroreactivity to microbial antigens in patients with inflammatory bowel disease. Gut 2007; 56: 1536-1542

22 Van Limbergen J, Russell RK, Nimmo ER, Törkvist L, Lees CW, Drummond HE, Smith L, Anderson NH, Gilllett PM, McGrocan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Sjöqvist U, Lördal M, Farrington SM, Dunlop MG, Wilson DC, Satsangi J. Contribution of the NOD1/CARD4 insertion/deletion polymorphism + 32656 to inflammatory bowel disease in Northern Europe. Inflamm Bowel Dis 2007; 13: 882-889

23 Franke A, Ruether A, Wedemeyer N, Karlsen TH, Nebel A, Schreiber S. No association between the functional CARD4 insertion/deletion polymorphism and inflammatory bowel diseases in the German population. Gut 2006; 55: 1679-1680

24 Tremelling M, Hancock L, Bredin F, Sharpstone D, Bingenham SA, Parkes M. Complex insertion/deletion polymorphism in NOD1 (CARD4) is not associated with inflammatory bowel disease susceptibility in East Anglia panel. Inflam Bowel Dis 2006; 12: 97-107

25 McGovern DP, Hysy P, Ahmad T, van Heel DA, Moffatt MF, Carey A, Cookson WO, Jewell DP. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease. Hum Mol Genet 2005; 14: 1245-1250

26 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634

27 Cochran WG. The combination of estimates from different experiments. Biometrics 1954; 10: 101-129

28 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558

29 Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22: 719-748

30 DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7: 177-188

31 Verma R, Ahuja V, Paul J. Frequency of single nucleotide polymorphisms in NOD1 gene of ulcerative colitis patients: a case-control study in the Indian population. BMC Med Genet 2009; 10: 82

32 Lakatos PL, Altorjai J, Mándi Y, Lakatos L, Tumpek J, Kovacs A, Molnar T, Tulassay Z, Miheller P, Patatka K, Szamosi T, Fischer S, Papp J, Papp M. Interaction between seroreactivity to microbial antigens and genetics in Crohn’s disease: is there a role for defensins? Tissue Antigens 2008; 71: 552-559

33 Molnar T, Hofner P, Nagy F, Lakatos PL, Fischer S, Lakatos L, Kovacs A, Altorjai J, Papp M, Patatka K, Demeter P, Tulassay Z, Nyári T, Miheller P, Papp J, Mandi Y, Lonovics J. NOD1 gene E266K polymorphism is associated with disease susceptibility but not with disease phenotype or NOD2/CARD15 in Hungarian patients with Crohn’s disease. Dig Liver Dis 2007; 39: 1064-1070

34 Van Limbergen J, Nimmo ER, Russell RK, Drummond HE, Smith L, Anderson NH, Davies G, Arnott ID, Wilson DC, Satsangi J. Investigation of NOD1/CARD4 variation in inflammatory bowel disease using a haplotype-tagging strategy. Hum Mol Genet 2007; 16: 2175-2186

35 McGovern DP, Butler H, Ahmad T, Paullucci M, van Heel DA, Negoro K, Hysy P, Ragoussis J, Travis SP, Cardon LR, Jewell DP. TUCAN (CARD8) genetic variants and inflammation-related bowel disease. Gastroenterology 2006; 131: 1190-1196

36 Ozsen SC, Dagli U, Kilig Y, Törtekin M, Celik Y, Ozkan M, Soykan I, Cetinkaya H, Ulker A, Ozden A, Bozdayı AM. NOD2/CARD15, NOD1/CARD4, and ICAM-1 gene polymorphisms in Turkish patients with inflammatory bowel disease. J Gastroenterol 2006; 41: 301-304

37 Zouali H, Lesage S, Merlin F, Cézard JP, Colombel JF, Belanche 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

38 Ogura Y, Bonen DK, Inohara N, Colene DL, Chen FF, Ramos R, Britton H, Ahmad T, Paullucci M, van Heel DA, Negoro K, Hysy P, Ragoussis J, Travis SP, Cardon LR, Jewell DP. TUCAN (CARD8) genetic variants and inflammation-related bowel disease. Gastroenterology 2006; 131: 1190-1196

39 Hampe J, Cutler A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lenard-Jones JE, Foelsch UR, Krawczek M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2 gene and Crohn’s disease in German and British populations. Lancet 2001; 357: 1925-1928

40 Kim JG, Lee SJ, Kagnoff MF. Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria.
that avoid recognition by toll-like receptors. *Infect Immun* 2004; 72: 1487-1495

41 **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

42 **Kühn R**, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; 75: 263-274

43 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn’s recurrence after ileal resection. *Gastroenterology* 1995; 108: 1617-1621

44 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn’s disease in the neoterminal ileum. *Lancet* 1991; 338: 771-774

45 **Inohara N**, Ogura Y, Chen FF, Muto A, Nuñez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem* 2001; 276: 2551-2554

46 **Girardin SE**, Jéhanno M, Mengin-Lecreulx D, Sansonetti PJ, Alzari PM, Philpott DJ. Identification of the critical residues involved in peptidoglycan detection by Nod1. *J Biol Chem* 2005; 280: 36648-36656