

Toll-like receptor 4 gene polymorphisms and susceptibility to colorectal cancer: a meta-analysis and review

Wang Yan Sheng, Zhang Yong, Zhu Yun, Hu Hong, Luo Lin Hai

The People's Hospital of Yuxi, Yuxi, China

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Corresponding author:

Luo Lin Hai
The People's Hospital of Yuxi
No.21 Nieer Road
653100 Yuxi
Hongta District, China
Phone: +86 08772014046
E-mail: Luolinhai_L@163.com

Abstract

Introduction: Many case-control studies have investigated the association between toll-like receptor 4 (TLR4) Asp299Gly and Thr399Ile polymorphisms and risk of colorectal cancer (CRC). However, published data are still conflicting.

Material and methods: A systematic search was conducted in the electronic databases of PubMed, MEDLINE, EMBASE, Web of Science and CNKI between 2000 and 2014. The associations between TLR4 polymorphisms and CRC susceptibility were assessed by pooled odds ratios (ORs) and 95% confidence intervals (95% CI) in fixed or random effects models.

Results: In total nine case-control studies were identified in this meta-analysis. For TLR4 Asp299Gly polymorphism, 9 studies included 1198 cases and 1290 controls. The GG genotype carriers had higher risk for developing CRC than AA + GA genotype carriers (OR = 1.95, 95% CI: 1.00–3.77, $p = 0.05$). No association was found in other genetic models ($p > 0.05$). Analysis stratified by ethnicity showed no association in any genetic models among the Asian or Caucasian population. For TLR4 Thr399Ile polymorphism, 6 studies contained 619 cases and 632 controls. The overall analysis showed significantly increased risk in TT homozygote carriers compared to CC homozygote (OR = 4.99, 95% CI: 1.41–17.65, $p = 0.01$) and C carriers (TC + CC) (OR = 4.50, 95% CI: 1.27–15.87, $p = 0.02$). In terms of analyses stratified by race, a significant association was found in each genetic model among the Asian population, rather than the Caucasian group.

Conclusions: The GG homozygote carriers of TLR4 Asp299Gly and TT homozygote carriers of TLR4 Thr399Ile polymorphisms might be correlated with an increased risk of CRC, suggesting they may serve as genetic risk factors for CRC.

Key words: colorectal carcinoma, toll-like receptor 4, polymorphism, meta-analysis.

Introduction

Colorectal carcinoma (CRC) is one of the primary causes of cancer death throughout the world and is still a major public health problem. Clinical studies indicate that the host immune system plays a key role in the development and progression of CRC [1]. According to global cancer statistics, it is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 [2]. Its incidence

rates continue to increase in economically transitioning countries [3]. Moreover, a large portion of successfully treated CRC patients develop a recurrence or metastasis within 5 years of diagnosis [4]. Hence, there is a real need to develop targeted prevention and early detection programs to reverse this situation.

Both environmental and genetic factors contribute to the tumorigenesis of CRC. Genome-wide association studies have shown that inherited genetic variation can contribute to CRC prognosis [5], and so far have successfully identified 20 susceptible single nucleotide polymorphisms (SNPs) in 18 risk loci for CRC, among which the toll-like receptor (TLR) gene polymorphisms have been highlighted [6]. TLRs are pattern-recognition receptors (PRRs) which have emerged as central regulators of intestinal homeostasis and CRC [7], and mediate the innate immune response to LPS challenge [8]. TLR4 is one of the major TLRs, and has been actively investigated in inflammation and cancer. It is involved in the regulation of inflammatory reactions and activation of the adaptive immune response to eliminate infectious pathogens and cancer debris [9, 10]. TLR4 is located on chromosome 9q32-q33, and contains four exons. There are two SNPs in TLR4 exon 3; one is an A/G substitution at 896 base pairs (bp), and the other is a C/T substitution at 1196 bp. Research has shown that TLR4 A896G has a protective effect on high house dust mite allergen exposure against specific sensitization [11]. A previous meta-analysis demonstrated that TLR4 polymorphisms are associated with gastric carcinogenesis and Crohn's disease [12, 13], while a recent study proved that these polymorphisms were not involved in the development of autoimmune diseases [14].

Recent studies suggest that gene variants in TLR4, which affect the extracellular domain of the receptor and may cause decreased ligand recognition [15], are critical for CRC susceptibility. However, the results remained inconsistent rather than conclusive. Therefore, we conducted this meta-analysis on all eligible published case-control studies to establish a relatively comprehensive picture of the relationship between these genetic variants and CRC risk.

Material and methods

Identification and eligibility of relevant studies

We searched for relevant papers published between 2000 and 2014 using the electronic databases of PubMed, MEDLINE, EMBASE, Web of Science and CNKI (China National Knowledge Infrastructure) with the following search terms: "toll-like receptor 4 or TLR4", "polymorphism",

"variant", "colorectal carcinoma or colorectal cancer" and "colon cancer" as well as their combinations. The corresponding Chinese terms were used in the Chinese library. No language restrictions were applied. All studies matching the eligibility criteria were retrieved, and references of the retrieved articles were also screened for original studies.

Criteria for article screening

Studies included had to meet all the following criteria: 1) case-control or cohort association studies; 2) evaluated the relationship of TLR4 (Asp299Gly and Thr399Ile) polymorphisms with CRC risk; 3) genotype distributions in the cases and controls were available for estimating the odds ratio (OR) with its 95% confidence interval (CI); and 4) the distribution of genotypes in the control group was consistent with Hardy-Weinberg equilibrium (HWE) [16].

The major exclusion criteria were: 1) only case population; 2) reviews, conference abstracts in which the genotype frequencies were not extractable; 3) the study did not have the outcomes of a comparison report; and 4) duplicate of previous articles.

Data extraction

All of the data were independently abstracted in duplicate by two authors using a data abstraction form to retrieve information on first author, year of publication, country, ethnicity, sample size, genotyping method, and the genotype frequencies in the cancer cases and controls. Disagreements between authors were resolved by consensus.

Statistical analysis

The crude OR and its 95% CI were used to assess the strength of the association between the TLR4 polymorphisms with the risk of CRC. The Z test was employed to determine the significance of the pooled ORs, and a *p* value less than 0.05 was considered statistically significant. This study examined the association between TLR4 Asp299Gly and Thr399Ile polymorphisms and CRC risk compared with that for the per-allele model (G vs. A for Asp299Gly, T vs. C for Thr399Ile), dominant model (GG + GA vs. AA for Asp299Gly, TT + TC vs. CC for Thr399Ile), recessive model (GG vs. GA + AA for Asp299Gly, TT vs. TC + CC for Thr399Ile), and additive model (GG vs. AA for Asp299Gly, TT vs. CC for Thr399Ile). To evaluate whether the association showed any ethnicity-specific effects, we analyzed the data for subgroups defined by ethnicity. The heterogeneity of the included articles was evaluated using Cochran's Q test and *I*² statistics. *P*-values less than 0.1 and *I*² less than 50% were considered

to be statistically significant. When a significant heterogeneity existed across the included studies ($p < 0.1$ and $I^2 > 50\%$), the random effect model was used. When there was no significant heterogeneity across the included studies ($p > 0.1$ and $I^2 < 50\%$), the fixed effect model was used. To assess whether our results were substantially influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and recalculating the significance of the result. Begg's funnel plot was used to examine the publication bias. Analyses were carried out using Review Manager 5.2 (The Cochrane Collaboration). All tests were two-sided.

Results

Characteristics of studies

The electronic database search identified 213 references. Of those, 159 records excluded for duplication and 54 articles were judged potentially relevant. Following screening of titles and abstracts for relevance, 31 full-text articles were comprehensively assessed against inclusion criteria. Overall, a total of 9 prospective cohort studies (7 in English and 2 in Chinese) containing 1448 CRC cases and 1640 controls were finally included in this review. Figure 1 shows the study flow.

Of the nine included articles, one was from Malaysia [17], one was from Tunisia [18], one was from Portugal [19], three were from China [20–22], one was from Croatia [23], one was from Russian [24] and one was from Spain [25]. The major characteristics of the eligible publications are reported in Table I. The genotype frequencies are listed in Table II.

Association of TLR4 Asp299Gly polymorphism and CRC risk

The evaluation of the association between these two polymorphisms and CRC risk is present-

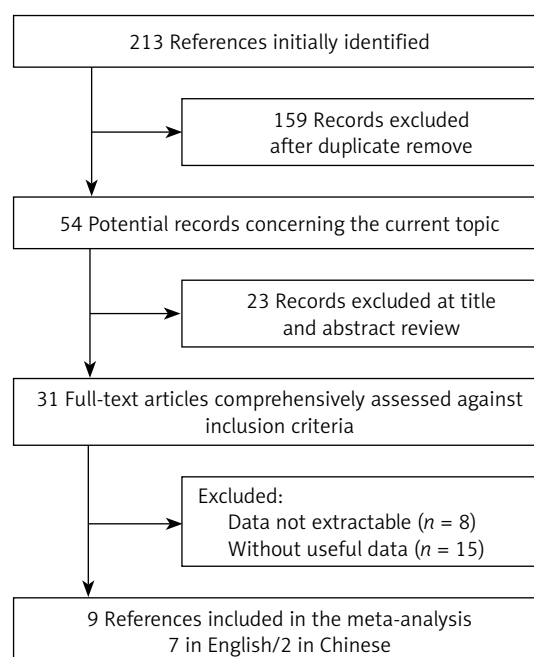


Figure 1. Flow chart of literature screening

ed in Table III. For TLR4 Asp299Gly polymorphism, a total of 9 studies including 1408 cancer cases and 1553 controls were selected in our meta-analysis. No significant heterogeneity was found among the included studies, and the fixed-effect model was chosen to analyze the data ($p > 0.1$). Overall, the frequency of G is higher in CRC than that in the healthy controls (6.8% vs. 5.3%). As shown in Figure 2, we found that GG homozygote carriers in the recessive model have an increased risk of CRC compared with A allele carriers (OR = 1.95, 95% CI: 1.00–3.77, $p = 0.05$) in a fixed-effect model. No association was found in other genetic models (G vs. A: OR = 1.19, 95% CI: 0.95–1.50, $p = 0.14$; GG + GA vs. AA: OR = 1.20, 95% CI: 0.94–1.54, $p = 0.14$; GG vs. AA: OR = 1.27, 95% CI: 0.52–3.15, $p = 0.60$). In an analysis stratified by ethnicity, no

Table I. Main characteristics of studies included in this meta-analysis

First author	Year	Country	Ethnicity	Cases	Controls	Genotyping method
Boraska Jelavic	2006	Croatia	Caucasian	89	88	PCR-RFLP
Guo	2006	China	Asian	110	160	PCR
Landi	2006	Spain	Caucasian	282	269	PCR-TaqMan
Davoodi	2011	Malaysia	Asian	60	50	PCR-RFLP
Yang	2011	China	Asian	102	87	PCR sequencing
Dai	2012	China	Asian	268	268	PCR
Omrane	2013	Tunisia	Caucasian	100	140	PCR
Pimentel-Nunes	2013	Portugal	Caucasian	193	278	PCR
Kutikhin	2014	Russian	Caucasian	244	300	PCR

Table II. Distributions of TLR4 (Asp299Gly and Thr399Ile) polymorphism genotypes and alleles among cancer patients and controls

Author	Genotypes						Alleles			
	Cases			Controls			Cases		Controls	
Asp299Gly	AA	GA	GG	AA	GA	GG	A	G	A	G
Boraska Jelavic	77	10	2	84	4	0	164	14	220	0
Guo	110	0	0	160	0	0	220	0	320	0
Landi	251	31	0	232	37	0	533	31	501	37
Davoodi	58	2	0	50	0	0	118	2	100	0
Yang	96	4	2	84	2	1	196	8	170	4
Dai	219	44	5	228	38	2	482	54	494	42
Omrane	87	13	0	120	18	2	187	13	258	22
Pimentel-Nunes	169	0	15	186	0	5				
Kutikhin	173	40	0	258	39	3	386	40	555	45
Thr399Ile	CC	TC	TT	CC	TC	TT	C	T	C	T
Boraska Jelavic	77	12	0	82	5	0	166	12	169	5
Davoodi	58	2	0	50	0	0	118	2	100	0
Yang	92	8	2	86	1	0	192	12	173	1
Dai	182	78	8	214	52	2	442	94	480	56
Omrane	94	6	0	123	17	0	194	6	263	17
Kutikhin	195	36	2	255	45	0	426	40	555	45

association was found in any genetic models among the Asian or Caucasian population (Table III).

Association of TLR4 Thr399Ile polymorphism and CRC risk

For Thr399Ile polymorphism, 6 articles including 852 CRC cases and 932 controls were retrieved in our study. The overall analysis showed significant increased risk in TT homozygotes carriers compared to CC homozygotes in the additive model (OR = 4.99, 95% CI: 1.41–17.65, $p = 0.01$) and C allele carriers in the recessive model (TC + CC) (OR = 4.50, 95% CI: 1.27–15.87, $p = 0.02$) (Figures 3 and 4). No association was observed in the dominant model (OR = 1.51, 95% CI: 0.85–2.67, $p = 0.16$) and allelic model (T vs. C: OR = 1.52, 95% CI: 0.88–2.61, $p = 0.13$) (Figures 5 and 6). When evaluating the effect of the polymorphism by ethnicity, we found a significant association in all genetic models among the Asian population (T vs. C: OR = 2.04, 95% CI: 1.45–2.88, $p < 0.0001$; TT + TC vs. CC: OR = 2.10, 95% CI: 1.44–3.06, $p = 0.0001$; TT vs. TC + CC: OR = 4.15, 95% CI: 1.04–16.62, $p = 0.04$; TT vs. CC: OR = 4.70, 95% CI: 1.17–18.88, $p = 0.03$) in a fixed-effect model, while no relationship was found among the Caucasian population ($p > 0.05$).

Sensitivity analyses and publication bias

A single study included in the meta-analysis was deleted each time to reflect the influence of

the individual data set on the pooled ORs. The corresponding pooled ORs were not materially changed, which confirmed the stability of our overall result.

Egger's test was conducted to assess the publication bias of the literature. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry (Figure 7). The statistical results still did not show publication bias ($p = 0.258$ for TLR4 Asp299Gly variant and $p = 0.342$ for Thr399Ile variant).

Discussion

TLRs are involved in innate immunity defense against microorganisms [26]. They are recognized as a link between innate and adaptive immunity and contribute to efficient combat of pathogens in the immune system [27]. Studies have shown that SNPs within TLR genes can cause altered susceptibility to disease [28, 29]. TLR4 is a central player in the response to infection by Gram-negative bacteria and thus an important candidate inflammatory gene [30]. In recent years, the impact of TLR4 polymorphisms on multiple types of cancer has been considered [31, 32]. The most intensively highlighted ones are Asp299Gly and Thr399Ile, which are usually co-segregated [33]. However, the results from different published studies have been inconsistent. Thus, we performed this meta-analysis to comprehensively analyze their associations.

In this meta-analysis, we selected a total of 9 articles. For TLR4 Asp299Gly polymorphism,

9 studies were selected to assess the association between Asp299Gly polymorphism and CRC risk. In the overall analysis, we observed that the GG genotype carriers had higher risk for developing CRC than AA + GA genotype carriers ($p = 0.05$). No association was found in other genetic models. In terms of analyses stratified by race, no significant association was found between the Asp299Gly variant and increased risk of CRC in any genetic models among the Caucasian population and Asian population ($p > 0.05$). However, the genetic models of the Asian group had higher risk for developing CRC than the Caucasian group. The TLR4 Asp299Gly polymorphism results in an aspartic acid to glycine replacement at codon 299 (D299G). It has been most investigated in different types of cancer, with controversial results [34, 35]. Omrane *et al.* showed a positive link between variant T Asp299Gly and CRC patients [18], while Davoodi *et al.* found that variant TLR4 Asp299Gly is not associated with risk of CRC [17]. This polymorphism has a distinct distribution in different populations due to evolutionary pressure, which may change the functionality of the receptor and explain the discrepancies between the studies [3].

For the Thr399Ile polymorphism, a total of 6 articles were retrieved in our study. The overall analysis showed significantly increased risk in TT homozygote carriers in the recessive model compared to CC homozygotes ($p = 0.01$) and C carriers (TC + CC) ($p = 0.02$). No association was observed in other models. When evaluating the effect of the polymorphism by ethnicity, we found a significant association in all genetic models among the Asian population, while no relationship was found among the Caucasian population. The Thr399Ile polymorphism leads to a threonine to isoleucine exchange at codon 399 (T399I). Guo *et al.* demonstrated that TLR4 D299G and T399I polymorphisms are associated with reduced activation of nuclear factor- κ B (NF- κ B) and pro-inflammatory cytokine expression [36]. These two variants of TLR4 disrupt the extracellular region in the ligand binding site of the receptor, and were associated with decreased responsiveness to LPS and an attenuated immune response causing secretion of low levels of pro-inflammatory cytokines [37]. Slattery *et al.* demonstrated that genetic variation in TLR4 may influence the development of colon cancer as well as influence survival after diagnosis of colon cancer [38]. However, in the Caucasian population, there is no significant difference between CRC subjects and controls for T399I genotypes, suggesting that TLR4 polymorphisms may have an ethnicity-specific association with susceptibility to CRC.

TLR4 activation affects the immune response against invading Gram-negative bacteria, and is involved in chronic inflammation, autoimmune

Table III. Summary of results from different comparative genetic models

Gene	Allelic model			Dominant model			Recessive model			Additive model			
	OR (95% CI)	Value of p	Ph/I ²	OR (95% CI)	Value of p	Ph/I ²	OR (95% CI)	Value of p	Ph/I ²	OR (95% CI)	Value of p	Ph/I ²	
Asp299Gly	G vs. A												
Total	9	1.19 (0.95–1.50)	0.14	0.18/33%	1.20 (0.94–1.54)	0.14	0.26/22%	1.95 (1.00–3.77)	0.05	0.38/6%	1.27 (0.52–3.15)	0.60	0.40/1%
Asian	4	1.40 (0.94–2.07)	0.10	0.70/0%	1.36 (0.89–2.09)	0.16	0.69/0%	2.25 (0.58–8.74)	0.24	0.80/0%	2.30 (0.59–8.97)	0.23	0.79/0%
Caucasian	5	1.14 (0.70–1.86)	0.60	0.06/59%	1.18 (0.72–1.91)	0.52	0.09/54%	1.86 (0.87–3.96)	0.11	0.15/43%	0.70 (0.18–2.66)	0.60	0.26/26%
Thr399Ile	T vs. C												
Total	6	1.52 (0.88–2.61)	0.13	0.02/61%	1.51 (0.85–2.67)	0.16	0.02/61%	4.50 (1.27–15.87)	0.02	0.97/0%	4.99 (1.41–17.65)	0.01	0.98/0%
Asian	3	2.04 (1.45–2.88)	< 0.0001	0.21/37%	2.10 (1.44–3.06)	0.0001	0.28/21%	4.15 (1.04–16.62)	0.04	0.97/0%	4.70 (1.17–18.88)	0.03	1.00/0%
Caucasian	3	1.13 (0.79–1.62)	0.51	0.12/49%	1.07 (0.49–2.32)	0.87	0.07/63%	6.49 (0.31–135.84)	0.23	NA	6.53 (0.31–136.89)	0.23	NA

NA – not applicable.

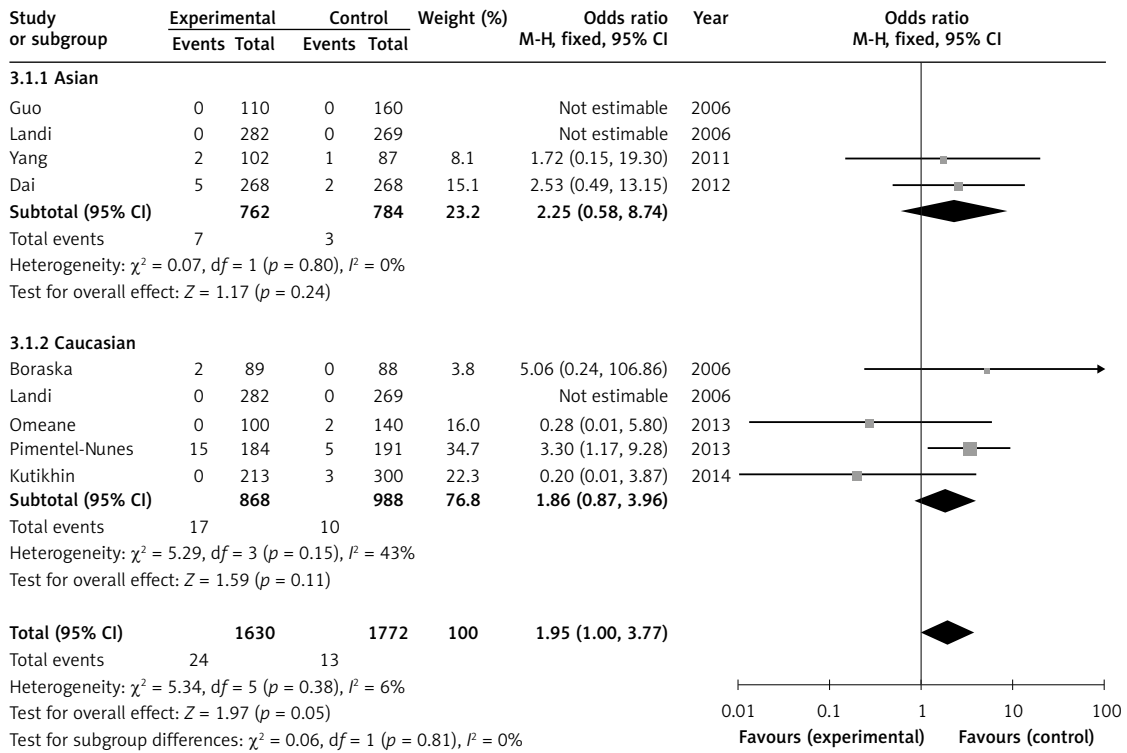


Figure 2. Forest plot of odds ratios (ORs) of TLR4 Asp299Gly polymorphism (GG vs. GA + AA) associated with CRC stratified by ethnicity

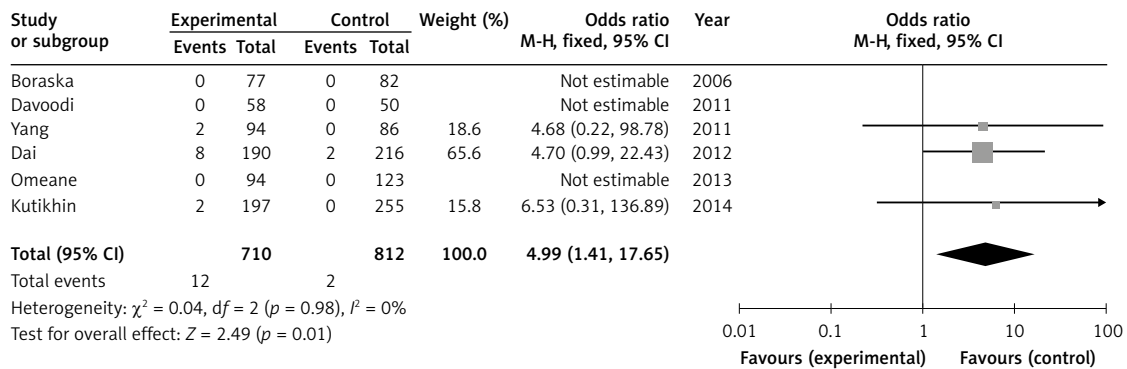


Figure 3. Forest plot of odds ratios (ORs) of TLR4 Thr399Ile polymorphism (TT vs. CC) associated with CRC risk

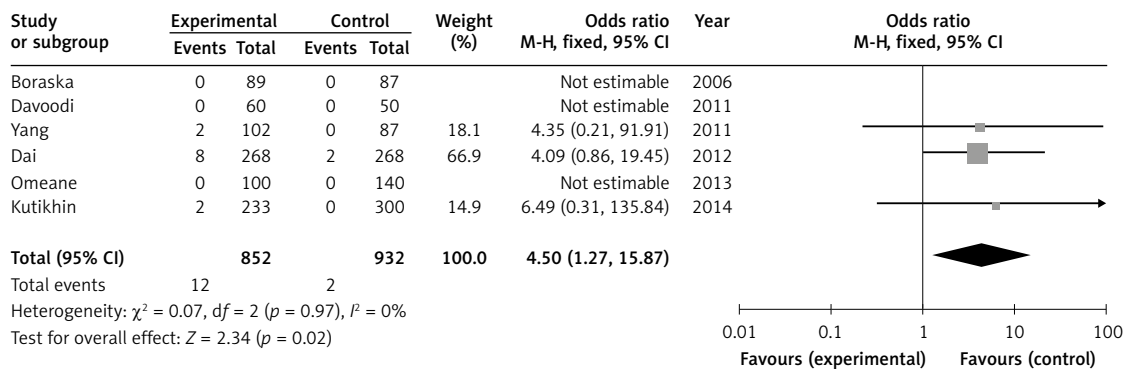


Figure 4. Meta-analysis of TLR4 Thr399Ile polymorphism (TT vs. TC + CC) with risk of CRC

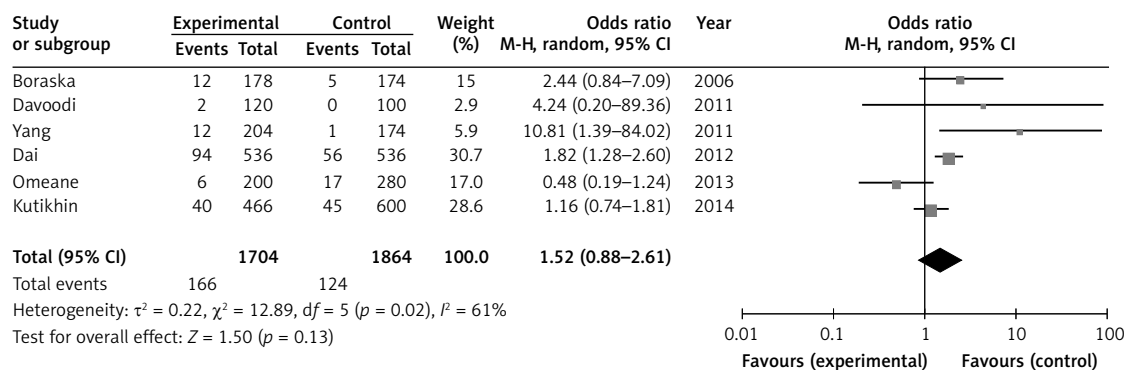


Figure 5. Forest plot of CRC associated with distribution of TLR4 Thr399Ile polymorphism (TT + TC vs. CC) by stratified analysis

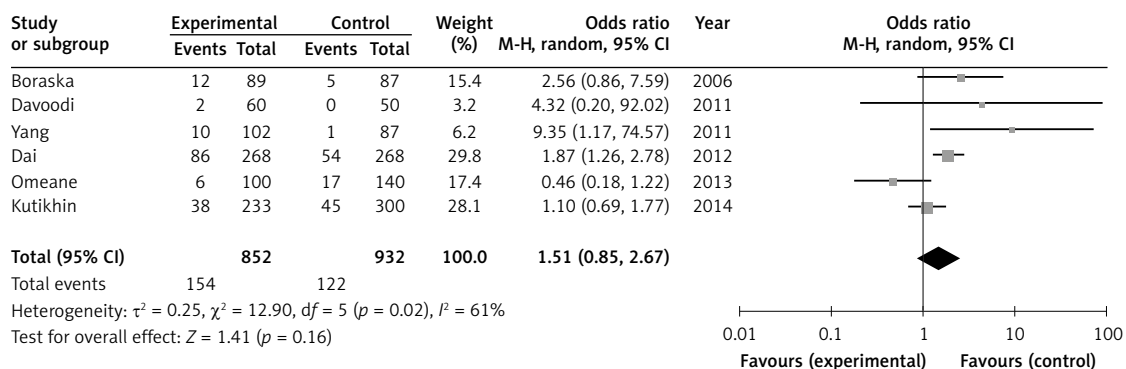


Figure 6. Association between T allele of TLR4 Thr399Ile polymorphism and CRC risk

diseases, and malignancies as well [39]. It may be exploited by bacterial toxins to gain access and entry into cells [40]. The TLR4 gene exhibits a differential pattern of expression not only between coronary artery disease patients and controls in this Asian Indian cohort [41], but also in CRC. Wang *et al.* suggest that high expression of TLR4 is associated with liver metastasis and is an independent predictor of poor prognosis in patients with CRC [42], while Nihon-Yanagi *et al.* find that TLR4 expression might not be involved in sporadic colorectal carcinogenesis [43]. A study proved that TLR4 D299G induces features of neoplastic progression in intestinal epithelial Caco-2 cells and associates with aggressive colon cancer in humans, implying a novel link between aberrant innate immunity and colonic cancerogenesis [44]. The TLR4 variant D299G promotes inflammation-associated colon cancer progression in the intestinal epithelium [45]. The role of Toll-like receptors in renal diseases has been discussed by Gluba *et al.* [46].

Several limitations of this meta-analysis should be addressed. Firstly, the original information was insufficient, so assessment of potential interactions is limited. Secondly, the subgroups may have a relatively low power based on a small number of studies. Thirdly, a more precise analysis should be conducted if individual information including other covariates such as age, sex and

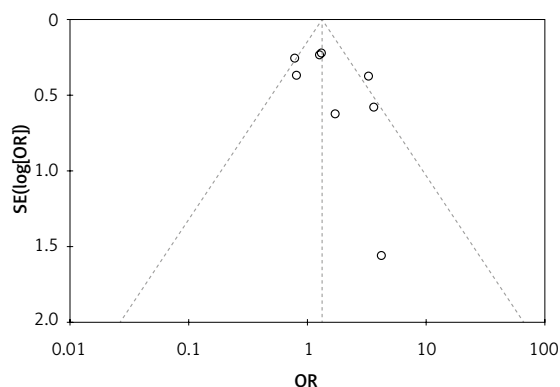


Figure 7. Begg's funnel plot for publication bias in selection of studies

smoking condition becomes available. Fourthly, the gene-gene interaction is important for the development of complex diseases including cancer [47].

In conclusion, the results from the present meta-analysis suggest that the GG genotype of TLR4 Asp299Gly and the TT genotype of TLR4 Thr399Ile polymorphisms might be correlated with an increased risk of CRC, indicating that they may serve as biomarkers of disease progression. However, further large, well-designed, comprehensive studies in various populations are needed to confirm our results. Furthermore, studies of gene-gene and gene-environment interactions be-

tween these polymorphisms and CRC risk should also be performed and considered.

Conflict of interest

The authors declare no conflict of interest.

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