RESEARCH ARTICLE

Associations Between *TLR9* Polymorphisms and Cancer Risk: Evidence from an Updated Meta-analysis of 25,685 Subjects

Guo-Xing Wan[&], Yu-Wen Cao[&], Wen-Qin Li, Yu-Cong Li, Wen-Jie Zhang, Feng Li*

Abstract

A meta-analysis incorporating 34 case-control studies from 19 articles involving 12,197 cases and 13,488 controls was conducted to assess the effects of three genetic variants of Toll-like receptor 9 (*TLR9*): rs187084, rs352140, and rs5743836. Studies on associations between *TLR9* polymorphisms and cancer risk were systematically searched in electronic databases. The reported odds ratios (OR) and 95% confidence intervals (CI) were pooled to assess the strength of any associations. The results showed that the rs187084 polymorphism was significantly associated with an increased risk of cancer (CC *vs* TC+TT: OR=1.14, 95% CI=1.02-1.28), specifically cervical cancer (C *vs* T: OR=1.19, 95% CI=1.05-1.34; TC *vs* TT: OR=1.32, 95% CI=1.10-1.58; CC *vs* TT: OR=1.31, 95% CI= 1.03-1.68; CC+TC *vs* TT: OR=1.32, 95% CI=1.11-1.56), and that this association was significantly positive in Caucasians (CC vs. TC+TT: OR=1.18, 95% CI=1.01-1.38). The rs352140 polymorphism had a protective effect on breast cancer (GA *vs* GG: OR=0.77, 95% CI=0.66-0.89), whereas the rs5743836 polymorphism was likely protective for digestive system cancers (CC+TC *vs* TT: OR=0.81, 95% CI=0.66-0.98). In conclusion, our results suggest that the rs187084 polymorphism may be associated with an elevated cancer risk, whereas polymorphisms of rs352140 and rs5743836 may play protective roles in the development of breast and digestive system cancers, respectively. From the results of this meta-analysis further large-scale case-control studies are warranted to verify associations between *TLR9* polymorphisms and cancer.

Keywords: Toll-like receptor 9 - polymorphisms - cancer risk - meta-analysis

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Introduction

Cancer posts a worldwide public health threat affecting all regions and ethnicities. Cancer is a polygenic and multifactorial disease with complex traits, and its exact pathogenesis remains to be elucidated (Zhang et al., 2013). The innate immune response, which is the primary first line of defense against invading pathogens, has been increasingly recognized as an essential component in the control and surveillance of malignant neoplasms (Bondar et al., 2013). This concept is supported by the observations that many human cancers arise from sites of infection, chronic irritation, and inflammationand by the fact that the tumor microenvironment is largely orchestrated by inflammatory cells, which are indispensable participants in the neoplastic process, fostering proliferation, survival, and migration of cancer cells (Mantovani et al., 2008).

Emerging evidence has suggested a role for inflammation-induced Toll-like receptors (TLRs) in cancer development, with some TLRs reported to be potential independent prognostic markers for tumorigenesis and tumor progression (Grimm et al., 2010; Castro et al., 2011; Kutikhin et al., 2011). TLRs constitute a family of evolutionarily conserved type I membrane receptors and play central roles in the recognition of the corresponding pathogen-associated molecular patterns. Until now, 13 related TLR genes have been identified and characterized (TLR1-TLR13) (Oldenburg et al., 2012), of which we chose *TLR9* as a model gene for this meta-analysis aimed at clarifying its associations with cancer risk.

Human *TLR9* is mapped at 3p21.3, a region frequently deleted in human cancers (Chuang et al., 2000). Unlike other members of the TLR gene family, which are membrane-bound pattern recognition receptors, TLR9 is localized intracellularly, mainly in the endocytic compartments, and is known to be expressed in multiple cancer cells and cell lines, including cervical, breast, colorectal, prostate, and gastric cancers (Fehri et al., 2014). Stimulation of TLR9 has been shown to activate human B cells and result in innate immune responses in preclinical tumor models and in patients through recognition of unmethylated cytosine-phosphate-guanine

*Department of Pathology and the Key Laboratories for Xinjiang Endemic and Ethnic Diseases (a joint venture with the Chinese Ministry of Education), Shihezi University School of Medicine, Shihezi, Xinjiang, China [&]Equal contributors *For correspondence: fengli7855@126.com*

(CpG) dinucleotide motifs located in bacterial, viral, and fungal DNA (Hemmi et al., 2000; Latz et al., 2004; Murad et al., 2007; Hao et al., 2014). Moreover, it has been established that *TLR9* mediates its functional effects via the myeloid differentiation primary response protein 88 (MyD88)-dependent pathway, leading to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, cytokine secretion, and inflammatory responses (Pandey et al., 2011).

Since it was first identified, *TLR9* has been found to be polymorphic on direct sequencing in three ethnic populations (Chen et al., 2012), and therefore, the associations between these polymorphisms and the risks of various cancers have been widely investigated across different ethnic populations. Among the studied single nucleotide polymorphisms (SNPs), three common variants: *TLR9* rs352140 (T1486C), rs187084 (G2848A), and rs5743836 (T1237C), have been frequently found to be associated with the risks of multiple tumors. Due to limited sample sizes, however, the reported results are inconsistent, obscuring their values. Although Zhang et al. (2013) found an increased cancer risk for *TLR9* rs352140 polymorphism in their pooled analysis with a reasonable sample size, this association should be verified by stratified analyses based on cancer types using a larger sample size. Individual studies with small sample sizes might have been underpowered to detect the overall effects, and therefore, a quantitative synthesis of the accumulated data from different studies is deemed necessary and important to provide evidence on the associations between the three selected *TLR9* SNPs (rs352140, rs187084, and rs5743836) and cancer risks. Furthermore, heterogeneity among these individual studies has also been quantified in the current meta-analysis.

Materials and Methods

Literature search

We systematically and electronically searched in the PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure (CNKI), and Chinese Biomedicine Database (CBD) up until June 2014 to look for relevant articles on genetic association studies in which the associations between *TLR9* polymorphisms and cancer risks were evaluated; articles on human studies and in English or Chinese were selected. We developed a search strategy using the following queries: ("*TLR9*" or "Toll-Like Receptor 9") and ("polymorphism" or "variant" or "genotype" or "SNP") and ("cancer" or "carcinoma" or "tumor" or "neoplasm"). The reference lists of major textbooks, review articles, and all of the included papers identified by the search were then individually and manually searched to find other potentially eligible studies.

Inclusion criteria

To be eligible for inclusion in the current meta-analysis, studies had to satisfy the following criteria: (1) studies focused on the association between *TLR9* polymorphisms and tumor risk; (2) sufficient information provided for estimating odds ratios (ORs) and their 95% confidence intervals (95%CIs); (3) all patients diagnosed with malignancies were confirmed by histology; (4) the frequencies of alleles or genotypes in the case and control groups could be extracted; (5) if studies had overlapping cases or controls, only the most recent and/or the largest studies with available data were included in the meta-analysis.

Exclusion criteria

Studies were excluded from the analysis if: (1) there was no control population; (2) the investigations were based on incomplete raw data; (3) the outcomes of comparisons were not reported or difficult to determine; (4) letters, reviews or editorial articles; (5) lack of necessary information for the meta-analysis.

Data extraction

Using a standardized form, data from the identified studies were extracted independently by two reviewers to evaluate their eligibility for inclusion by screening the titles and abstracts of each identified reference, and the papers were categorized based on the full texts. For each included study, the following information was collected: first author, year of publication, country, genotyping method, sample size, polymorphisms, cancer type, allele or genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE). Any discrepancies between the two reviewers were resolved following a discussion and consultation with a third reviewer.

Statistical analysis

Individual or pooled ORs and 95% CIs were calculated for the strength of the association between *TLR9* polymorphisms and cancer risks using the Review Manager Version 5.2 software (Cochrane Collaboration, Oxford, England; http://www.cochrane.org/software/revman.htm). The between-study heterogeneity was evaluated by the Q test, with $P<0.1$ indicating evidence of heterogeneity (Cochran et al., 1950; Higgins et al., 2003). The random-effects model was selected if the between-study heterogeneity was significant across included studies (DerSimonian et al., 1986), otherwise, the fixed-effects model (the Mantel-Haenszel method) was used (Mantel et al., 1959). Sources of heterogeneity were evaluated by stratification analysis, according to the study characteristics. Beggar's test was used to assess the possible presence of publication bias (Higgins et al., 2002). All analyses were performed using Stata 12.0 software (Stata Corp., College Station, TX, USA). All generated *P* values were two tailed, with *P* values <0.05 considered significant.

Results

Characteristics of included studies

A total of 47 potentially relevant publications focusing on the association between *TLR9* polymorphisms and cancer risk were retrieved based on the search strategy. We reviewed the titles, abstracts, and full texts of all retrieved articles through defined criteria. Finally, 19 articles were included in this study (Nieters et al., 2006; Etokebe et al., 2009; Hold et al., 2009; Mollaki et al.,

2009; Ashton et al., 2010; Pandey et al., 2011; Zeng et al., 2011; Chen et al., 2012; Carvalho et al., 2012; Li et al., 2012; Mandal et al., 2012; Miedema et al., 2012; Noack et al., 2012; Roszak et al., 2012; Xie et al., 2012; Lai et al., 2013; Resler et al., 2013; Singh et al., 2013; Wang et al., 2013). Since more than one case-control study was included in ten articles (Nieters et al., 2006; Hold et al., 2009; Mollaki et al., 2009; Ashton et al., 2010; Carvalho et al., 2012; Li et al., 2012; Miedema et al., 2012; Roszak et al., 2012; Lai et al., 2013; Resler et al., 2013), they were considered as separate studies in the meta-analysis. In total, 34 case-control studies from 19 articles, including 17 in English and 2 in Chinese, were included, resulting in 12197 cases and 13488 controls (rs187084, 4148 cases and 4501 controls; rs352140, 3775 cases and 3769 controls; rs5743836, 4274 cases and 5218 controls) being included in the final pooled analyses, among which there were 11, 11, and 12 case-control studies for the rs187084, rs352140, and rs5743836 polymorphisms, respectively. The study populations in the 19 included articles consisted of 9 Asian and 10 Caucasian studies. The distribution of genotypes in the controls of all included studies was consistent with Hard-Weinberg equilibrium except for the studies conducted by Mandal et al. (2012), Pandey et al. (2011), and Lai et al. (2013). Detailed characteristics of the included studies are summarized in Table 1. Table 2 and Figure. 1 describe the heterogeneity test for the investigated SNPs in the overall and subgroup analyses with an appropriate effect model.

Quantitative synthesis

TLR9 rs187084: The aggregated ORs and heterogeneity test results for the association between the rs187084 polymorphism and cancer risk are presented in Table 2 and Figure. 1. Ultimately, 11 case-control studies were pooled into the meta-analysis, including 3 studies focusing on cervical cancer and 8 focusing on other cancers. Of these, 6 studies were conducted in Asian populations, and

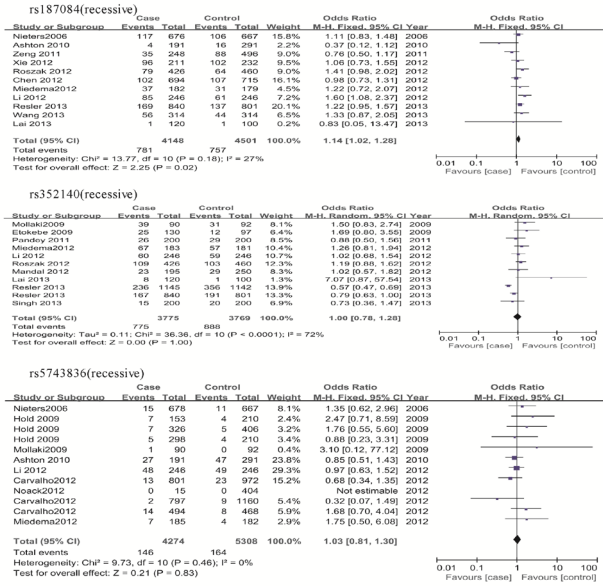

Figure 1. Forest Plots of Cancer Risk Associated with Variants of *TLR9* in a Recessive Model

Table 1. Characteristics of the Included Studies in the Meta-Analysis

First author	Year	Country	Ethnicity	Genotype-case VR Ho/Ht/WT Ho	Genotype-control VR Ho/Ht/WT Ho	Source of control	Genotype method	Cancer type	TLR9 variants	HWE test
Chen	2012	China	Asian	102/346/246	107/319/289	population	PCR-RFLP	Cervical cancer	rs187084	Y
Mandal	2012	India	Asian	23/118/54	29/142/79	population	PCR-RFLP	Prostate cancer	rs352140	N
Hold	2009	Poland	Caucasian	7/58/261	5/85/316	population	Taqman	Gastric cancer	rs5743836	Y
	2009	USA	Caucasian	7/29/117	4/57/149	population	Taqman	Esophageal cancer	rs5743836	Y
	2009	USA	Caucasian	5/69/224	4/57/149	population	Taqman	Gastric cancer	rs5743836	Y
Pandey	2011	India	Asian	26/115/59	29/112/59	population	PCR-RFLP	Cervical cancer	rs352140	N
Resler	2013	USA	Caucasian	169/381/290	137/362/302	population	KASPAR	Breast cancer	rs187084	Y
	2013	USA	Caucasian	167/406/267	191/391/219	population	KASPAR	Breast cancer	rs352140	Y
	2013	USA	Caucasian	236/572/337	356/560/226	population	KASPAR	Breast cancer	rs352140	Y
Roszak	2012	Poland	Caucasian	79/206/141	64/203/193	hospital	PCR-RFLP	Cervical cancer	rs187084	Y
	2012	Poland	Caucasian	109/230/87	103/235/122	hospital	PCR-RFLP	Cervical cancer	rs352140	Y
Lai	2013	China	Asian	1/1/118	1/0/99	hospital	PCR-RFLP	Cervical cancer	rs187084	N
	2013	China	Asian	8/14/98	1/2/97	hospital	PCR-RFLP	Cervical cancer	rs352140	N
Singh	2013	India	Asian	15/89/96	20/97/83	population	PCR-RFLP	Bladder cancer	rs352140	Y
Etokebe	2009	Yugoslavia	Caucasian	25/60/45	12/49/36	hospital	Taqman	Breast cancer	rs352140	Y
Xie	2012	China	Asian	96/85/30	102/109/21	hospital	SNaPshot	HCC	rs187084	Y
Wang	2013	China	Asian	56/164/94	44/148/122	population	PCR-RFLP	Gastric carcinoma	rs187084	Y
Li	2012	China	Asian	85/112/49	61/116/69	population	PCR-RFLP	Colorectal Carcinoma	rs187084	Y
	2012	China	Asian	60/118/68	59/114/73	population	PCR-RFLP	Colorectal Carcinoma	rs352140	Y
	2012	China	Asian	48/115/83	49/109/88	population	PCR-RFLP	Colorectal Carcinoma	rs5743836	Y
Ashton	2010	Australia	Caucasian	4/49/138	16/88/187	population	sequencing	Endometrial cancer	rs187084	Y
	2010	Australia	Caucasian	29/79/85	47/128/116	population	sequencing	Endometrial cancer	rs5743836	Y
Zeng	2011	China	Asian	35/124/89	88/243/165	population	PCR-RFLP	Gastric cancer	rs187084	Y
Nieters	2006	Germany	Caucasian	117/332/227	106/331/230	population	PCR-RFLP	lymphoma	rs187084	Y
	2006	Germany	Caucasian	15/156/507	11/181/475	population	PCR-RFLP	lymphoma	rs5743836	Y
Carvalho	2012	Portugal	Caucasian	2/244/551	9/217/934	hospital	Bi-PASA	lymphoma	rs5743836	Y
	2012	Italy	Caucasian	14/135/345	8/81/379	hospital	Bi-PASA	lymphoma	rs5743836	Y
	2012	USA	Caucasian	13/209/579	23/275/674	hospital	Taqman	lymphoma	rs5743836	Y
Mollaki	2009	Greece	Caucasian	39/49/2	31/50/11	hospital	PCR-RFLP	lymphoma	rs352140	Y
	2009	Greece	Caucasian	1/50/39	0/31/61	hospital	PCR-RFLP	lymphoma	rs5743836	Y
Miedema	2012	Netherlands	Caucasian	67/85/31	57/88/36	population	AS-PCR	ALL	rs352140	Y
	2012	Netherlands	Caucasian	37/88/57	31/98/50	population	AS-PCR	ALL	rs187084	Y
	2012	Netherlands	Caucasian	7/45/133	4/40/138	population	AS-PCR	ALL	rs5743836	Y
Noack	2012	Germany	Caucasian	‘0/4/11	0/61/343	population	TPA	lymphoma	rs5743836	Y

*VR,variant;WT,wild-tlype;Ht,heterozygote; VR Ho,variant homozygote;WT Ho,wide-type homozygote;Y, in agreement with HWE; (Hardy-Weinberg equilibrium); N, in disagreement with HWE;HCC, Hepatocellular carcinoma; ALL, Acute lymphoblastic leukemia

Table 2. Associations between Polymorphisms in *TLR9* and Cancer Risk

TLR9 variants	Cancer type	Sample size (case/control)	Allele contrast OR(95%CI)	P_h	Ht vs. WT Ho OR(95%CI)	P_h	VR Ho vs. WT Ho OR(95%CI)	P_h	Dominant model OR(95%CI)	P_h	Recessive model OR(95%CI)	P_h
rs187084	overall	4148/4501	1.08 (0.98-1.21)	<0.01	1.08 (0.94-1.25)	0.05	1.16 (0.94-1.43)	0.02	1.10 (0.94-1.28)	<0.01	1.14 (1.02-1.28)	0.18
	CC	1240/1275	1.19 (1.05-1.34)	0.34	1.32 (1.10-1.58)	0.83	1.31 (1.03-1.68)	0.27	1.32 (1.11-1.56)	0.63	1.13 (0.90-1.42)	0.3
	Others	2908/3226	1.05 (0.92-1.20)	<0.01	1.00 (0.85-1.19)	0.07	1.09 (0.83-1.44)	0.01	1.02 (0.85-1.24)	0.01	1.14 (1.00-1.30)	0.12
	Asian	1833/2103	1.11 (0.94-1.30)	0.04	1.13 (0.89-1.44)	0.08	1.13 (0.79-1.62)	0.02	1.15 (0.89-1.48)	0.04	1.09 (0.93-1.29)	0.17
	Caucasian	2315/2398	1.06 (0.90-1.24)	0.01	1.06 (0.93-1.20)	0.11	1.19 (0.90-1.58)	0.08	1.05 (0.85-1.30)	0.03	1.18 (1.01-1.38)	0.25
rs352140	overall	3775/3769	1.06 (0.88-1.28)	<0.01	1.04 (0.84-1.30)	<0.01	1.08 (0.74-1.58)	<0.01	1.09 (0.84-1.42)	<0.01	1.00 (0.78-1.28)	<0.01
	CC	746/760	1.45 (0.87-2.43)	<0.01	1.45 (0.83-2.54)	0.05	1.40 (0.73-2.67)	0.1	1.64 (0.85-3.14)	0.01	1.17 (0.90-1.53)	0.15
	BC	2115/2040	0.83 (0.64-1.08)	<0.01	0.77 (0.66-0.89)	0.26	0.71 (0.42-1.21)	<0.01	0.76 (0.56-1.02)	0.02	0.79 (0.53-1.18)	<0.01
	Others	914/969	1.07 (0.94-1.22)	0.17	1.08 (0.87-1.35)	0.16	1.17 (0.88-1.56)	0.1	1.12 (0.81-1.54)	0.09	1.10 (0.88-1.39)	0.57
	Asian	961/996	1.10 (0.82-1.46)	<0.01	1.11 (0.80-1.53)	0.08	1.04 (0.78-1.40)	0.23	1.16 (0.79-1.69)	0.01	1.00 (0.77-1.30)	0.36
	Caucasian	2814/2773	1.03 (0.80-1.33)	<0.01	1.00 (0.74-1.36)	<0.01	1.12 (0.65-1.94)	<0.01	1.04 (0.72-1.50)	<0.01	1.02 (0.72-1.44)	<0.01
rs5743836	overall	4274/5218	1.12 (0.93-1.35)	<0.01	1.12 (0.87-1.44)	<0.01	1.04 (0.81-1.33)	0.41	1.10 (0.86-1.42)	<0.01	1.03 (0.81-1.30)	0.46
	lymphoma	2875/3763	1.33 (0.96-1.85)	<0.01	1.44 (0.96-2.16)	<0.01	1.00 (0.67-1.51)	0.18	1.43 (0.96-2.13)	<0.01	0.97 (0.65-1.46)	0.22
	DSC	1023/982	0.95 (0.81-1.11)	0.79	0.85 (0.70-1.05)	0.39	1.18 (0.78-1.77)	0.6	0.81 (0.66-0.98)	0.21	1.13 (0.77-1.64)	0.45
	Others	376/473	0.97 (0.77-1.21)	0.14	0.96 (0.71-1.30)	0.31	0.90 (0.55-1.48)	0.23	0.96 (0.72-1.29)	0.19	0.95 (0.60-1.52)	0.3

*VR, variant; WT, wild-type; Ht, heterozygote; VR Ho, variant homozygote; WT Ho, wide-type homozygote. The results were in bold, if the 95% CI; excluded 1 or p<0.05; CC, cervical cancer; DSC, digestive system cancer; BC, breast cancer; Ph, p value of Q-test for heterogeneity test, and Random effects model was used when P value for heterogeneity test<0.1; otherwise, fixed effects model was used in the analysis

5 studies in Caucasian populations. Overall, a significantly increased risk of cancer for the rs187084 polymorphism was found in the recessive model (CC *vs* TC+TT: OR=1.14, 95% CI=1.02-1.28). Similarly, in the subgroup analysis based on ethnicity, we observed a significant positive association between the rs187084 polymorphism and cancer risk in Caucasians in the recessive model (CC *vs* TC+TT: OR=1.18, 95% CI=1.01-1.38) but not in Asian populations. In the stratified analyses by specific tumor types, an elevated risk among studies of cervical cancer (allele contrast, C *vs* T: OR=1.19, 95% CI=1.05-1.34; Ht *vs* WT Ho, TC *vs* TT: OR=1.32, 95% CI=1.10-1.58; VR Ho *vs* WT Ho, CC *vs* TT: OR=1.31, 95% CI= 1.03-1.68; dominant model, CC+TC *vs* TT: OR=1.32, 95% CI=1.11-1.56), and other cancers in the recessive model (CC vs. TC+TT: OR=1.14, 95% CI=1.00-1.30) were noted. The details of the results are listed in Table 2.

TLR9 rs352140: The *TLR9* rs352140 polymorphism was investigated in 11 case-control studies (3, 3, and 5 studies on cervical, breast, and other cancers, respectively). There were 5 studies of Asian and 6 studies of Caucasian populations. The results of the overall meta-analysis did not suggest any significant associations between the *TLR9* rs352140 polymorphism with cancer risk in all genetic models. Similarly, in terms of the stratified analysis by ethnicity, no significant association was revealed. However, the subgroup analyses for different cancer types indicated that the *TLR9* rs352140 polymorphism was significantly associated with a decreased risk of breast cancer (Ht *vs* WT Ho, GA *vs* GG: OR=0.77, 95% CI=0.66-0.89), whereas no significant association was observed in any genetic model for cervical cancer and other cancers (Table 2).

TLR9 rs5743836: Twelve case-control studies, including 6 on lymphoma, 4 on digestive system cancers, and 2 on other cancers, were pooled into the meta-analysis. The overall analysis showed no significant associations between the *TLR9* rs5743836 polymorphism and cancer risk. Since only 1 of the 12 included studies for this association was conducted in an Asian population, subgroup analysis by ethnicity was not performed. However, in the subgroup analysis based on tumor type, a significantly decreased risk of digestive system cancers was observed in the dominant model (CC+TC *vs* TT: OR=0.81, 95% CI=0.66-0.98), whereas no significant association was suggested in any genetic model for lymphoma and other cancers (Table 2).

Table 3. Sensitivity Analysis by Sequential Omission

Study omitted	CC *vs* TC+TT model p	OR(95%CI)	Effect model
Nieters 2006	0.03	1.14 (1.01-1.30)	F
Ashton 2010	0.01	1.16 (1.03-1.30)	F
Zeng 2011	<0.01	1.18 (1.05-1.32)	F
Xie 2012	0.02	1.15 (1.02-1.29)	F
Roszak 2012	0.08	1.11 (0.99-1.25)	F
Chen 2012	0.01	1.17 (1.03-1.32)	F
Miedema 2012	0.03	1.14 (1.01-1.28)	F
Li 2012	0.1	1.10 (0.98-1.24)	F
Resler 2013	0.09	1.12 (0.98-1.27)	F
Wang 2013	0.05	1.13 (1.00-1.27)	F
Lai 2013	0.02	1.14 (1.02-1.28)	F

*F, fixed effect model; OR, odds ratio; 95%CI, 95% confidence interval

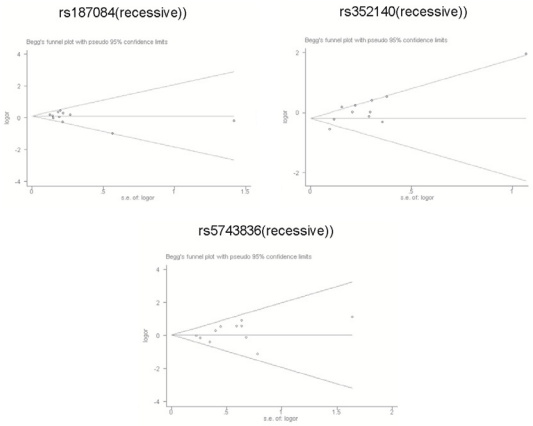

Figure 2. Begg's Funnel Plots for Publication Bias Test on the Association of *TLR9* Polymorphisms with Cancer Risk in a Recessive Model

Sensitivity analysis and publication bias

Sensitivity analysis was performed by sequential omission of individual studies to investigate the influence of each study on the overall OR. The significance of the pooled OR in the analysis for rs352140 and rs5743836 was not affected excessively in any of the genetic models. However, in the sensitivity analysis, we found a

slight fluctuation in the *p* value around 0.05, indicating a significant association of the rs187084 polymorphism CC vs. TC+TT with cancer risk (Table 3). Nevertheless, a significant association of this polymorphism with the risk of cancer was suggested by most results of sequential omission in the sensitivity analysis. Publication bias of the literature was assessed by Beggar's test. The graphical funnel plots for the three studied SNPs were all symmetrical, suggesting no evident publication bias (recessive model: *P*=0.484 for rs187084, *P*=0.186 for rs352140, *P*=0.697 for rs5743836). The funnel plots for publication bias are shown in Figue. 2.

Discussion

Accumulative evidence has indicated that *TLR9* plays an important role in both innate and adaptive immune responses, constituting a possible link between infections, chronic inflammation, and tumor development (Chen et al., 2012; Zhang et al., 2013). Numerous studies have reported that the expression of *TLR9* at the transcriptomic or proteomic level can be detected in a wide spectrum of cancers, including bladder, lung, cervical, breast, prostate, colorectal, and gastric cancers. *TLR9* is capable of recognizing unmethylated CpG dinucleotide motifs located in the DNA of bacteria, viruses, and fungi, resulting in the activation of MyD88 and interleukin-1 receptor family members and subsequent activation of the transcription factor NF-κB, leading to the transcription of pro-inflammatory genes, which may participate in inflammation-induced tumor growth and progression (Aderem et al., 2000; Huang et al., 2005; Wang et al., 2013).

If an individual's ability to properly respond to TLR ligands is impaired by certain SNPs within the *TLR9* gene, the individual may become susceptible to cancer development. Several functional *TLR9* gene polymorphisms have been identified and hypothesized to be predisposing genetic factors for various malignancies in recent years, of which the most intensively studied polymorphisms are rs187084 (T1486C), rs352140 (G2848A), and rs5743836 (T1237C). However, the reported associations of *TLR9* polymorphisms with cancer risk are inconsistent, which prompted us to perform this quantitative analysis to estimate and explain their diversity. To the best of our knowledge, this is the most comprehensive meta-analysis of the association between *TLR9* gene polymorphisms and tumor risks to date. Our meta-analysis included a total of 34 case-control studies derived from 19 publications involving 12197 cases and 13488 controls, and provided a quantitative assessment of the associations between the three widely studied polymorphisms in the *TLR9* gene and cancer risk.

We here found that the *TLR9* rs187084 polymorphism is associated with a significant increase in overall cancer risk, whereas the rs5743836 and rs352140 polymorphisms do not appear to have an influence on cancer risk. In addition to a noteworthy positive association between the rs187084 polymorphism and the risk of cervical cancer, a weak risk effect on overall cancer risk was also revealed particularly in Caucasians. Although the current meta-analysis suggested the importance of the *TLR9* rs187084 polymorphism on cancer risk, this association may need to be confirmed in further investigation with a larger sample size of less heterogeneity.

For the rs187084 polymorphism, 4148 cases and 4501 controls from 11 case-control studies were pooled into the meta-analysis. In the overall analysis, we found a significant association between the rs187084 polymorphism and increased cancer risk in the recessive model, which is inconsistent with the results of a previous meta-analysis by Zhang et al. (2013), which failed to show any significant association by any genetic model. However, this may be due to the relatively small sample size (1370 cases and 1382 controls) in their study. In terms of stratified analyses by cancer types, we observed a significantly increased risk for cervical cancer, which is different from the results of some previous individual studies regarding this association. Similar to our results, both Roszak et al. (2012) and Chen et al. (2012) found that the rs187084 heterozygote TC is associated with a significantly increased risk of cervical cancer, although no such association was detectable by Lai et al. (2013). The likely reason for the negative finding in the previous study may be the insufficient power to detect the effect owing to a relatively small sample size.

For the rs352140 polymorphism, previous studies have found that this synonymous polymorphism in exon 2 of the *TLR9* gene may affect the mRNA expression of *TLR9*, and thereby influence cancer risk. However, to date, no studies have demonstrated significant associations between this SNP and breast (Etokebe et al., 2009; Resler et al., 2013), bladder (Singh et al., 2013), prostate (Mandal et al., 2012), colorectal (Li et al., 2009), and cervical cancers (Pandey et al., 2011; Lai et al., 2013), except for one study, which reported an increased risk for cervical cancer (Roszak et al., 2012). Consistent with most of these individual studies, in this meta-analysis incorporating 11 case-control studies with 3775 cases and 3769 controls, we found no significant evidence supporting an association between cancer risk and the rs352140 polymorphism. The subgroup analyses based on cancer type, however, showed a significantly decreased risk of breast cancer in persons carrying the GA genotype compared with those carrying the GG genotype, suggesting that the rs352140 polymorphism may play a role, although modest, in breast cancer development. In contrast to our result, Zhang et al. (2013) reported an association of the rs352140 polymorphism with an increased cancer risk. Two potential reasons may account for this disparity. First, this discrepancy indicates that tumor specificity is likely significant for the assessment regarding the association. Second, the discrepancy may be due to the relatively small sample size in the study by Zhang et al. (2013) which may have insufficient statistical power to detect the exact effect, or which may generate a fluctuated risk estimate.

For the rs5743836 polymorphism, 12 case-control studies with a total of 4274 cases and 5218 controls were included in this meta-analysis. The rs5743836 polymorphism develops because of the substitution of tyrosine with cytosine at nucleotide position-1237 within the proximal promoter region of *TLR9* (Hold et al., 2009).

Zhang et al. (2013) demonstrated no association between rs5743836 polymorphism and cancer risk in their pooled analysis, and similarly, we did not observe any significant associations between rs5743836 polymorphism and cancer risk in our overall analysis. However, in the stratified analyses by cancer types, a significantly reduced risk was observed for C allele carriers in digestive system cancers, but not in lymphoma and other cancers. Conversely, the rs5743836 polymorphism has been reported to show no apparent association with gastric cancer risk in a previous study (Hold et al., 2009). These discrepancies may be due to different carcinogenic mechanisms of different cancers and/or due to the higher power of the quantitative approach employed here.

In light of differences in genetic backgrounds, which may contribute to the associations between polymorphisms and tumor susceptibility, we here conducted stratified analyses by ethnicity. A significantly increased risk for rs187084 was found in Caucasians but not in Asians, suggesting a possible role of genetics and/or environmental factors. For rs352140, however, the subgroup analysis failed to reveal any significant associations, which is inconsistent with the analysis by Zhang et al. (2013). We speculate that, due to the larger sample size in the current meta-analysis, better estimates were obtained compared with individual analyses using smaller sample sizes. Moreover, no significant association between the rs5743836 polymorphism and cancer risk was found in Caucasians, while there was only one available study conducted in Asians included in our meta-analysis, and subgroup analysis could hence not be performed. Hence, the current findings warrant future investigations into ethnic differences that may validate the risk of the polymorphisms for cancers, especially in Asians and Caucasians.

In the present meta-analysis, the heterogeneity of the subgroups reduced significantly compared to the relatively large heterogeneity that existed in the overall analysis. Accordingly, it may be hypothesized that the relatively large heterogeneity existed partially owing to differences in tumor types and ethnicities. Moreover, sensitivity analysis was performed by sequential omission of individual studies, and the stability of the results regarding the *TLR9* rs352140 and rs5743836 polymorphisms and cancer risk was confirmed. Although a slight fluctuation of the *P* value around 0.05 indicating a significant association of rs187084 polymorphism with the risk of cancer was observed for CC vs. TC+TT in the sensitivity analysis, most results of the sequential omissions supported the significance of this polymorphism. Moreover, no evidence of publication bias was revealed for the studied polymorphisms using Beggar's test, suggesting that our results were reliable.

Despite of the considerable efforts to explore associations between *TLR9* polymorphisms and cancer risks, there are limitations in interpreting the results of the current meta-analysis. First, the included studies in our analysis only reported positive findings, and studies with negative findings may not have been published, thus resulting in inevitable publication bias. Second, our results are based on published, but unadjusted estimates, and a more precise analysis should be conducted by adjusting for other factors, such as age, smoking, drinking, and environmental factors. Third, the published controls are not uniformly defined, and the possibility cannot be ruled out that some individuals may develop cancers in subsequent years after the investigation. Hence, non-differential misclassification bias is possible. Fourth, there is a lack of available studies regarding these associations in different ethnicities. In studies on the rs5743836 polymorphism, most participants were Caucasians, while only one case-control studies was conducted on an Asian population, which would limit the comprehensiveness and veracity of the results. Furthermore, it was difficult to perform subgroup analysis for each cancer type, and we hence grouped some rarely examined cancers as "other cancers" in this analysis. Additionally, the lack of original data on genotypes and environmental risk factors for the included studies limited our evaluation on the potential gene-environment interactions.

In summary, the results of our meta-analysis demonstrated that the *TLR9* rs187084 polymorphism is associated with an increased cancer risk, specifically of cervical cancer, and among Caucasians. Conversely, the *TLR9* rs352140 and rs5743836 polymorphisms may play protective roles against the development of breast and digestive system cancers, respectively. Nevertheless, carcinogenesis is a multifactorial and multistep process involving multiple genes, and the findings presented herein warrant future investigations to validate these associations using well-designed studies and larger sample sizes.

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