Pathology & Oncology Research (2020) 26:307–315
https://doi.org/10.1007/s12253-018-0465-x

ORIGINAL ARTICLE

The Relationship of the *TLR9* and *TLR2* Genetic Polymorphisms with Cervical Cancer Risk: a Meta-Analysis of Case-Control Studies

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Received: 13 January 2018 / Accepted: 23 August 2018 / Published online: 13 September 2018

Abstract

This meta-analysis aimed to assess the association of common *TLR9* and *TLR2* gene polymorphisms (*TLR9* 1486 T/C, *TLR9* G2848A, and *TLR2*–196 to −174 del/ins) with cervical cancer risk. Studies were searched in Scopus, Pubmed, Embase, and CNKI until December 2017. Both fixed-effects and random-effects models were applied to combine odds ratio (OR) and 95% confidence intervals (95% CI). A total of 11 studies including 7856 participants were identified. The pooled estimation revealed an increased risk of cervical cancer in Caucasian subjects carrying the C allele of the *TLR9* 1486 T/C polymorphism (OR = 1.46, 95% CI: 1.11–1.92, $p = 0.007$), while there was a decreased risk in Mixed subjects carrying the C allele (OR = 0.35, 95% CI: 0.15–0.82, $p = 0.016$). Concerning the *TLR9* G2848A polymorphism, the A allele was associated with an increased risk of cervical cancer in Caucasians (OR = 1.19, 95% CI: 1.02–1.40, $p = 0.030$), whereas Asian and Mixed subjects showed no significant associations. No significant associations were demonstrated between the *TLR2*–196 to −174 del/ins polymorphism and cervical cancer. Our findings suggest that the *TLR9* 1486 T/C and G2848A polymorphisms contribute to cervical cancer risk, but there is no association of the *TLR2*–196 to −174 del/ins polymorphism with cervical cancer.

Keywords TLR9 · TLR2 · Meta-analysis · Cervical cancer · Polymorphism

Background

Cervical cancer is the third most prevalent cancer in women worldwide and as such represents a significant global health burden [1]. It is estimated that in 2012, approximately 528,000 women developed cervical cancer and that 266,000 died from the disease [1]. Persistent infection with oncogenic human papillomavirus (HPV) has been established as a major contributor to cervical cancer. However, HPV infections alone are not sufficient for the development of cervical cancer, as transient HPV infections are extremely common in the general population and relatively few women infected with HPV progress to cervical cancer [2]. Familial aggregation studies and evaluation of inherited genetic variations suggest that host genetic factors may also contribute to cervical cancer pathogenesis.

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Toll-like receptors (TLRs) are type I transmembrane glycoproteins that recognize a diverse array of microbial pathogen-associated molecular patterns (PAMPs) and endogenous damage associated molecular patterns (DAMPs) [3]. Signals transduced through the TLRs cause synthesis and secretion of pro-inflammatory cytokines and co-stimulatory molecules, which has a critical role in innate and adaptive immunity. To date, 13 different TLRs have been identified in mammals, 10 of which are found in humans. Beyond their role as innate immune receptors for pathogenic invaders, there is considerable evidence that TLRs are highly important in cancer biology [3]. Previous studies examined TLR expression in cell lines bearing episomal or integrated copies of the HPV genome and in cervical cancer tissues [4–8]. High levels of TLR2 and TLR9 in cervical cancer tissues have been associated with disease severity, progression and poor clinical outcome [4, 9, 10].

In recent years, efforts have been put into investigating the association between cervical cancer and genetic variants of *TLR9* and *TLR2*. The aim of this meta-analysis is to evaluate the role of common *TLR9* and *TLR2* gene polymorphisms (*TLR9* 1486 T/C, *TLR9* G2848A, *TLR2*–196 to −174 del/ins) in risk of cervical cancer. Separate analyses were conducted so as to investigate race-specific effects.

Methods

Literature Search

The primary literature search was conducted up to the end of December 2017 using the Scopus, Pubmed, Embase, and China National Knowledge Infrastructure (CNKI) research databases. The following key words were used in the literature search: cervical cancer, gene, toll-like receptor, TLR2, TLR9, polymorphism, and susceptibility. We screened citations of all potentially eligible articles without language restrictions. Reference lists of all retrieved papers were manually searched for potentially eligible papers. Institutional review board approval and patient consent were not required because of the nature of this study as a meta-analysis.

Study Selection

The present meta-analysis included case-control studies that fulfilled the following inclusion criteria: (1) written in English or Chinese; (2) provided cases of cervical cancer and control subjects without cervical cancer; (3) reported risk estimates and/or presentation of data necessary for calculating odds ratios (ORs) and 95% confidence intervals (CIs); (4) use of validated molecular methods for genotyping; and (5) published in a peer-reviewed journal. In studies with overlapping cases or controls, we included the most recent and/or the largest study with extractable data in the meta-analysis. We excluded studies that were published only as abstracts or conference reports.

Data Extraction

For each included study, data were extracted using standardized forms. Data extracted from each study included: first author, year of publication, geographical location, ethnic group of the study population, diagnostic criteria for cervical cancer, age of cases and controls, genotyping method, source of controls, analysis for subgroups of interest, and the genotype distribution of cases and controls. Ethnicity was classified as Caucasian, Asian and Mixed. Two experienced investigators independently extracted data, and disagreements were resolved through consensus. All data were extracted from published articles, and we did not contact individual authors for further information.

Statistical Analyses

All statistical analyses for this meta-analysis were conducted on Stata version 10.0.

The combined ORs were calculated for the allele contrast, the dominant, recessive, and homozygote models using the meta-analysis technique. No value was added to cells with zero counts. We evaluated between-study heterogeneity using the χ^2-based Q statistic and the I^2 statistic for the extent of heterogeneity. Tests for heterogeneity were performed for each meta-analysis, with significance set at $p < 0.10$. The pooled ORs were calculated by the fixed-effect model in case of no heterogeneity [11]. Otherwise, a random-effect model was used [12]. The influence of individual studies on the summary OR was evaluated by reestimating and plotting the summary OR in the absence of each study. We used a Galbraith plot to evaluate the potential source of heterogeneity. Publication bias was assessed by means of a funnel plot of lnOR versus the inverse of the standard error of lnOR for individual studies. The Egger' s test was used to formally evaluate publication bias.

Results

Study Characteristics

Figure 1 showed the selection process of studies included in our meta-analysis. Electronic database searches resulted in 271 potentially relevant studies, of which 258 did not meet inclusion criteria. Of the 13 studies reviewed in full-text, two were excluded because they were irrelevant papers. We finally included 11 case-control studies with a total of 3543 cervical cancer cases and 4313 controls [13–23]. They were published between 2009 and 2017. Tables 1 and 2 summarize the characteristics of these studies. Seven of the studies were conducted in Asian populations [13–15, 17, 19, 21, 23], two in Caucasian populations [16, 20], and two in Mixed populations [18, 22]. All studies used validated variant detection methods, such as PCR or PCR-RFLP, with the majority employing multiplex PCR protocols to identify the *TLR9* and *TLR2* genotypes along with control genes for reaction quality control. A summary of the meta-analysis results regarding the associations of *TLR9* and *TLR2* gene polymorphisms with cervical cancer risk is shown in Tables 3-5.

Meta-Analysis for the *TLR9* and *TLR2* Polymorphisms

The studies investigating the *TLR9* 1486 T/C polymorphism reported on a total of 2390 cases and 2531 controls. The minor allele frequency (MAF) was 24.3% in Asians, 36.0% in Caucasians, and 49.0% in Mixed populations. When all studies were included there was no evidence for association between the *TLR9* 1486 T/C polymorphism and cervical cancer under dominant model (CC + TC vs. TT, OR = 1.11, 95% CI: 0.79–1.56, Z = 0.61, $p = 0.545$) (Fig. 2), homozygous model (CC vs. TT, OR = 1.19, 95% CI: 0.94–1.51, Z = 1.44, $p = 0.149$), recessive model (CC vs. TC + TT, OR = 1.07, 95% CI: 0.86–1.33, Z = 0.62, $p = 0.533$), and allele contrast (C allele vs. T allele, OR = 1.04, 95% CI: 0.88–1.23, Z = 0.44, $p =$

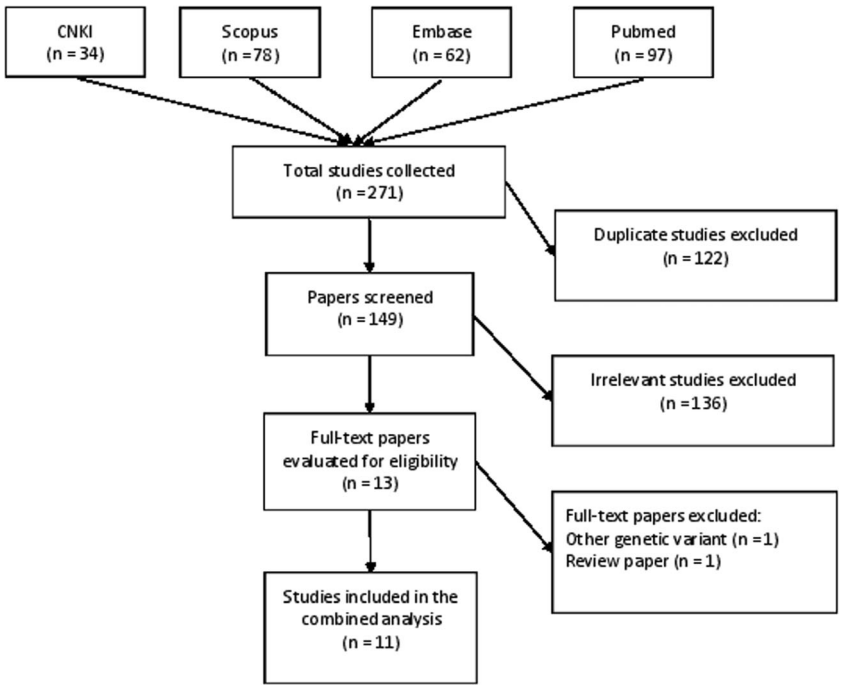

Fig. 1 Flowchart showing literature search and study selection

0.658). In subgroup analysis by ethnicity, an increased risk of cervical cancer was observed in Caucasian subjects under dominant model (OR = 1.46, 95% CI: 1.11–1.92, Z = 2.71, $p = 0.007$) (Fig. 2), homozygous model (OR = 1.69, 95% CI: 1.14–2.51, Z = 2.60, $p = 0.009$) and allele contrast (OR = 1.33, 95% CI: 1.10–1.61, Z = 2.90, $p = 0.004$), whereas in Mixed subjects there was a decreased risk of cervical cancer under dominant model (OR = 0.35, 95% CI: 0.15–0.82, Z = 2.41, $p = 0.016$) (Fig. 2) and homozygous model (OR = 0.32, 95% CI: 0.12–0.84, Z = 2.32, $p = 0.020$). In Asians, there was no association between the *TLR9* 1486 T/C polymorphism and cervical cancer risk (Table 3).

Table 1 Characteristics of association studies investigating the association between *TLR9* polymorphisms and cervical cancer risk

Author	Year	Country	Ethnicity	Diagnosis criteria	Controls		Cases		Age		Genotyping method
					1486 T/C	G2848A	1486 T/C	G2848A	Controls	Cases	
Pandey	2011	India	Asians	Histologically confirmed	NA	200	NA	200	47.0 ± 9.8	48.3 ± 10.0	PCR-RFLP
Chen	2012	China	Asians	Histologically confirmed	715	NA	694	NA	54.6 ± 11.8	54.6 ± 12.8	PCR-RFLP
Roszak	2012	Poland	Caucasians	Histologically confirmed	460	460	426	426	51.9 ± 9.8	51.8 ± 9.7	PCR-RFLP
Lai	2013	China	Asians	Histologically confirmed	100	100	120	120	NA	NA	PCR-RFLP
Bodelon	2014	USA	Mixed	Identified through the CSS	1100	1100	876	876	18–74	18–74	Illumina Goldengate multiplex platform
Bi	2014	China	Asians	Histologically confirmed	100	100	102	102	NA	NA	PCR-RFLP
Zidi	2016	Tunisia	Caucasians	Cervical biopsy	NA	260	NA	130	53 ± 1.2	52 ± 0.9	PCR-RFLP
Xu	2016	China	Asians	Histologically confirmed	NA	330	NA	253	51.3 ± 8.8	45.8 ± 9.7	Taqman assay
Martínez-Campos	2017	Mexico	Mixed	Histologically confirmed	56	NA	172	NA	37.3 ± 13.0	50.9 ± 13.1	Predesigned 5′ endonulease assay
Jin	2017	China	Asians	Histologically confirmed	NA	842	NA	420	57.5 ± 16.1	58.8 ± 15.2	PCR-RFLP

CSS Cancer Surveillance System, *NA* not available, *PCR-RFLP* polymerase chain reaction-restriction fragment length polymorphism, *TLR9* toll-like receptor 9, *USA* United States of America

Table 2 Characteristics of association studies investigating the association between the *TLR2*–196 to −174 del/ins polymorphism and cervical cancer risk

Author	Year	Country	Ethnicity	Diagnosis criteria	Controls	Cases	Age		Genotyping method
							Controls	Cases	
Pandey	2009	India	Asians	Histologically confirmed	150	150	45.6 ± 9.6	47.9 ± 10.4	PCR
Bi	2014	China	Asians	Histologically confirmed	100	102	NA	NA	PCR
Zidi	2016	Tunisia	Caucasians	Cervical biopsy	260	130	53 ± 1.2	52 ± 0.9	PCR

NA not available, *PCR* polymerase chain reaction, *TLR2* toll-like receptor 2

With respect to the *TLR9* G2848A polymorphism eight studies (2527 cases and 3392 controls) were eligible. The overall meta-analyses showed that the *TLR9* G2848A polymorphism was associated with cervical cancer under dominant model (AA + GA vs. GG, OR = 1.34, 95% CI: 1.02–1.77, Z = 2.08, $p = 0.038$) and homozygous model (AA vs. GG, OR = 1.60, 95% CI: 1.04–2.46, Z = 2.14, $p = 0.033$) (Table 4). In subgroup analysis by ethnicity, Caucasian subjects showed significance under dominant model (OR = 1.29, 95% CI: 1.00–1.67, Z = 1.97, $p = 0.049$), homozygous model (OR = 1.43, 95% CI: 1.04–1.95, Z = 2.21, $p = 0.027$), and allele contrast (OR = 1.19, 95% CI: 1.02–1.40, Z = 2.17, $p = 0.030$) (Fig. 3), whereas Asian and Mixed subjects showed no significant associations (Table 4).

The studies investigating the *TLR2*–196 to −174 del/ins polymorphism reported on a total of 382 cases and 510 controls. There was no evidence of an association between the *TLR2*–196 to −174 del/ins polymorphism and cervical cancer risk under dominant model (del/del + del/ins vs. ins/ins, OR = 1.19, 95% CI: 0.89–1.60, Z = 1.17, $p = 0.224$), homozygous model (del/del vs. ins/ins, OR = 1.65, 95% CI: 0.28–9.64, Z = 0.56, $p = 0.577$), recessive model (del/del vs. del/ins + ins/ins, OR = 1.52, 95% CI: 0.26–8.79, Z = 0.47, $p = 0.637$), and allele contrast (del vs. ins, OR = 1.13, 95% CI: 0.89–1.43, Z = 1.02, $p = 0.310$) (Fig. 4). Subgroup analysis by ethnicity did not find any associations in Asians or Caucasians (Table 5).

Heterogeneity, Publication Bias and Sensitivity Analyses

Significant heterogeneity between studies was observed for the *TLR9* polymorphisms (Tables 3, 4 and 5). Concerning

Table 3 Meta-analysis of *TLR9* 1486C/T polymorphism

Contrast	Population	Study (n)	Heterogeneity		Association		Overall effect	
			I^2 (%)	*p* value	OR	95% CI	Z-value	*p* value
CC + TC vs. TT	All	5	60.2	0.040	1.11	0.79–1.56	0.61	0.545
	Asians	3	0.0	0.897	1.22	1.00–1.50	1.93	0.054
	Caucasians	1	NA	NA	1.46	1.11–1.92	2.71	0.007
	Mixed	1	NA	NA	0.35	0.15–0.82	2.41	0.016
CC vs. TT	All	4	71.1	0.016	1.19	0.94–1.51	1.44	0.149
	Asians	2	0.0	0.840	1.12	0.81–1.53	0.67	0.500
	Caucasians	1	NA	NA	1.69	1.14–2.51	2.60	0.009
	Mixed	1	NA	NA	0.32	0.12–0.84	2.32	0.020
CC vs. TC + TT	All	4	35.7	0.198	1.07	0.86–1.33	0.62	0.533
	Asians	2	0.0	0.909	0.98	0.73–1.31	0.15	0.878
	Caucasians	1	NA	NA	1.41	0.98–2.02	1.87	0.062
	Mixed	1	NA	NA	0.66	0.34–1.28	1.23	0.219
C vs. T	All	6	62.4	0.021	1.04	0.88–1.23	0.44	0.658
	Asians	3	0.0	0.382	1.06	0.92–1.23	0.86	0.389
	Caucasians	1	NA	NA	1.33	1.10–1.61	2.90	0.004
	Mixed	2	83.7	0.013	0.84	0.48–1.46	0.63	0.528

CI confidence interval, *NA* not available, *OR* odds ratio, *TLR9* toll-like receptor 9

Table 4 Meta-analysis of *TLR9* G2848A polymorphism

Contrast	Population	Study (n)	Heterogeneity		Association		Overall effect	
			I^2 (%)	*p* value	OR	95% CI	Z-value	*p* value
AA + GA vs. GG	All	7	68.2	0.004	1.34	1.02–1.77	2.08	0.038
	Asians	5	77.2	0.001	1.41	0.93–2.13	1.63	0.103
	Caucasians	2	0.0	0.367	1.29	1.00–1.67	1.97	0.049
AA vs. GG	All	6	71.0	0.004	1.60	1.04–2.46	2.14	0.033
	Asians	4	81.1	0.001	1.81	0.84–3.89	1.52	0.128
	Caucasians	2	0.0	0.727	1.43	1.04–1.95	2.21	0.027
AA vs. GA + GG	All	6	68.5	0.007	1.45	1.00–2.09	1.96	0.050
	Asians	4	78.7	0.003	1.61	0.82–3.14	1.38	0.166
	Caucasians	2	0.0	0.449	1.27	0.98–1.65	1.83	0.068
A vs. G	All	8	81.8	0.003	1.21	0.99–1.49	1.85	0.065
	Asians	5	88.3	0.001	1.33	0.89–2.00	1.38	0.168
	Caucasians	2	0.0	0.826	1.19	1.02–1.40	2.17	0.030
	Mixed	1	NA	NA	1.09	0.96–1.24	1.36	0.174

CI confidence interval, *NA* not available, *OR* odds ratio, *TLR9* toll-like receptor 9

the *TLR9* 1486C/T polymorphism, the Galbraith plot showed that the study by Roszak et al. and the study by Martínez-Campos et al. largely accounted for the heterogeneity (Fig. 5a). For the *TLR9* G2848A polymorphism, the study by Lai et al. and the study by Jin et al. were the major sources of heterogeneity (Fig. 5b). There was no evidence of publication bias on funnel plots (not shown). The Egger's test additionally showed no evidence of publication bias ($p = 0.401$ for *TLR9* 1486 T/C, $p = 0.627$ for *TLR9* G2848A, $p = 0.314$ for *TLR2*–196 to −174 del/ins). In order to determine the influence of individual studies on the pooled OR, sensitivity was used to recalculate the pooled OR by ruling out each of the involved study in turn. No individual study was found to be significantly biasing the pooled results for the *TLR9* and *TLR2* polymorphisms (not shown).

Discussion

Cervical cancer is a leading cause of death in women worldwide, accounting for 7.5% of all female cancer deaths. Epidemiological studies and genomic analyses have suggested that genetic factors influencing the host immune response may be involved in the pathogenesis of cervical cancer.

Table 5 Meta-analysis of *TLR2*–196 to −174 del/ins polymorphism

Contrast	Population	Study (n)	Heterogeneity		Association		Overall effect	
			I^2 (%)	*p* value	OR	95% CI	Z-value	*p* value
del/del + del/ins vs. ins/ins	All	3	0.0	0.385	1.19	0.89–1.60	1.17	0.224
	Asians	2	47.2	0.169	1.18	0.81–1.71	0.84	0.400
	Caucasians	1	NA	NA	1.22	0.76–1.96	0.82	0.414
del/del vs. ins/ins	All	2	61.8	0.106	1.65	0.28–9.64	0.56	0.577
	Asians	1	NA	NA	5.59	0.64–48.63	1.56	0.119
	Caucasians	1	NA	NA	0.86	0.41–1.81	0.40	0.688
del/del vs. del/ins + ins/ins	All	2	61.6	0.107	1.52	0.26–8.79	0.47	0.637
	Asians	1	NA	NA	5.14	0.59–44.52	1.49	0.137
	Caucasians	1	NA	NA	0.80	0.38–1.66	0.60	0.547
del vs. ins	All	3	19.7	0.288	1.13	0.89–1.43	1.02	0.310
	Asians	2	57.7	0.124	1.17	0.87–1.58	1.02	0.305
	Caucasians	1	NA	NA	1.07	0.73–1.57	0.33	0.741

CI confidence interval, *NA* not available, *OR* odds ratio, *TLR9* toll-like receptor 9

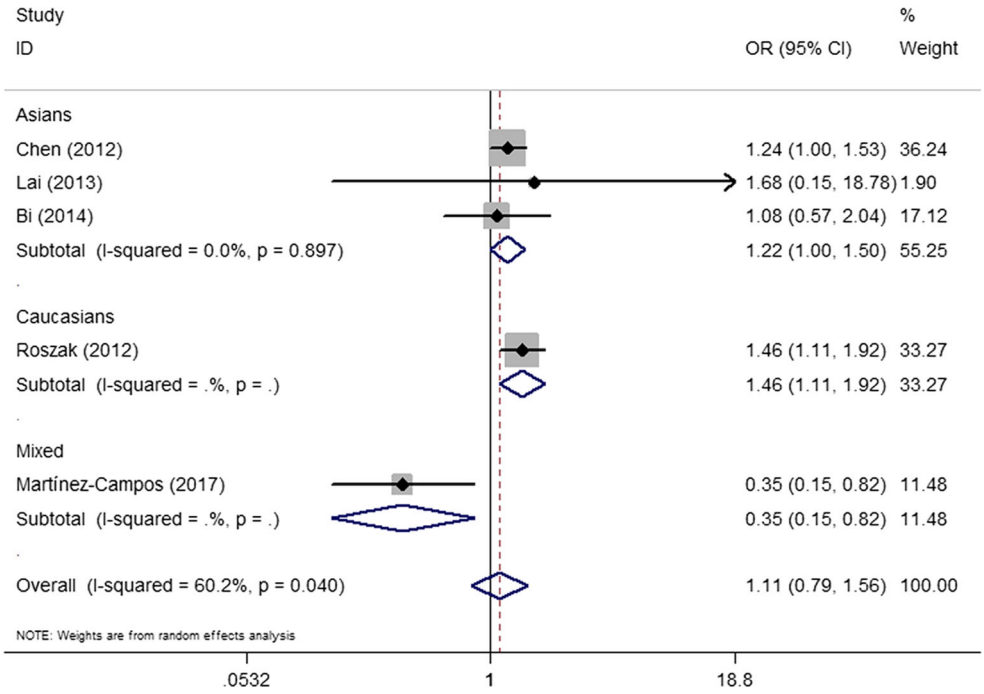

Fig. 2 Forest plot for the association between the *TLR9* 1486 T/C polymorphism and cervical cancer risk in a dominant model (CC + TC vs. TT)

TLRs play important roles in the signaling of many pathogen-related molecules and endogenous proteins associated with immune activation. *TLR* genes are expressed in the female genital tract and there is evidence that they contribute to the clearance of the HPV infection [24, 25]. Genetic association studies have evaluated the association between cervical cancer and the common genetic polymorphisms of *TLR2* (−196 to −174 del/ins) and *TLR9* (1486 T/C and G2848A). However, there are inconsistencies in the results of these studies. We performed a meta-analysis of 11 case-control studies with a total of 3543 cases and 4313 controls to investigate the association. The principal findings in the present meta-analysis were the following: (1) the *TLR9* 1486C/T polymorphism was associated with an increased risk of cervical cancer in Caucasian subjects, while it was associated a decreased risk of cervical cancer in Mixed subjects; (2) the *TLR9* G2848A polymorphism was associated with an increased risk of cervical cancer in Caucasian subjects but not in Asian and Mixed subjects; and (3) there was no association of the *TLR2*–196 to −174 del/ins polymorphism with cervical cancer.

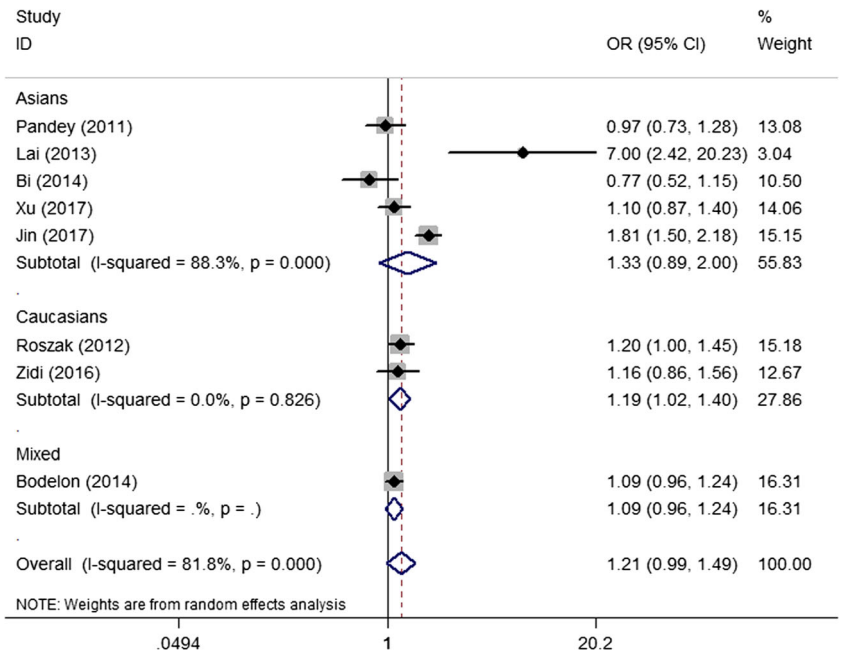

Fig. 3 Forest plot for the association between the *TLR9* G2848A polymorphism and cervical cancer risk in allele contrast (A vs. G)

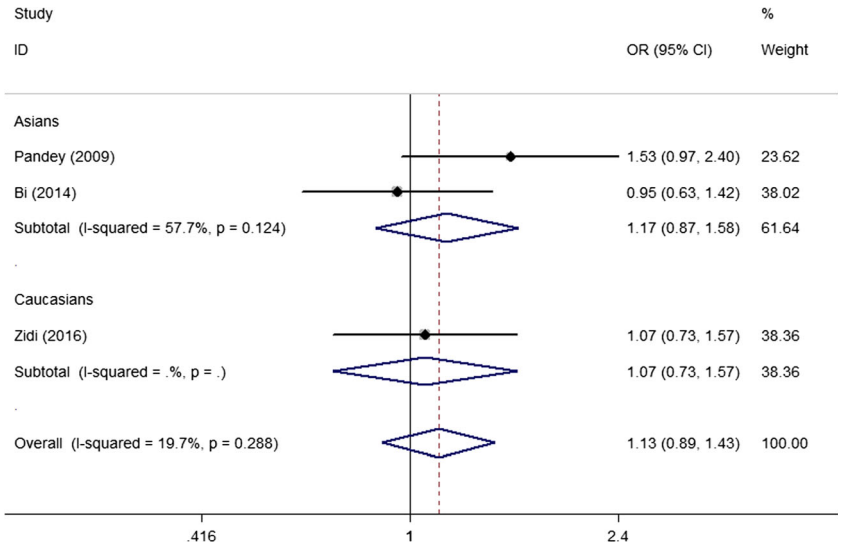

Fig. 4 Forest plot for the association between the *TLR2*–196 to −174 del/ins polymorphism and cervical cancer risk in allele contrast (del vs. ins)

Our results for the *TLR9* polymorphisms were not consistent with those of the meta-analysis by Mu et al. which had a smaller sample size [26]. Concerning the *TLR9* G2848A polymorphism, Mu et al. did not find any significant association with cervical cancer risk in the overall analyses ($n = 5$). It was noted that they only assessed the relation in all study participants but did not further perform subgroup analysis by ethnicity to evaluate race-specific effects. In our study, a significant association of the *TLR9* G2848A polymorphism with cervical cancer was seen for the meta-analysis restricted to studies of Caucasian subjects, but not for Asian and Mixed subjects. Due to limited availability of data ($n = 4$), Mu et al. did not perform ethnicity-specific analysis for the *TLR9* 1486C/T polymorphism. In contrast, in subgroup analysis by ethnicity, we found that the association of cervical cancer with the *TLR9* 1486C/T polymorphism was in the opposite direction between Caucasians and Mixed subjects. Compared with the meta-analysis by Mu et al., strengths of our study were including a larger number of cases and controls, subgroup analyses according to ethnicity, evaluation of robustness of overall results, and assessment of sources for heterogeneity. As far as we known, this is the first meta-analysis examining the association between the *TLR2*–196 to −174 del/ins polymorphism and cervical cancer risk. Our data contributed to a growing line of evidence that the *TLR2*–196 to −174 del/ins polymorphism was not associated with risk of cervical cancer.

We found race-specific effects of the *TLR9* polymorphisms on cervical cancer risk. This may be attributable to several reasons, including different genetic backgrounds, heterogeneity in the populations, different sample sizes, and gene-environment regulatory interactions. Previous meta-analyses have also shown that results from polymorphism associations

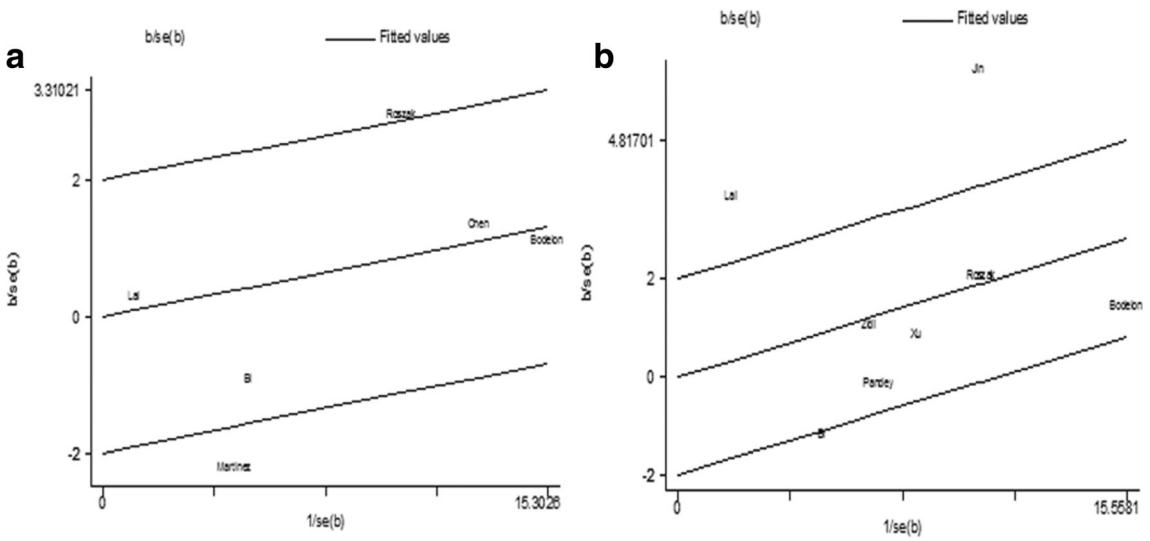

Fig. 5 **a** Galbraith plot for investigating the source of heterogeneity between studies assessing the *TLR9* 1486 T/C polymorphism. **b**. Galbraith plot for investigating the source of heterogeneity between studies assessing the *TLR9* G2848A polymorphism

with cancer risk differ when the analysis was stratified by ethnicity [27–29]. It is important to recognize that subgroup analysis according to ethnicity should not be overlooked, since it may provide important information on effect size in different populations and is especially helpful when the overall result is negative.

Publication bias is one threat to validity that researchers conducting meta-analysis studies confront. We have attempted to review published studies of the association of *TLR9* and *TLR2* gene polymorphisms with cervical cancer risk through several iterations of search criteria; it is possible, however, that we have missed some eligible studies. It is thought that studies showing positive results are more likely to be published in international journals. However, this form of potential bias was unlikely to be an issue in this meta-analysis, because association studies reporting negative results for the *TLR9* and *TLR2* polymorphisms were also included. In addition, Egger's test and funnel plots did not show any evidence of publication bias.

This meta-analysis has several limitations. Firstly, the number of studies that contributed to the study population pool was limited. We expect that as more studies become available, a more comprehensive estimation of the relationship of *TLR9* and *TLR2* with cervical cancer will be obtained. Secondly, this meta-analysis was based on unadjusted risk estimates as we were unable to retrieve data on various potential confounders including age at onset, socio-economic status, and clinical manifestations from the original publications. The existence of effect modifiers may have produced heterogeneity between studies. Thirdly, more detailed genotyping of the *TLR9* and *TLR2* genes or haplotype analysis might have yielded additional insight into the relationship between cervical cancer and genetic variations of *TLR9* and *TLR2*, but such information could not be collected from all original studies. Despite these limitations, this meta-analysis has several advantages: (1) the quality of the included studies was sufficient according to our well-designed selection criteria; (2) sensitivity analysis indicated that our results were statistically robust; and (3) no evidence of publication bias was found.

In conclusion, our findings suggest that the *TLR9* 1486 T/C and G2848A polymorphisms contribute to the risk of cervical cancer, but there is no association of the *TLR2*–196 to −174 del/ins polymorphism with cervical cancer. Additional case-control studies using much larger sample sizes are needed to further substantiate and enrich the present findings.

Funding This work was supported in part by the National Natural Science Foundation of China (31560324). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with Ethical Standards

Conflict of Interests The authors declare that there are no competing interests to disclose.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386
2. Patel JP, Lee EH, Mena CI, Walker CN (2017) Effects of metformin on endothelial health and erectile dysfunction. Transl Androl Urol 6(3):556–565
3. Yu L, Chen S (2008) Toll-like receptors expressed in tumor cells: targets for therapy. Cancer Immunol Immunother 57(9):1271–1278
4. Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, Mansour M, Vincent I, Gissmann L, Iftner T, Sideri M, Stubenrauch F, Tommasino M (2007) TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. J Immunol 178(5):3186–3197
5. Lee JW, Choi JJ, Seo ES, Kim MJ, Kim WY, Choi CH, Kim TJ, Kim BG, Song SY, Bae DS (2007) Increased toll-like receptor 9 expression in cervical neoplasia. Mol Carcinog 46(11):941–947
6. Fehri E, Ennaifer E, Ardhaoui M, Ouerhani K, Laassili T, Bel Haj Rhouma R, Guizani I, Boubaker S (2014) Expression of toll-like receptor 9 increases with progression of cervical neoplasia in Tunisian women–a comparative analysis of condyloma, cervical intraepithelial neoplasia and invasive carcinoma. Asian Pac J Cancer Prev 15(15):6145–6150
7. Ghosh A, Dasgupta A, Bandyopadhyay A, Ghosh T, Dalui R, Biswas S, Banerjee U, Basu A (2015) A study of the expression and localization of toll-like receptors 2 and 9 in different grades of cervical intraepithelial neoplasia and squamous cell carcinoma. Exp Mol Pathol 99(3):720–724
8. de Matos LG, Cândido EB, Vidigal PV, Bordoni PH, Lamaita RM, Carneiro MM, da Silva-Filho AL (2017) Association between toll-like receptor and tumor necrosis factor immunological pathways in uterine cervical neoplasms. Tumori 103(1):81–86
9. Hasimu A, Ge L, Li QZ, Zhang RP, Guo X (2011) Expressions of toll-like receptors 3, 4, 7, and 9 in cervical lesions and their correlation with HPV16 infection in Uighur women. Chin J Cancer 30(5):344–350
10. DeCarlo CA, Rosa B, Jackson R, Niccoli S, Escott NG, Zehbe I (2012) Toll-like receptor transcriptome in the HPV-positive cervical cancer microenvironment. Clin Dev Immunol 2012:785825
11. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22(4): 719–748
12. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7(3):177–188
13. Pandey S, Mittal RD, Srivastava M, Srivastava K, Singh S, Srivastava S, Mittal B (2009) Impact of toll-like receptors [TLR] 2 (−196 to −174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in north Indian women. Gynecol Oncol 114(3):501–505
14. Pandey S, Mittal B, Srivastava M, Singh S, Srivastava K, Lal P, Mittal RD (2011) Evaluation of toll-like receptors 3 (c.1377C/T) and 9 (G2848A) gene polymorphisms in cervical cancer susceptibility. Mol Biol Rep 38(7):4715–4721
15. Chen X, Wang S, Liu L, Chen Z, Qiang F, Kan Y, Shen Y, Wu J, Shen H, Hu Z (2012) A genetic variant in the promoter region of toll-like receptor 9 and cervical cancer susceptibility. DNA Cell Biol 31(5):766–771
16. Roszak A, Lianeri M, Sowińska A, Jagodziński PP (2012) Involvement of toll-like receptor 9 polymorphism in cervical cancer development. Mol Biol Rep 39(8):8425–8430
17. Lai ZZ, Ni-Zhang PXL, Song L (2013) Toll-like receptor 9 (TLR9) gene polymorphisms associated with increased susceptibility of

human papillomavirus-16 infection in patients with cervical cancer. J Int Med Res 41(4):1027–1036
18. Bodelon C, Madeleine MM, Johnson LG, Du Q, Galloway DA, Malkki M, Petersdorf EW, Schwartz SM (2014) Genetic variation in the TLR and NF-κB pathways and cervical and vulvar cancer risk: a population-based case-control study. Int J Cancer 134(2): 437–444
19. Bi X, Xu X, Yu X, Wang N, Yin F, Wang Y (2014) Study on TLRs polymorphisms and cervical cancer susceptibility. Prog Obstet Gynecol 23(7):520–523
20. Zidi S, Sghaier I, Gazouani E, Mezlini A, Yacoubi-Loueslati B (2016) Evaluation of toll-like receptors 2/3/4/9 gene polymorphisms in cervical Cancer evolution. Pathol Oncol Res 22(2): 323–330
21. Xu X, Yin C, Yan Z, Hu Y, Yang H (2017) The correlation of TLR9 gene polymorphisms with occurence and development of cervical cancer in Yunnan Han population. J Guizhou Med Univ 42(1):21–25
22. Martínez-Campos C, Bahena-Román M, Torres-Poveda K, Burguete-García AI, Madrid-Marina V (2017) TLR9 gene polymorphism -1486T/C (rs187084) is associated with uterine cervical neoplasm in Mexican female population. J Cancer Res Clin Oncol 143(12):2437–2445
23. Jin Y, Qiu S, Shao N, Zheng J (2017) Association of toll-like receptor gene polymorphisms and its interaction with HPV infection in determining the susceptibility of cervical cancer in Chinese Han population. Mamm Genome 28(5–6):213–219
24. Nasu K, Narahara H (2010) Pattern recognition via the toll-like receptor system in the human female genital tract. Mediat Inflamm 2010:976024
25. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki AB (2011) Association between toll-like receptor expression and human papillomavirus type 16 persistence. Int J Cancer 128(4):879–886
26. Mu X, Zhao J, Yuan X, Zhao X, Yao K, Liu Y, Zhao X (2015) Gene polymorphisms of toll-like receptor 9 -1486T/C and 2848G/a in cervical Cancer risk. Int J Gynecol Cancer 25(7):1173–1178
27. Ding C, Yu H, Yu H, Qin H (2012) TP53 codon 72 polymorphism with hepatocellular carcinoma: a metaanalysis. J Int Med Res 40(2): 446–454
28. Wang HL, Lu X, Yang X, Xu N (2016) Association of MBL2 exon1 polymorphisms with high-risk human papillomavirus infection and cervical cancers: a meta-analysis. Arch Gynecol Obstet 294(6):1109–1116
29. Tan SC, Ismail MP, Duski DR, Othman NH, Ankathil R (2017) FAS c.-671A>G polymorphism and cervical cancer risk: a case-control study and meta-analysis. Cancer Genet 211:18–25