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Association of TLR4 gene 2026A/G (rs1927914), 896A/G (rs4986790), and 1196C/T (rs4986791) polymorphisms and cancer susceptibility

Meta-analysis and trial sequential analysis

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Abstract

Background: This study was performed to assess the association of TLR4 gene 2026A/G (rs1927914), 896A/G (rs4986790), and 1196C/T (rs4986791) polymorphisms and cancer susceptibility based on published case-control studies.

Methods: Web of Science, PubMed, Embase, CBM, WanFang Data, CNKI, and VIP database were used for article retrieving. Then, these articles were screened according to the study inclusion and exclusion criteria. The data was extracted, and the study quality was evaluated according to the principle of Newcastle-Ottawa Scale. Meta-analysis was performed by RevMan 5.4 and Stata MP-17 software. Trial sequential analysis was performed by TSA 0.9.5.10 Beta software.

Results: Eighty-seven case-control studies including 25,969 cases and 32,119 controls were included in the meta-analysis. The diseases involved in case groups include prostate cancer, lung cancer, gastric cancer, hepatocellular carcinoma, colorectal cancer, etc. A versus G model of rs1927914, A versus G model of rs4986790 and C versus T model of rs4986791 showed that odds ratio (OR) = 1.08, OR = 0.85, and OR = 0.74 respectively. All the 3 comparisons were statistically significant. Sensitivity analysis showed that the results were stable. Publication bias analysis and trial sequential analysis showed that no significant publication bias was found in the results of the meta-analysis, and the probability of false positives was small.

Conclusion: People with A allele of rs1927914, G allele of rs4986790, or T allele of rs4986791 have higher risks of cancer. The results of meta-analysis are stable and have less probability of false positives.

Abbreviations: 95% CI = 95% confidence interval, NF-κB = nuclear factor kappa-B, OR = odds ratio, SNP = single nucleotide polymorphism, TLR4 = toll-like receptor 4, TSA = trial sequential analysis.

Keywords: cancer, gene polymorphism, meta-analysis, susceptibility, toll-like receptor 4, trial sequential analysis

1. Introduction

With the development of society, the prevalence of cancer is increasing. Cancer is a kind of disease caused by malignant proliferation of cells. It is invasive and has become one of the important diseases threatening people's life quality and life span. Cancer is also a multi-factorial disease. In addition to lifestyle, occupation and biological, physical and chemical factors in the environment, the influence of genetic factors has emerged as another crucial factor.[1]

Single nucleotide polymorphism (SNP) refers to the change of genetic information at the nucleotide locus of a gene.[2] It was previously believed that missense mutations altering the amino acid composition of translated proteins would lead to changes in genetic traits, susceptibility to disease and other outcomes.[2,3] However, a recent study published in Nature showed that many synonymous mutations are also harmful to organisms, rather than neutral or near neutral.[4] Both nonsense mutations and missense mutations may lead to changes in individual genetic traits to varying degrees, thus changing factors such as external performance or susceptibility to disease.[4] Therefore, the study of the relationship between gene polymorphisms and disease susceptibility can provide strong evidence for the genetic diagnosis and prevention of diseases and has important clinical significance.

Toll-like receptor 4 (TLR4) is one of the receptors that closely related to immunity.[5] SNPs of genes encoding TLR4 often lead to a series of changes in the body's immune system, leading to certain diseases, such as immune related diseases like asthma,[6] rheumatoid arthritis,[7] systemic lupus

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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erythematosus,[8] and even intracranial aneurysm,[9] hypertension,[10] and endometriosis.[11]

More and more case-control studies have confirmed that the SNPs of TLR4 gene 2026A/G (rs1927914), 896A/G (rs4986790), and 1196C/T (rs4986791) are related to the susceptibility and prognosis of prostate cancer,[12] lung cancer,[13] gastric cancer,[14] cervical cancer,[15] and many other cancers. However, most of the studies only focused on the same cancer type. Moreover, most of the patients included in a single study were confined to the same hospital, lowering the representativeness of the results. Therefore, focusing on case-control studies on the relationship between SNPs of TLR4 gene 2026A/G (rs1927914), 896A/G (rs4986790), and 1196C/T (rs4986791) and cancer susceptibility, meta-analysis and trial sequential analysis (TSA) were carried out in this paper. In this way, the secondary research results with data of multi centers and large samples were obtained, to give hints for clinical practice and basic research.

2. Methods

2.1. Study inclusion criteria

The study design of the included articles should be a publicly published case-control study. It should assess the association between TLR4 gene 2026A/G, 896A/G, and 1196C/T polymorphisms and cancer susceptibility. The case group consisted of patients with clinically and pathologically diagnosed cancer, and the control group consisted of healthy people. All the people included in the study were not restricted by race, gender or age. Moreover, the articles should contain the numbers of people in the case and control groups with each genotype. The data quality should be reliable, and the results should be clearly expressed.

2.2. Study exclusion criteria

Articles with incomplete analytical data or unavailable after contacting the original author, were not included in this meta-analysis. Articles that were repeatedly published and retrieved, as well as articles whose original research object was not human, were also excluded.

2.3. Retrieval strategy

Web of Science, PubMed, Embase, CBM, WanFang Data, CNKI, and VIP database were used for retrieving. The retrieval time interval was from the date of database establishment to May 31, 2022. Articles in the reference list were also reviewed to find eligible case-control studies. The retrieval was carried out by combining free words with subject words. The key words used include TLR4, TLR4, cancer, carcinoma, malignant tumor, neoplasm, lymphoma, SNP, polymorphism, variant, SNP, etc. Taking PubMed as an example, its retrieval strategy is shown in Table 1.

2.4. Article screening and data extraction

The 2 researchers independently screened the articles, extracted the data, and performed independent cross examination on them. Disputes were settled through discussion with a third party. When screening articles, the title was read firstly to exclude apparently unrelated articles. Then, the abstract and full text was further read to determine whether it could be included. If necessary, the author will be contacted by email to obtain the key information that has not been mentioned in the original study. The content of data extraction includes the basic information of each included study: the first author, publication year, country, number of included people in case group and control group, number of cases and controls corresponding to each genotype, type of cancer studied, etc.

2.5. Study quality evaluation

According to the principle of Newcastle-Ottawa Scale, the general qualities of included case-control studies were evaluated independently by 2 researchers.[16] Disputes were settled through discussion with a third party.

2.6. Statistical methods

Meta-analysis was performed by RevMan 5.4 (RevMan, College Station, TX) and Stata MP-17 software (Stata, London, UK). TSA was performed by TSA 0.9.5.10 Beta software. The test level of heterogeneity test is $\alpha = 0.10$. If the result of the heterogeneity test shows $P < .10$, it indicates that there is heterogeneity, and the random effects model is used. If the result of heterogeneity test shows $P > .10$, it means that there is no heterogeneity, and the fixed effect model is used. The heterogeneity judgment method of sensitivity analysis is the same as the above. Odds ratio (OR), 95% confidence interval (95% CI) and P value were recorded. The test level of meta-analysis and TSA analysis is $\alpha = 0.05$. The publication bias was tested by Begg's test, and the test level was $\alpha = 0.05$.

2.7. Institutional review board statement

Ethical approval is not applicable as data were derived from published articles.

3. Results

3.1. Article retrieving and quality evaluation results

According to the meta-analysis article retrieving and screening process recommended by Preferred Reporting Items for Systematic Reviews and Meta-Analyses, 87 case-control studies from more than 30 countries were included in this meta-analysis (Fig. 1).[12–15,17–76] Among them, there were 25,969 people in the case group and 32,119 in the control group. The diseases involved in case groups include prostate cancer, lung cancer, gastric cancer, hepatocellular carcinoma, colorectal cancer, etc. Study quality evaluation showed that all original studies had clear case groups and control groups with reliable inclusion and diagnostic criteria. The basic characteristics of the original studies included in the meta-analysis are shown in Tables 2–4.

Table 1

Retrieval strategy of PubMed.

#1	"toll-like receptor 4"[Mesh]
#2	TLR4
#3	#1 OR #2
#4	cancer
#5	"carcinoma"[Mesh]
#6	malignant tumor
#7	neoplasm
#8	lymphoma
#9	#4 OR #5 OR #6 OR #7
#10	polymorphism
#11	variant
#12	#10 OR #11
#13	#3 AND #9 AND #12

3.2. Meta analysis results

1.3.2. Cancer susceptibility characteristics of rs1927914 A versus G model Forest plot of rs1927914 A versus G model was shown as Figure 2. Heterogeneity test showed that $\chi^2 = 24.04$, $P = .06 < .10$, indicating heterogeneity exists. In this way, statistical analysis was performed using random effects model. The results of meta-analysis showed that OR = 1.08 > 1, indicating individuals carrying allele A have a higher risk of developing cancer than individuals carrying allele G. Besides, 95% CI = [1.01, 1.15] which did not pass through 1, and $P = .02 < .05$, indicating the results were statistically significant.

2.3.2. Cancer susceptibility characteristics of rs4986790 A versus G model Forest plot of rs4986790 A versus G model was shown as Figure 3. Heterogeneity test showed that $\chi^2 = 79.95$, $P < .0001 < .10$, indicating heterogeneity exists. In this way, statistical analysis was performed using random effects model. The results of meta-analysis showed that OR = 0.85 < 1, indicating individuals carrying allele A have a lower risk of developing cancer than individuals carrying allele G. Besides, 95% CI = [0.75, 0.96] which did not pass through 1, and $P = .007 < .05$, indicating the results were statistically significant.

3.3.2. Cancer susceptibility characteristics of rs4986791 C versus T model Forest plot of rs4986791 C versus T model was shown as Figure 4. Heterogeneity test showed that $\chi^2 = 58.37$, $P = .004 < .10$, indicating heterogeneity exists. In this way, statistical analysis was performed using random effects model. The results of meta-analysis showed that OR = 0.74 < 1, indicating individuals carrying allele C have a lower risk of developing cancer than individuals carrying allele T. Besides, 95% CI = [0.63, 0.86] which did not pass through 1, and $P = .0001 < .05$, indicating the results were statistically significant.

4.3.2. Summary of meta-analysis results The results of meta-analysis were shown as Table 5. For AA versus GG, AA+AG versus GG and A versus G models of rs1927914, all the OR value was higher than 1, and the results were statistically significant. This indicates that considering TLR4 gene 2026A/G (rs1927914) polymorphism, individuals with the A allele have a higher risk of cancer.

For AA versus AG+GG, AA versus AG and A versus G models of rs4986790, all the OR value was lower than 1, and the

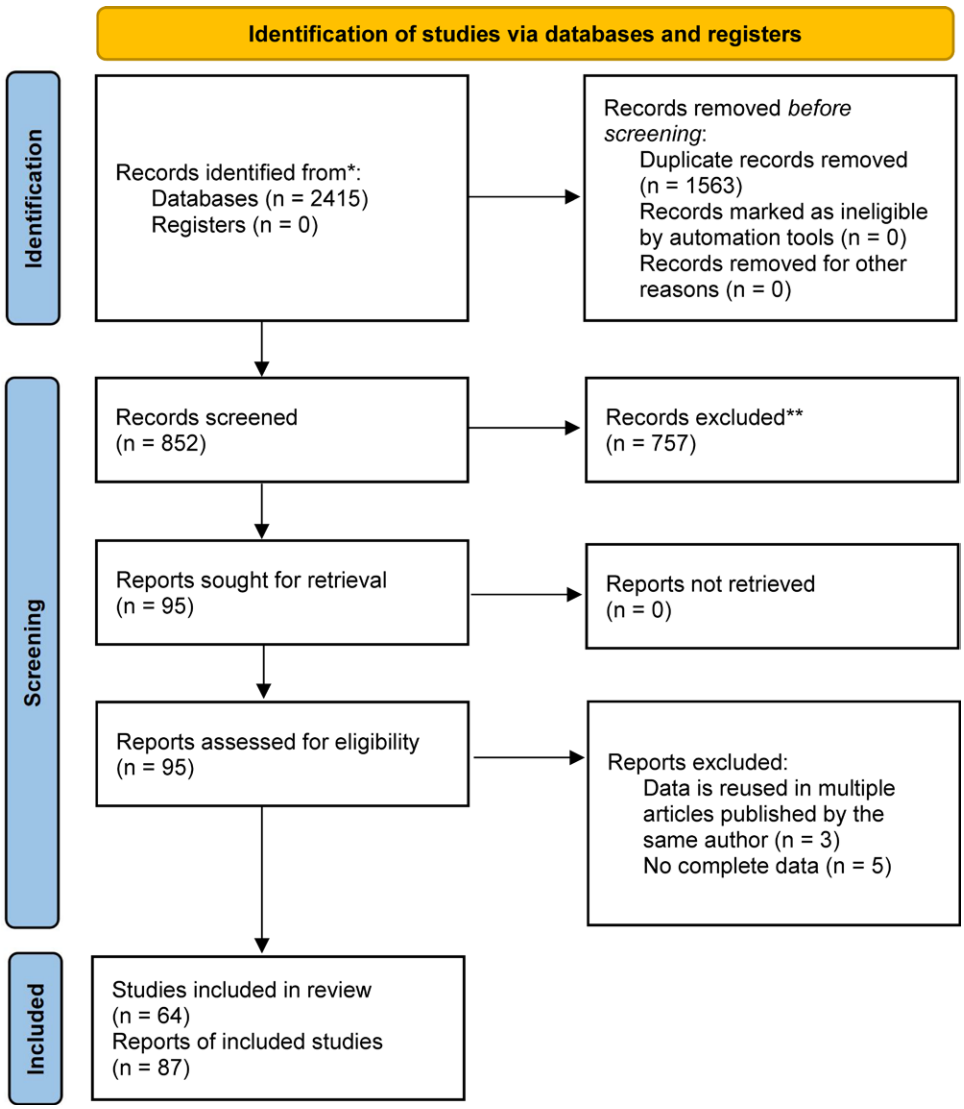

Figure 1. Article screening process.

Table 2

Study characteristics included in meta-analysis for rs1927914.

First author	Year	Country	Case			Control			Cancer type
			AA	AG	GG	AA	AG	GG	
Zheng SL	2004	Sweden	625	596	154	341	354	81	Prostate cancer
Chen YC	2005	USA	297	301	60	290	288	91	Prostate cancer
Song J	2009	Korea	48	87	7	69	54	14	Prostate cancer
Huang H	2010	China	339	450	157	358	476	153	Gastric cancer
Hart K	2011	Norway	196	197	42	196	183	52	Lung cancer
Minmin S	2011	China	94	87	35	74	118	36	Hepatocellular carcinoma
Liu GP [a]	2014	China	18	36	14	46	63	23	Colon cancer
Liu GP [b]	2014	China	51	73	19	46	63	23	Rectal cancer
Zhu L [a]	2014	China	80	102	33	216	302	109	Colon cancer
Zhu L [b]	2014	China	136	186	65	216	302	109	Rectal cancer
Shi G	2017	China	19	11	4	10	31	8	Hepatocellular carcinoma
Kou RH	2019	China	195	237	48	169	238	73	Esophageal carcinoma
Wu H	2020	China	225	351	124	233	346	121	Lung cancer
Li A	2021	China	178	220	82	150	250	80	Rectal cancer
Li Z	2021	China	173	233	65	149	241	81	Gastric cancer
Zhang HM	2022	China	103	169	32	94	154	56	Lung cancer

The lower case letters after the first author are used to distinguish different types of cancer in the same article.
AA = adenine/adenine, AG = adenine/guanine, GG = guanine/guanine.

Table 3

Study characteristics included in meta-analysis for rs4986790.

First author	Year	Country	Case			Control			Cancer type
			AA	AG	GG	AA	AG	GG	
Zheng SL	2004	Sweden	1241	136	1	693	79	5	Prostate cancer
Chen YC	2005	USA	588	66	3	605	59	5	Prostate cancer
Stephan H	2005	Mixed	83	4	0	313	45	0	Lymphoma
Boraska Jelavic T	2006	Croatia	77	10	2	84	4	0	Colorectal cancer
Forrest M S	2006	USA	794	106	3	1254	172	6	Lymphoma
Nieters A	2006	Germany	590	84	1	596	71	1	Lymphoma
Hold GL [a]	2007	UK	414	79	3	581	47	2	Gastric cancer
Hold GL [b]	2007	UK	97	10	0	194	16	1	Oesophageal cancer
Cheng I	2007	USA	439	66	1	456	48	2	Prostate cancer
Santini D	2008	Italy	159	11	1	140	11	0	Gastric cancer
Pandey S	2009	India	114	35	1	123	26	1	Cervical cancer
Purdue MP	2009	USA	1195	133	6	1126	131	8	Non-Hodgkin lymphoma
Ashton KA	2010	Australia	163	25	3	258	31	2	Endometrial cancer
Balistreri CR	2010	Italy	49	1	0	111	13	1	Prostate cancer
Gast A	2011	Germany	665	91	0	659	73	3	Malignant melanoma
Theodoropoulos GE	2012	Greece	201	57	3	412	63	5	Breast cancer
Yang ZH	2012	China	205	29	2	250	33	4	Nasopharyngeal cancer
Dai Q	2012	China	219	44	5	228	38	2	Colorectal cancer
Priyadarshini A	2013	India	157	32	9	173	20	7	Prostate cancer
Shen Y	2013	China	431	2	3	519	1	2	Bladder cancer
Pimentel-Nunes P	2013	Portugal	169	0	15	186	0	5	Colorectal cancer
Omrane I	2014	Tunisia	87	13	0	120	18	2	Colorectal cancer
Gu X	2014	China	149	7	1	413	21	1	Non-Hodgkin lymphoma
Companioni O	2014	Mixed	316	45	0	1134	133	3	Gastric cancer
Kopp TI	2015	Denmark	839	76	0	1577	141	1	Colorectal cancer
Winchester DA	2015	USA	768	94	5	741	82	7	Prostate cancer
Zidi S	2016	Tunisia	116	6	8	207	46	7	Cervical cancer
Semlali A	2016	Saudi Arabia	106	7	1	92	7	1	Colorectal cancer
Winchester DA	2017	USA	555	64	0	465	58	4	Prostate cancer
Ragaa AR	2017	Egypt	118	22	5	121	9	0	Colon cancer
Li ZH	2017	China	78	14	4	74	15	3	Prostate cancer
Abdelhabib S	2017	KSA	115	8	0	101	14	0	Breast cancer
Seyed VH	2017	Iran	150	0	0	150	0	0	Colorectal cancer
Nilesh OP	2019	India	70	37	3	107	32	2	Cervical cancer
Moaaz M	2020	Egypt	99	24	4	125	15	1	Colorectal cancer
Asghari A	2021	Iran	81	9	0	83	6	1	Colorectal cancer

The lower case letters after the first author are used to distinguish different types of cancer in the same article.
AA = adenine/adenine, AG = adenine/guanine, GG = guanine/guanine.

Table 4

Study characteristics included in meta-analysis for rs4986791.

First author	Year	Country	Case			Control			Cancer type
			CC	CT	TT	CC	CT	TT	
Boraska Jelavic T	2006	Croatia	77	12	0	82	5	0	Colorectal cancer
Garza-Gonzalez E	2007	Mexico	77	1	0	179	10	0	Gastric cancer
Santini D	2008	Italy	155	15	1	147	4	0	Gastric cancer
Trejo-de la OA	2008	Mexico	57	4	0	193	9	0	Gastric cancer
Pandey S	2009	India	127	21	2	133	16	1	Cervical cancer
Srivastava K	2010	India	195	32	5	232	24	1	Gallbladder cancer
Balistreri CR	2010	Italy	48	2	0	118	7	0	Prostate cancer
Rigoli L	2010	Mexico	57	13	0	81	6	0	Gastric cancer
Davoodi H	2011	Malaysia	58	2	0	50	0	0	Colorectal cancer
Theodoropoulos GE	2012	Greece	253	8	0	466	14	0	Breast cancer
de Oliveira JG	2012	Brazil	165	9	0	219	6	0	Gastric cancer
Yang ZH	2012	China	188	45	3	254	32	1	Nasopharyngeal cancer
Agundez JA	2012	Spain	143	12	0	341	47	2	Hepatocellular cancer
Dai Q	2012	China	182	78	8	214	52	2	Colorectal cancer
Singh V	2013	India	163	35	2	173	26	1	Bladder cancer
Priyadarshini A	2013	India	158	32	8	157	37	6	Prostate cancer
de Oliveira JG	2013	Brazil	191	9	0	234	6	0	Gastric cancer
Yang CX	2013	China	202	0	0	201	1	0	Breast cancer
Shen Y	2013	China	433	1	2	517	3	2	Bladder cancer
Kutikhin AG [a]	2014	Russia	55	11	0	255	45	0	Gastric cancer
Kutikhin AG [b]	2014	Russia	100	23	2	255	45	0	Rectal cancer
Kutikhin AG [c]	2014	Russia	195	36	2	255	45	0	Colorectal cancer
Kutikhin AG [d]	2014	Russia	69	9	1	144	24	0	Ovarian cancer
Companioni O	2014	Mixed	309	45	0	1124	134	5	Gastric cancer
Omrane I	2014	Tunisia	94	6	0	123	17	0	Colorectal cancer
Zeljic K	2014	Serbia	77	16	0	90	13	1	Oral cancer
Qadri Q	2014	Kashmir	114	16	0	182	18	0	Gastric cancer
Kurt H	2016	Turkey	156	4	0	91	9	0	Lung cancer
Rybka J	2016	Poland	52	6	1	104	18	0	Acute myeloid leukaemia
Jin Y	2017	China	237	147	36	535	262	45	Cervical cancer
Ragaa AR	2017	Egypt	111	31	3	119	11	0	Colon cancer
Li ZH	2017	China	92	3	1	89	2	1	Prostate cancer
Nilesh P	2018	India	110	0	0	141	0	0	Cervical cancer
Moaaz M	2020	Egypt	97	27	3	131	9	1	Colorectal cancer
Asghari A	2021	Iran	85	5	0	87	2	1	Colorectal cancer

The lower case letters after the first author are used to distinguish different types of cancer in the same article.
CC = cytosine/cytosine, CT = cytosine/thymine, TT = thymine/thymine.

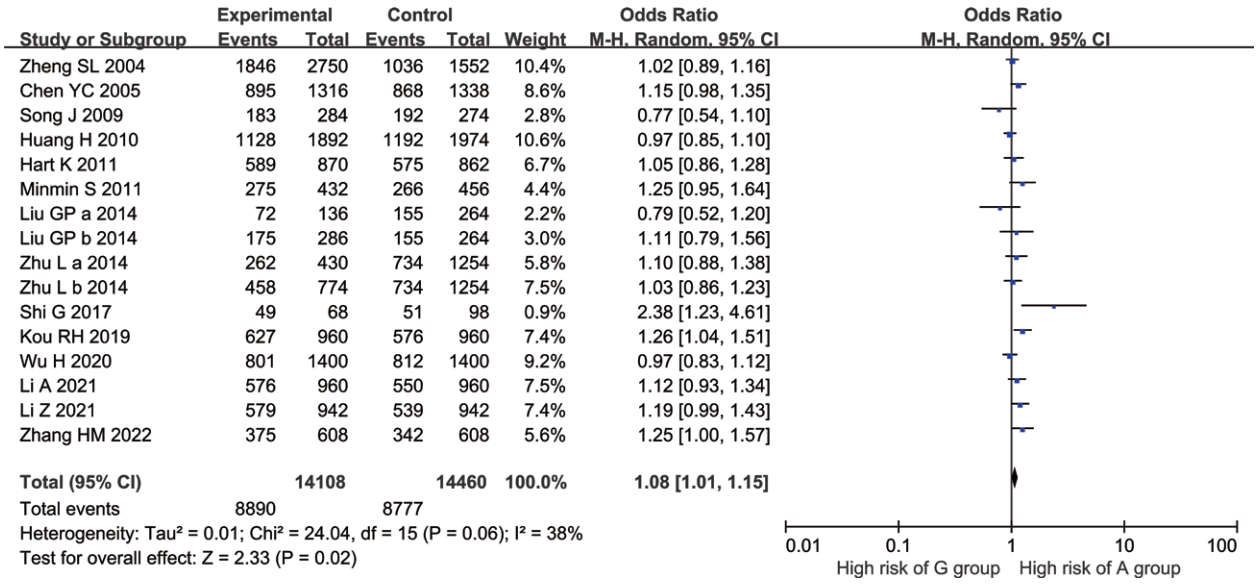

Figure 2. Cancer susceptibility characteristics of rs1927914 A versus G model.

results were statistically significant. This indicates that considering TLR4 gene 896A/G (rs4986790) polymorphism, individuals with the G allele have a higher risk of cancer.

For all the models of rs4986790, all the OR value was lower than 1, and the results were statistically significant. This indicates that considering TLR4 gene 1196C/T (rs4986791)

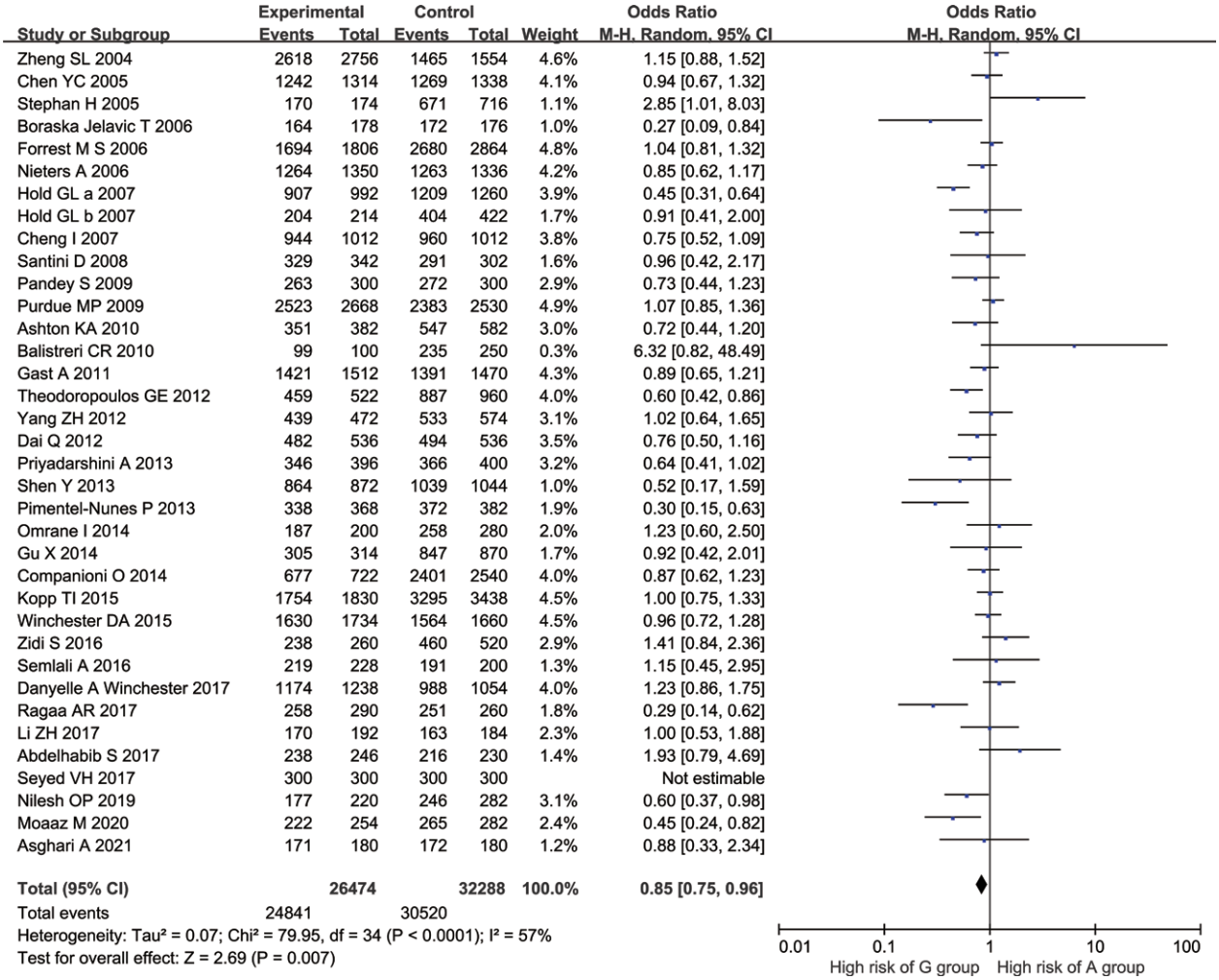

Figure 3. Cancer susceptibility characteristics of rs4986790 A versus G model.

polymorphism, individuals with the T allele have a higher risk of cancer.

3.3. Sensitivity analysis

Taking the allele model as examples, the sensitivity analysis was carried out by one-by-one exclude method. The results of sensitivity analysis showed that for the A versus G model of rs1927914, the OR value was 1.09 at the highest and 1.07 at the lowest after excluding a single study, and the results were statistically significant. For the A versus G model of rs4986790, the OR value was 0.87 at the highest and 0.83 at the lowest after excluding a single study, and the results were statistically significant. For the C versus T model of rs4986791, the OR value was 0.76 at the highest and 0.71 at the lowest after excluding a single study, and the results were statistically significant. Sensitivity analysis showed that the results of meta-analysis were relatively stable, and the results were less affected by changes in a single original study.

3.4. Publication bias analysis

Taking the allele model as examples, Begg's test was used to analyze publication bias. For A versus G model of rs1927914, publication bias analysis results showed that $Z = 1.13$, $P = .260 > .05$, indicating no publication bias analysis exists. The funnel plot showed that the included original studies were generally distributed in a symmetrical funnel shape along the symmetry axis, indicating that there was no obvious publication bias (Fig. 5A). These conclusions were the same for A versus G model of rs4986790 ($P = .460 > .05$) and C versus T model of rs4986791 ($P = .477 > .05$), and the funnel plots were shown as Figure 5B and C. This shows that the reliability of the meta-analysis conclusion is less affected by publication bias.

3.5. TSA results

Taking the allele model as examples, the results of TSA were shown in Figure 6. The results showed that although the accumulated information did not reach the required information size, the Z curve had intersected with the boundary value, indicating that the association between the current gene and the high risk of cancer has been confirmed. Therefore, more tests are generally not required for further verification, and the possibility of false positives is small.

4. Discussion

Cancer is a serious threat to human life quality and longevity. In the past 2 decades, a large number of original clinical studies and related secondary studies have been published, revealing the association of TLR4s with cancer susceptibility and prognosis. Our meta-analysis showed that the individual carrying A allele in TLR4 Gene 2026A/G (rs1927914) polymorphism, G allele

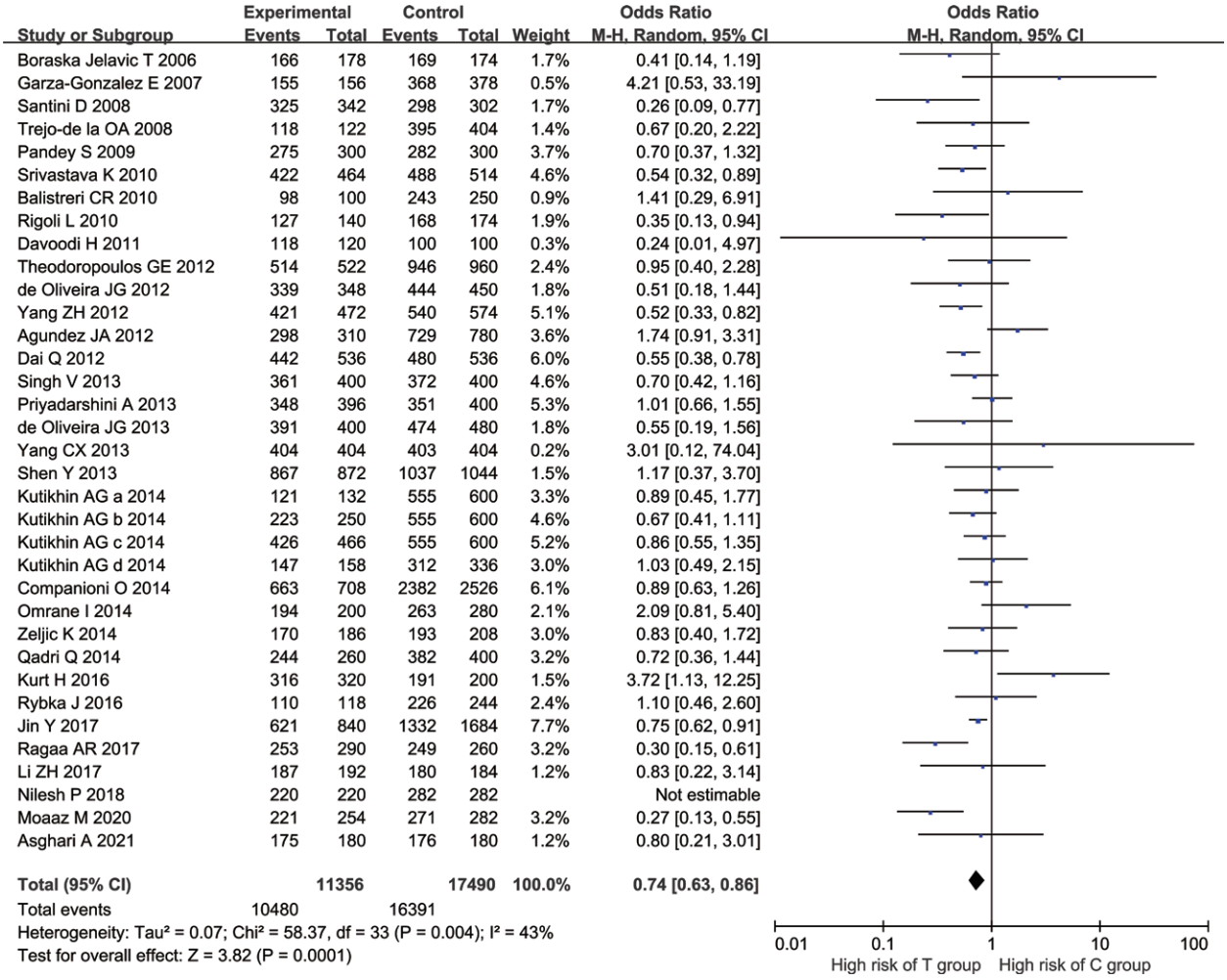

Figure 4. Cancer susceptibility characteristics of rs4986791 C versus T model.

Table 5

Summary of meta-analysis results.

	rs1927914			rs4986790			rs4986791		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
aa vs bb	1.21	1.05, 1.38	.007	0.85	0.64, 1.13	.27	0.50	0.36, 0.69	.0001
aa+ab vs bb	1.16	1.02, 1.31	.02	0.87	0.65, 1.15	.32	0.53	0.38, 0.73	.0001
aa vs ab+bb	1.09	0.97, 1.21	.14	0.84	0.74, 0.96	.008	0.74	0.62, 0.87	.0004
aa vs ab	1.06	0.94, 1.19	.38	0.85	0.74, 0.97	.01	0.76	0.64, 0.90	.001
ab vs bb	1.13	0.98, 1.31	.09	1.14	0.83, 1.56	.42	0.64	0.45, 0.91	.01
a vs b	1.08	1.01, 1.15	.02	0.85	0.75, 0.96	.007	0.74	0.63, 0.86	.0001

For rs1927914, a represents A, b represents G; For rs4986790, a represents A, b represents G; For rs4986791, a represents C, b represents T.
CI = confidence interval; OR = odds ratio.

in 896A/G (rs4986790) polymorphism, or T allele in 1196C/T (rs4986791) polymorphism had an increased risk for cancer. Sensitivity analysis showed that the results of the meta-analysis were slightly affected by a single study, and the results were stable. Publication bias analysis and TSA showed that no significant publication bias was found in the results of the meta-analysis, and the probability of false positives was small. In conclusion, the A allele of rs1927914, G allele of rs4986790, and T allele of rs4986791 are the susceptibility genes to cancer, and the results are reliable.

TLRs are a class of pattern recognition receptors that can interact with other pattern recognition receptor families and activate a variety of pathogen-associated molecular patterns to initiate a sequence of signal transduction.[77] It is closely related to inflammatory responses, and the variation of related genes will affect several pathways of the body, thus resulting in a series of changes in health status or the occurrence of diseases. The effects of an inflammatory immune response have 2 aspects: on the 1 hand, they improve an organism's ability to fight against infection; on the other hand, a persistently inflammatory environment may make it easier for tumor cells to escape the immune system.[78] That is to say, TLRs appear to represent a potential link between infections, persistent inflammation, and the emergence of tumors in the context of cancer. In this way, TLRs are one of the prime choices to determine how inflammation plays a part in cancer.

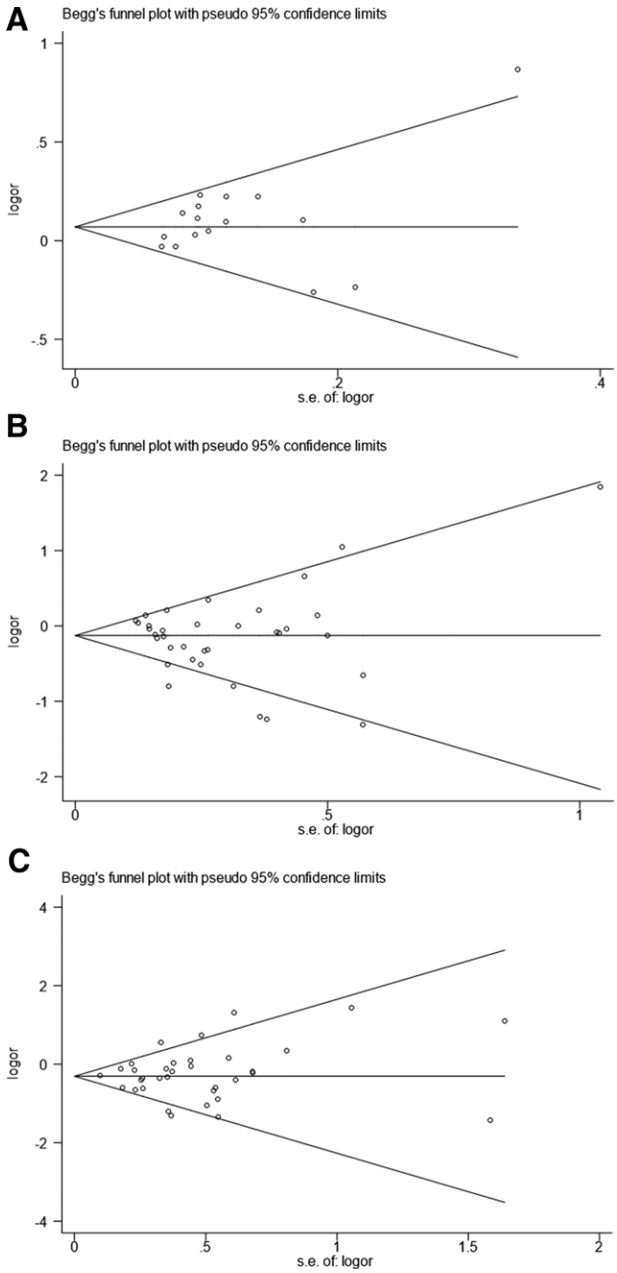

Figure 5. Funnel plots of publication bias analysis.

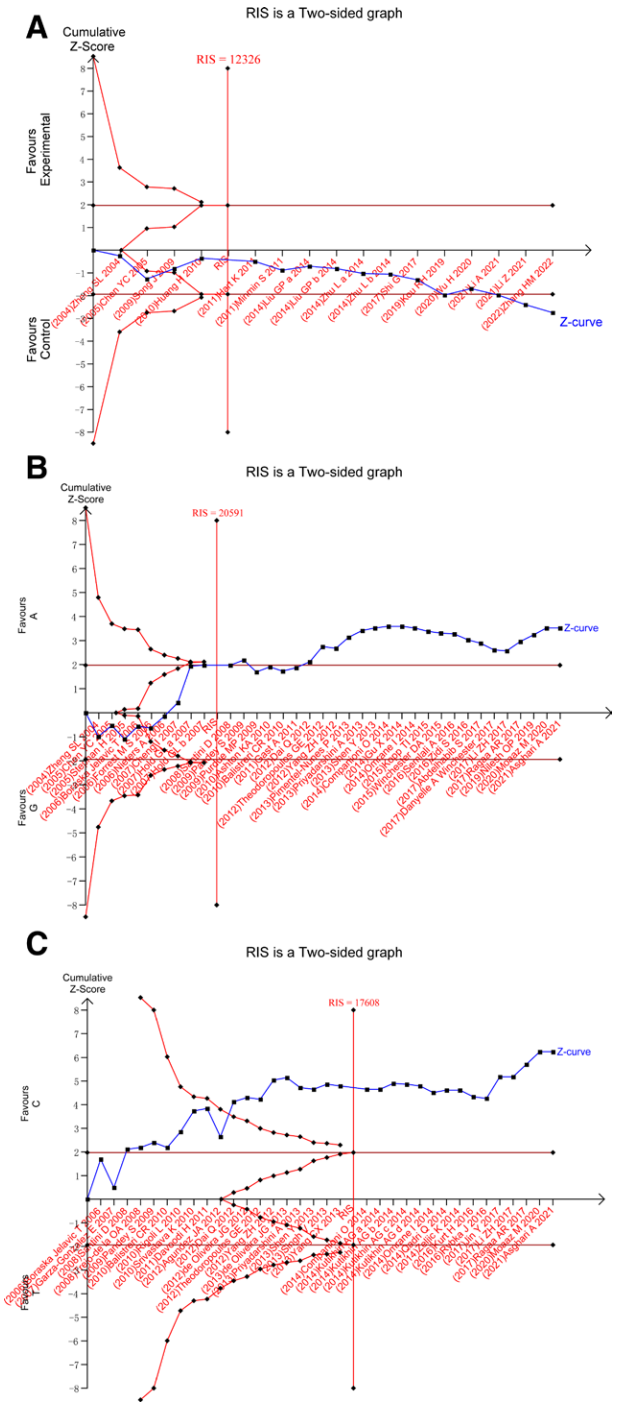

Figure 6. Trial sequential analysis results.

Two key pathways are used to transmit the signals that TLR4 mediates: one uses the adapter protein myeloid differentiation factor 88, and the other uses the adaptor-inducing interferon protein, which contains a toll/interleukin-1 receptor domain.[79] TLR4 can mediate reactions to host molecules like oxidized low-density lipoprotein, amyloid peptide, heat shock proteins, and those made in response to tissue injury. Recognition of their ligands causes a series of signaling events, the first of which is represented by the activation of the interleukin-1 receptor family. This is followed by the activation of the transcription factor nuclear factor kappa-B (NF-κB) with the transcription of pro-inflammatory genes.[80] Besides, a specific collection of genes implicated in proinflammatory, antiviral, and antibacterial responses begin to be transcribed when TLR4 activates myeloid differentiation factor 88, in this way NF-κB activation is promoted which leads to the production of inflammatory cytokines.[5,79,81] It has been hypothesized that activation of NF-κB is a key mediator of inflammation-induced tumor growth and progression. Numerous studies have shown that NF-κB is an important regulator of Snail expression and that it is particularly important for the spread of carcinoma.[82,83] The development and spread of cancer may be aided by the production of inflammatory mediators via the NF-κB pathway that is activated by the TLRs.[80,84,85] Besides, it has been suggested that innate immune activation brought on by TLR-mediated identification of pathogens or endogenous chemicals, such as those created by cell and DNA damage, can foster the growth of cancer in an inflammatory environment.[86] Therefore, the polymorphisms of TLR4 gene may affect the above pathways, thus leading to changes in cancer susceptibility, or affecting the prognosis of cancer by affecting metastasis and invasion ability.

However, our study also has some limitations. First of all, most of the meta-analysis of each group has high heterogeneity. In view of this situation, we used random effects model for statistical analysis. Then, for the meta-analysis of some loci, the countries where the original research were conducted were mostly confined to Asia, especially China. At the same time, the original research involved a large number of cancer types, resulting in a small number of original studies for each cancer type. Therefore, subgroup analyses were not performed. Finally, if personal data containing additional factors, such as age, sex, and smoking status, becomes available, a more accurate analysis should be carried out.

In summary, people with A allele of rs1927914, G allele of rs4986790, or T allele of rs4986791 were more susceptibility to cancer. The results are basically stable and there is less chance of false positives. To support our findings, additional sizable, thoughtful, comprehensive research across a range of groups is required.

Author contributions

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