

Review Article

Association between the NFκB1-94ins/del ATTG polymorphism and cancer risk: a meta-analysis and trial sequential analysis

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Abstract: Objective: This study aimed to perform a comprehensive meta-analysis and trial sequential analysis to clarify the association between the NFκB1 -94ins/del ATTG promoter polymorphism and cancer risk. Methods: A total of 42 studies including 16814 cases and 23367 controls were analyzed in this meta-analysis. We used pooled odds ratios (ORs) to evaluate the strength of the association, and 95% confidence intervals (CIs) to identify precision of the estimate. Results: We found that the NFκB1 promoter -94ins/del ATTG polymorphism was significantly associated with cancer risk in all five genetic models (Homozygote model, OR=1.33, 95% CI=1.12-1.59; Heterozygote model, OR=1.15, 95% CI=1.03-1.29; Dominant model, OR=1.21, 95% CI=1.07-1.38; Recessive model, OR=1.18, 95% CI=1.05-1.32; Allele model, OR=1.14, 95% CI=1.05-1.24). Subgroup analyses revealed a significant association between the polymorphism and cancer risk in Asian population and hospital-based studies. Stratified analysis was also performed in genotyping method, and significant associations were detected in all subgroups individually. We found the association was cancer-specific in cancer type subgroup analysis. The trial sequential analysis demonstrated statistical significance in favor of the NFκB1 promoter -94ins/del ATTG polymorphism increasing cancer risk, and the number of participants enrolled in this meta-analysis reached the low-bias heterogeneity adjusted information size. Conclusion: Our meta-analysis and TSA results suggested that the association between -94ins/del polymorphism in the promoter of NFκB1 and cancer risk is statistically significant and the association might be ethnic-specific.

Keywords: NFκB1, gene polymorphism, cancer, meta-analysis, trial sequential analysis

Introduction

Cancer is a terminal complex disease with high morbidity and mortality that results from the interactions between inherited and environmental factors [1]. In the past decades, many genes were found as influence factors of cancer [2]. Although the oncogenesis has been widely studied, the complex etiology of cancer is not yet fully clarified. Genetic susceptibility is a known possible explanation for the interindividual variation in cancer risk and contribute to the development of cancer [3].

Nuclear factor κB (NFκB) is a nuclear protein which was first identified by Sen and Baltimore

in 1986 [4]. NFκB is known as a transcription factor that plays significant roles in various physiological process such as inflammation, cell survival, cell adhesion, differentiation, angiogenesis and apoptosis [5-8]. There are five members of the NFκB family present in mammals: NFκB1 (p50), RelA (p65), c-Rel, RelB and NFκB2 (p52) [9]. The human NFκB1 gene that is located on chromosome 4q24 encodes protein p50 which can regulate inflammation and cancer development [10-13]. -94ins/del ATTG (rs28362491) is a common four nucleotides polymorphism in the promoter region of NFκB1 gene [13], which including three genotypes: wild homozygous (ins/ins), variant homozygous (del/del), and heterozygous (ins/del) [14]. The

association between the NFκB1 -94ins/del ATTG polymorphism and cancer susceptibility has been investigated by many studies which had inconsistent results [15-23]. In addition, some published meta-analysis focused on the association between the NFκB1 -94ins/del ATTG polymorphism and cancer risk also obtained conflicting results [24-26].

To explore the association between the NFκB1 -94ins/del ATTG promoter polymorphism and cancer in a better manner, we collected all available data to perform a comprehensive meta-analysis and performed a trial sequential analysis in the hope of providing more precise evidence.

Materials and methods

Search strategy

A systematic search on PubMed, EMBASE and Web of Science was performed to identify all published potentially appropriate studies (till May 20th, 2016). The key words were (“genetic polymorphism”, “polymorphism”, “SNP”, “single nucleotide polymorphism”, “gene mutation”, or “genetic variant”), (“neoplasm”, “cancer”, “tumor”, “carcinoma”, or “carcinogenesis”), and (“NFκB1”, “nuclear factor kappa B1”, “NF kappa B1”, or “nuclear factor κ B1”. Additional publications were identified when we searched the reference list of original articles manually. A flow diagram of the study selection process is presented in **Figure 1**.

Inclusion criteria

(1) Studies were case-control studies. (2) Studies estimated the association between NFκB1 -94ins/del ATTG polymorphism and cancer risk. (3) The information from studies was performed in detail for calculation of odds ratio (OR) with 95% confidence interval (CI). (4) Data involved in different studies were not overlapping (if any, we selected the study with the largest samples).

Exclusion criteria

(1) Studies were not case-control studies. (2) Studies consisted no usable reported data. (3) Studies did not relate to cancer risk. (4) Studies had overlapped data.

Data extraction

Two investigators (Yuxiao Zheng and Xiao Li) independently extracted all useful information involved in eligible studies according to the inclusion criteria performed above. The review of result was carried out by a third investigator (Gong Cheng). The following information was recorded for each selected study: name of first author, year of publication, ethnicity, genotyping method, source of controls, frequencies of the genotypes in cases and controls, cancer type and Hardy-Weinberg equilibrium (HWE) of genotype distribution among controls. We considered studies which consisted more than one type of cancer as individual datasets only in subgroup analyses according to cancer type.

Statistical analysis

We used pooled odds ratio (OR) with corresponding 95% confidence interval (CI) to evaluate the strength of association between NFκB1 promoter -94ins/del ATTG polymorphism and cancer risk. Z test was performed to determine the statistical significance of the pooled OR, and a *P* value of < 0.05 was considered statistically significant. We used the Homozygote model (ins/ins vs. del/del), Heterozygote model (ins/del vs. del/del), Recessive model (ins/ins vs. del/del+ins/del), Dominant model (ins/ins+ins/del vs. del/del), and Allele (ins vs. del) model to examine NFκB1 -94ins/del ATTG genotypes. In addition to the overall comparison, we also performed subgroup analysis stratified by ethnicity, genotyping method, source of controls, cancer type.

The statistical heterogeneity between studies involved in this meta-analysis was evaluated using Q test which based on Cochran's chi-square and *I*² values. We calculated the summary OR by the random-effects model when *P* value of heterogeneity was < 0.05 and *I*² > 50% which indicated the presence of heterogeneity [27]; Otherwise, the fixed-effects model using the Mantel-Haenszel method was used [28]. We performed allele counting to regulate the allele frequencies of the NFκB1 promoter -94ins/del ATTG polymorphism from the individual study. HWE was evaluated using the goodness-of-fit test (chi-square or Fisher exact test) for the control groups in this investigation. The one-way sensitivity analyses (we cut out a single study in the meta-analysis each time to

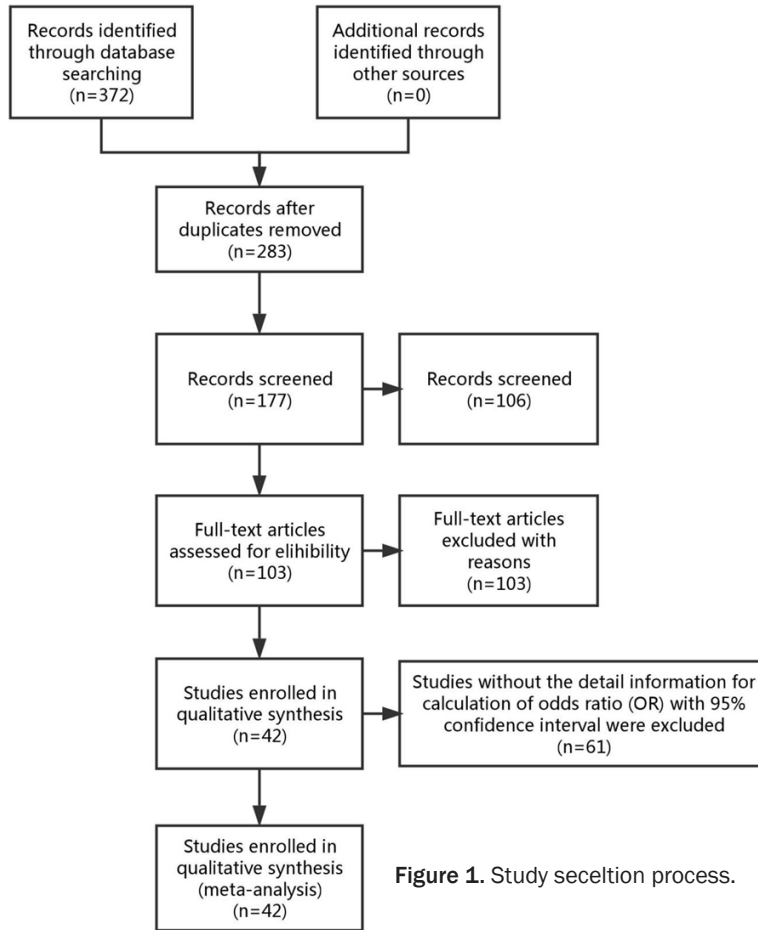


Figure 1. Study selection process.

reflect the influence of the individual data set to the pooled OR) were performed to evaluate the stability of the results. Egger's test and Begg's funnel plots were used to assess the potential publication bias; P value < 0.05 and asymmetric plot suggest a potential publication bias. Statistical analyses were performed using STATA 12.0 software (Stata Corp, College Station, TX, USA).

Trial sequential analysis

Trial sequential analysis (TSA) was used to assess the increased type I error caused by few enrolled data and repeated significance testing when updating with new published studies [29-34]. We cumulated sample size of studies included in this meta-analysis and then performed trial sequential analysis to estimate the information-size of meta-analysis. The latter, called trial sequential monitoring boundaries, reduce type I errors [29, 31, 33, 35]. We treated the addition of each individual study in

a cumulative meta-analysis as an interim meta-analysis to elucidate whether additional trials are needed. If the TSA result showed no cross connection between Z-curve and the boundary and the required information size was not large enough, that means we should enroll more trials and collect more evidence to reach a conclusion [36-38]. In conclusion, TSA was used to reduce the risk of type I error to evaluate whether we should enroll more samples from more eligible studies in further trials.

Results

Characteristics of eligible studies

A total of 42 studies including 16814 cases and 23367 controls were analyzed in this meta-analysis. Figure 1 described the process for study identification and selection, and the characteristics of all involved studies were presented in

Table 1. Newcastle-Ottawa Scale was performed to estimate the strength of the evidence of included studies and the result was shown in Supplementary Table 1. We treated the study by Bu [23] as two independent studies in subgroup analysis of cancer type because two different cancer types were involved in this article. Finally, 3 bladder cancer, 2 breast cancer, 5 colorectal cancer, 3 gastric cancer, 2 hepatocellular carcinoma, 2 lung cancer, 2 nasopharyngeal carcinoma, 2 non-small cell lung cancer, 2 oral squamous cell carcinoma, 3 ovarian cancer, 4 prostate cancer, 1 cervical carcinoma, 1 cervical squamous cell carcinoma, 1 cutaneous melanoma, 1 epithelial ovarian cancer, 1 esophageal squamous cell carcinoma, 1 gastroenteropancreatic neuroendocrine tumors, 1 liver cancer, 1 melanoma, 1 multiple myeloma, 1 osteosarcoma, 1 papillary thyroid carcinoma and 1 renal cell carcinoma studies were enrolled in this meta-analysis. Among those eligible studies, 29 [16-18, 20, 22-23,

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Table 1. Main characteristics of studies involved in this meta-analysis

Author	Year	Ethnicity	Genotyping method	SOC	Cases			Controls			Cancer Type	HWE
					del/del	ins/del	ins/ins	del/del	ins/del	ins/ins		
Lin [39]	2006	Asian	PCR-RFLP	HB	50	103	59	58	100	43	Oral Squamous Cell Carcinoma	Y
Bu-1 [23]	2007	Caucasian	PCR-RFLP	HB	34	84	67	67	255	116	Melanoma	N
Bu-2 [23]	2007	Caucasian	PCR-RFLP	HB	81	323	63	67	256	116	Colorectal Cancer	N
Lewander [15]	2007	Asian	PCR-RFLP	HB	42	101	50	79	266	113	Colorectal Cancer	N
Riemann [62]	2007	Caucasian	Others	HB	30	124	88	48	141	118	Bladder Cancer	Y
Lo [40]	2008	Asian	PCR-RFLP	HB	31	89	62	34	62	20	Gastric Cancer	Y
Barnik [41]	2009	Caucasian	PCR-RFLP	HB	2	30	18	12	58	30	Gastroenteropancreatic Neuroendocrine Tumors	N
Tang [63]	2009	Asian	PCR-RFLP	HB	26	92	89	46	108	74	Bladder Cancer	Y
Zhang [43]	2009	Asian	PCR-RFLP	HB	14	57	46	31	68	44	Prostate Cancer	Y
Zhou [42]	2009	Asian	PCR-RFLP	HB	22	67	74	42	90	71	Nasopharyngeal Carcinoma	Y
Andersen [22]	2010	Caucasian	Taqman	PB	62	195	121	102	347	307	Colorectal Cancer	Y
Zhou [44]	2010	Asian	PCR-RFLP	HB	20	105	108	64	166	135	Cervical Squamous Cell Carcinoma	Y
Fan [18]	2011	Asian	PCR-RFLP	HB	17	84	78	44	103	76	Ovarian Cancer	Y
Lin [20]	2012	Asian	Taqman	HB	100	246	116	168	271	81	Oral Squamous Cell Carcinoma	Y
Vangsted [45]	2012	Caucasian	Taqman	PB	55	163	110	253	778	665	Multiple Myeloma	Y
Arisawa [49]	2013	Asian	PCR-RFLP	HB	68	239	172	103	435	342	Gastric Cancer	N
Cai [17]	2013	Asian	Taqman	HB	153	473	401	153	562	379	Renal Cell Carcinoma	N
Cheng [16]	2013	Asian	PCR-RFLP	HB	29	64	42	168	271	81	Hepatocellular Carcinoma	Y
Ebrahim [64]	2013	Asian	Others	HB	18	122	96	35	106	62	Breast Cancer	Y
Huang [50]	2013	Asian	Taqman	PB	225	459	372	210	491	355	Lung Cancer	Y
Huo [47]	2013	Asian	PCR-RFLP	HB	22	82	83	47	103	71	Epithelial Ovarian Cancer	Y
Kopp [65]	2013	Caucasian	Taqman	PB	54	152	128	64	161	109	Prostate Cancer	Y
Li [19]	2013	Asian	PCR-RFLP	HB	151	269	189	93	324	223	Bladder Cancer	Y
Suzairi [46]	2013	Asian	PCR-RFLP	HB	75	127	35	83	138	16	Colorectal Cancer	N
Umar [48]	2013	Asian	PCR-RFLP	HB	27	132	131	22	129	160	Esophageal Squamous Cell Carcinoma	Y
Gao [53]	2014	Asian	Taqman	HB	40	102	68	79	160	171	Liver Cancer	N
Hua [51]	2014	Asian	Others	HB	127	182	92	83	230	120	Gastric Cancer	Y
Liu [67]	2014	Asian	Taqman	PB	152	438	316	224	512	336	Nasopharyngeal Carcinoma	Y
Ottulu [54]	2014	Caucasian	PCR-RFLP	HB	16	44	35	6	47	46	Non-small Cell Lung Cancer	Y
Wang [66]	2014	Asian	PCR-RFLP	HB	171	210	93	123	216	162	Breast Cancer	N
Wang [68]	2014	Asian	PCR-RFLP	HB	106	186	60	171	209	79	Papillary Thyroid Carcinoma	Y
Zhang [52]	2014	Asian	PCR-RFLP	HB	419	312	205	1064	790	542	Hepatocellular Carcinoma	N
Chen [70]	2015	Asian	Taqman	HB	95	195	120	122	235	85	Ovarian Cancer	Y
Cui [59]	2015	Asian	PCR-RFLP	HB	99	246	198	186	355	212	Prostate Cancer	Y
Han [60]	2015	Asian	PCR-RFLP	HB	534	339	63	567	331	38	Prostate Cancer	Y
Kopp [56]	2015	Caucasian	PCR-RFLP	PB	146	449	320	253	787	679	Colorectal Cancer	N
Li [58]	2015	Asian	PCR-RFLP	HB	46	114	60	66	106	50	Osteosarcoma	Y
Pallavi [55]	2015	Asian	PCR-RFLP	HB	42	133	110	113	104	73	Cervical Carcinoma	N
Wang [69]	2015	Asian	PCR-RFLP	HB	89	219	113	131	205	89	Non-small Cell Lung Cancer	Y
Zhang [57]	2015	Asian	PCR-RFLP	HB	32	252	434	76	290	352	Lung Cancer	Y
Escobar [71]	2016	Mixed	PCR-RFLP	HB	19	37	61	31	41	44	Cutaneous Melanoma	N
Lu [61]	2016	Asian	PCR-RFLP	HB	221	351	115	253	339	95	Ovarian Cancer	Y

HB: hospital-based study; PB: population-based study; SOC: source of controls; HWE: Hardy Weinberg equilibrium.

39-61] of them were obeyed HWE while 13 studies [15, 19, 23, 62-71] were not.

Meta-analysis results

Overall, the statistically significant association between the NFκB1 promoter -94ins/del ATTG

polymorphism and cancer risk across the five genetic models in the overall population were evaluated by the pooled ORs (Homozygote model, OR=1.33, 95% CI=1.12-1.59; Heterozygote model, OR=1.15, 95% CI=1.03-1.29; Dominant model, OR=1.21, 95% CI=1.07-1.38; Recessive model, OR=1.18, 95% CI=1.05-1.32;

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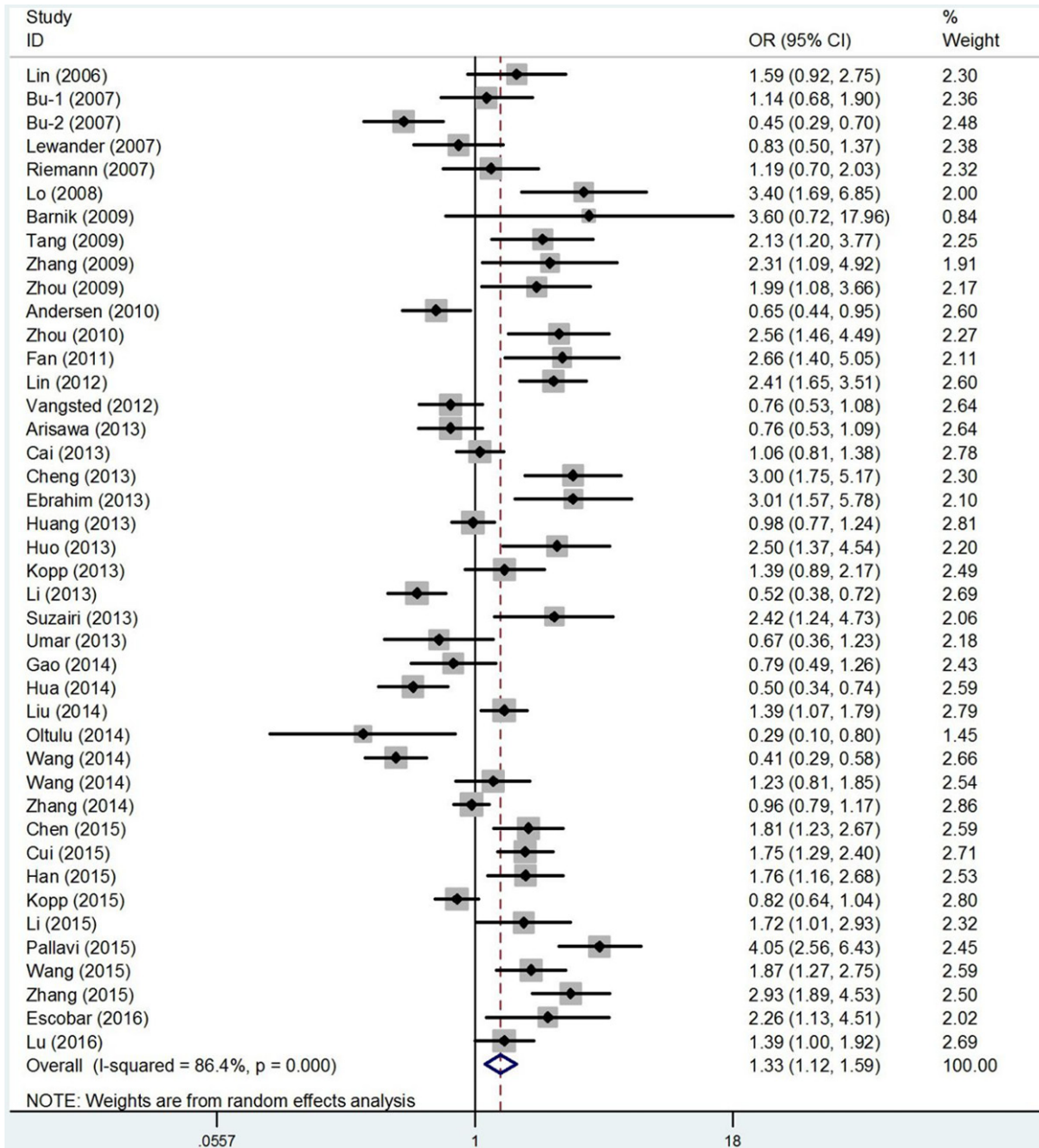


Figure 2. Forest plot of cancer risk associated with NFκB1 promoter -94ins/del ATTG polymorphism (for Homozygote model) among all studies.

and Allele model, OR=1.14, 95% CI=1.05-1.24). Ethnic subgroup analyses showed cancer risk significant increases in all five models among Asians but not among Caucasians. The results of SOC subgroup analysis revealed that the association between the NFκB1 promoter -94ins/del ATTG polymorphism and hospital-based study is statistically significant. Stratified analysis was also performed in genotyping method, and significant associations were detect-

ed in all subgroups individually. The results of cancer type subgroup analysis demonstrated significant association existed between the NFκB1 -94ins allele and oral squamous cell carcinoma, bladder cancer, gastroenteropancreatic neuroendocrine tumors, prostate cancer, nasopharyngeal carcinoma, cervical squamous cell carcinoma, ovarian cancer, hepatocellular carcinoma, lung cancer, epithelial ovarian cancer, osteosarcoma, cervical carcinoma

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Table 2. Meta-analysis of the NFκB1 -94ins/del ATTG promoter polymorphism and cancer risk

Variables	N*	Cases/ Controls	ins/ins versus del/del		ins/del versus del/del		ins/ins+ins/del versus del/del		ins/ins versus ins/del+del/del		ins allele versus del allele	
			OR (95% CI)	I ² (%)	OR (95% CI)	I ² (%)	OR (95% CI)	I ² (%)	OR (95% CI)	I ² (%)	OR (95% CI)	I ² (%)
Total	42	16814/23367	1.33 (1.12-1.59)	86.4	1.15 (1.03-1.29)	73.8	1.21 (1.07-1.38)	82.3	1.18 (1.05-1.32)	81.9	1.14 (1.05-1.24)	86.7
Ethnicities												
Asian	32	13703/17363	1.48 (1.22-1.81)	87.2	1.20 (1.15-1.37)	78.1	1.29 (1.11-1.50)	84.9	1.27 (1.13-1.42)	78.7	1.20 (1.09-1.32)	87.1
Caucasion	9	2994/5888	0.83 (0.63-1.09)	67.8	0.97 (0.82-1.15)	29	0.92 (0.77-1.09)	35.4	0.86 (0.69-1.08)	78.6	0.92 (1.81-1.04)	65.8
Mixed	1	117/116	2.26 (1.13-4.51)	/	1.47 (0.71-3.04)	/	1.88 (0.99-3.57)	/	1.78 (1.06-3)	/	1.69 (1.16-2.47)	/
Genotyping Method												
PCR-RFLP	30	10824/15044	1.43 (1.14-1.81)	87.5	1.20 (1.04-1.38)	74.9	1.28 (1.09-1.51)	83.6	1.24 (1.07-1.43)	82.9	1.19 (1.07-1.33)	87.7
Taqman	9	5111/7380	1.15 (0.89-1.48)	80.7	1.06 (0.92-1.22)	46.4	1.09 (0.92-1.28)	65.4	1.09 (0.89-1.33)	83.1	1.06 (0.93-1.21)	82.5
Others	3	879/943	1.18 (0.43-3.25)	91.3	1.14 (0.46-2.84)	90.6	1.16 (0.45-2.99)	92.1	1.02 (0.69-1.51)	73.5	1.02 (0.66-1.59)	90.3
SOC												
HB	36	12897/16734	1.43 (1.16-1.76)	87	1.19 (1.04-1.36)	76.5	1.27 (1.09-1.48)	83.8	1.24 (1.09-1.41)	81.4	1.18 (1.07-1.30)	87
PB	6	3971/6633	0.96 (0.75-1.21)	72.3	1.01 (0.90-1.14)	6.9	0.99 (0.84-1.16)	51	0.94 (0.79-1.13)	76.3	0.97 (0.85-1.10)	77.4
Cancer Type												
Oral Squamous Cell Carcinoma	2	674/721	2.06 (1.39-3.05)	33	1.42 (1.10-1.83)	0	1.59 (1.24-2.03)	3.9	1.67 (1.29-2.17)	0	1.42 (1.22-1.67)	6.9
Melanoma	1	185/438	1.14 (0.68-1.90)	/	0.65 (0.40-1.05)	/	0.80 (0.51-2.03)	/	1.56 (1.09-2.26)	/	1.15 (0.90-1.47)	/
Colorectal Cancer	5	2190/3609	0.82 (0.55-1.21)	77.8	0.95 (0.82-1.11)	0	0.89 (0.77-1.03)	0	0.85 (0.59-1.22)	85.6	0.89 (0.77-1.03)	68.6
Bladder Cancer	3	1058/1175	1.07 (0.45-2.53)	90.1	1.00 (0.46-2.18)	88.8	1.04 (0.46-2.33)	90.7	1.04 (0.73-1.48)	72.7	1.03 (0.70-1.51)	89
Gastric Cancer	3	1062/1429	1.03 (0.43-2.46)	90.9	0.84 (0.48-1.46)	82.1	0.90 (0.47-1.76)	88.8	1.11 (0.67-1.84)	84.4	1.02 (0.67-1.55)	91.2
Gastroenteropancreatic Neuroendocrine Tumors	1	50/100	3.60 (0.72-17.96)	/	3.10 (0.65-14.78)	/	3.27 (0.70-15.23)	/	1.31 (0.64-2.69)	/	1.35 (0.82-2.23)	/
Prostate Cancer	4	1930/2166	1.70 (1.38-2.10)	0	1.17 (1.01-1.35)	0	1.30 (1.09-1.55)	24	1.45 (1.23-1.70)	0	1.26 (1.15-1.39)	0
Nasopharyngeal Carcinoma	2	1069/1275	1.49 (1.12-1.97)	12.8	1.28 (1.02-1.61)	0	1.36 (1.10-1.68)	0	1.26 (0.99-1.59)	26.9	1.25 (1.03-1.51)	40.8
Cervical Squamous Cell Carcinoma	1	233/365	2.56 (1.46-4.49)	/	2.02 (1.16-3.54)	/	2.26 (1.33-3.85)	2.26	1.47 (1.05-2.06)	/	1.49 (1.17-1.91)	/
Ovarian Cancer	3	1276/1352	1.73 (1.26-2.39)	41.7	1.25 (0.95-1.64)	44.5	1.38 (1.05-1.82)	49.8	1.47 (1.20-1.79)	8.9	1.29 (1.12-1.48)	30.9
Mutipule Myeloma	1	328/1696	0.76 (0.53-1.06)	/	0.96 (0.69-1.35)	/	0.87 (0.63-1.20)	/	0.78 (0.61-1.00)	/	0.85 (0.72-1.01)	/
Renal Cell Carcinoma	1	1027/1094	1.06 (0.81-1.36)	/	0.84 (0.65-1.09)	/	0.93 (0.73-1.18)	/	1.21 (1.01-1.40)	/	1.08 (0.95-1.22)	/
Hepatocellular Carcinoma	2	1071/2916	1.65 (0.54-5.04)	93.3	1.08 (0.83-1.39)	30	1.26 (0.72-2.19)	82	1.50 (0.60-3.75)	93.4	1.27 (0.74-2.19)	92.8
Breast Cancer	2	710/704	1.09 (0.16-7.67)	96.4	1.21 (0.39-3.78)	90.8	1.18 (0.28-5.00)	94.7	0.89 (0.30-2.64)	94.9	0.96 (0.39-2.34)	96.6
Lung Cancer	2	1774/1774	1.67 (0.57-4.88)	94.7	1.31 (0.56-3.05)	91.2	1.50 (0.55-4.07)	94.3	1.30 (0.89-1.91)	87.1	1.25 (0.81-1.95)	94.5
Epithelial Ovarian Cancer	1	187/221	2.50 (1.37-4.54)	/	1.70 (0.95-3.05)	/	2.03 (1.17-3.51)	/	1.69 (1.13-2.52)	/	1.58 (1.19-2.10)	/
Esophageal Squamous Cell Carcinoma	1	290/311	0.67 (0.36-1.23)	/	0.83 (0.45-1.54)	/	0.74 (0.41-1.33)	/	0.78 (0.56-1.07)	/	0.82 (0.64-1.05)	/
Liver Cancer	1	210/410	0.79 (0.49-1.26)	/	1.26 (0.80-1.98)	/	1.01 (0.66-1.55)	/	0.67 (0.47-0.95)	/	0.83 (0.65-1.05)	/
Non-small Cell Lung Cancer	2	516/524	0.78 (0.12-4.90)	91	0.81 (0.19-3.48)	86.6	0.78 (0.15-3.92)	89.8	1.01 (0.50-2.03)	78.6	0.96 (0.45-2.02)	90.5
Papillary Thyoid Carcinoma	1	352/459	1.23 (0.81-1.85)	/	1.44 (1.05-1.96)	/	1.38 (1.02-1.85)	/	0.99 (0.68-1.43)	/	1.15 (0.95-1.41)	/
Osteosarcoma	1	220/222	1.72 (1.01-2.93)	/	1.54 (0.97-2.44)	/	1.60 (1.04-2.47)	/	1.29 (0.84-1.99)	/	1.31 (1.01-1.71)	/
Cevical Carcinoma	1	285/290	4.05 (2.56-6.43)	/	3.44 (2.22-5.33)	/	3.69 (2.47-5.53)	/	1.87 (1.31-2.67)	/	2.15 (1.70-2.72)	/
Cutaneous Melanoma	1	117/116	2.26 (1.13-4.51)	/	1.47 (0.71-3.04)	/	1.88 (0.99-3.57)	/	1.18 (1.05-1.32)	/	1.69 (1.16-2.47)	/

*: Number of comparisons/No data; HB: hospital-based study; PB: population-based study; SOC: source of controls; HWE: Hardy Weinberg equilibrium.

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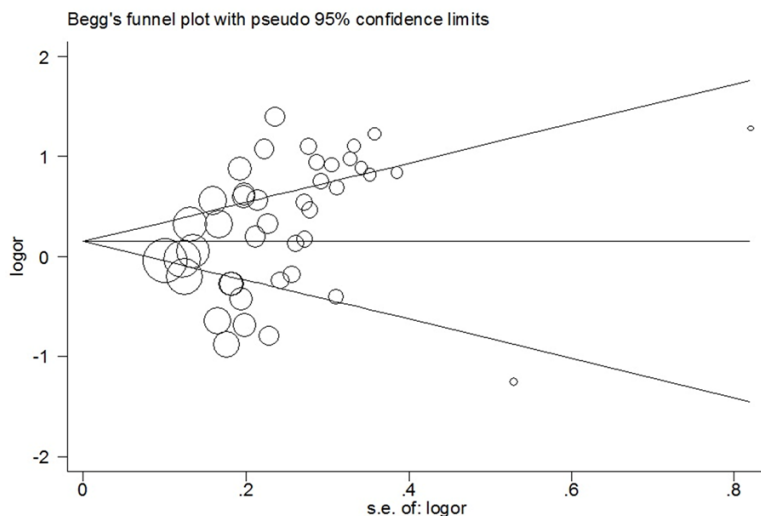


Figure 3. Funnel plot for publication bias test (for Homozygote model). Each circle represents an individual study for the indicated association.

and cutaneous melanoma, while the association were not revealed in colorectal cancer, multiple myeloma and esophageal squamous cell carcinoma in all 5 models. **Figure 2** reveals the association between -94ins/del polymorphism in the promoter of NFκB1 and cancer risk is statistically significant (data was extracted from Homozygote model). All the results of overall meta-analysis and subgroup analysis were showed in **Table 2**.

Evaluation of publication bias

We performed Begg's funnel plot and Egger's test to evaluate publication bias, and no evidence of publication bias was found for all analyses. The funnel plot analysis was showed in **Figure 3**.

Trial sequential analysis

The result of TSA with a type I error of 5% on NFκB1 promoter -94ins/del ATTG polymorphism and cancer risk was provided in **Figure 4**. 42 studies which marked with black squares were enrolled in TSA, and the result demonstrated that the cumulative Z-curve (blue line with black squares) crosses the monitoring boundary (red lines with black diamonds). Additionally, the cumulative Z-curve also crossed the line represents the low-bias heterogeneity adjusted information size (39651 patients) which was estimated by assuming a 10% relative risk reduction (RRR). The TSA result dem-

onstrated statistical significance in favor of the NFκB1 promoter -94ins/del ATTG polymorphism can increase cancer risk and the number of participants enrolled in this meta-analysis reached the low-bias heterogeneity adjusted information size.

Discussion

Studies involving NFκB has grown tremendously in the past decades since it was discovered in 1986 by Sen and Baltimore [4]. NFκB is one of approximately 2000 estimated transcription factors in human [72, 73], and is the key transcription factor involved in the inflammatory pathway. Thus, NFκB is constitutively active in most cancers [74].

NFκB1/p50 is one of the five family members (the rest are RelA/p65, c-Rel, RelB and NFκB2/P52) of NFκB transcription factors family [75], which is among the major signaling pathways participated in the cellular response to environmental stress [13]. NFκB1 plays a significant role in inhibiting cell apoptosis by regulating the level of survival genes including bcl-2 homologue A1 [76], PAI-2 [77], and IAP gene family [78]. In addition, previous studies have suggested that NFκB1 signaling pathway is involved in the process of cellular proliferation by increasing IL-5 [79], promoting MAPK phosphorylation [13] and modulating cyclin D1 expression [80]. In recent years, a -94ins/del ATTG polymorphism in the promoter region of NFκB1 was reported in association with the risk of numerous cancers. Accumulated evidence illustrated that the insertion allele that can inhibit apoptosis and promote cellular proliferation by upregulating the expression of NFκB1 [14, 19, 62], which was implicated in the mechanism mentioned above.

Several meta-analyses had studied on the association between the NFκB1 promoter -94ins/del ATTG polymorphism and cancer risk in the past decade. However, the results of these meta-analyses are not completely consistent. For example, results of meta-analyses from Yang et al [81] and Duan et al [82] showed

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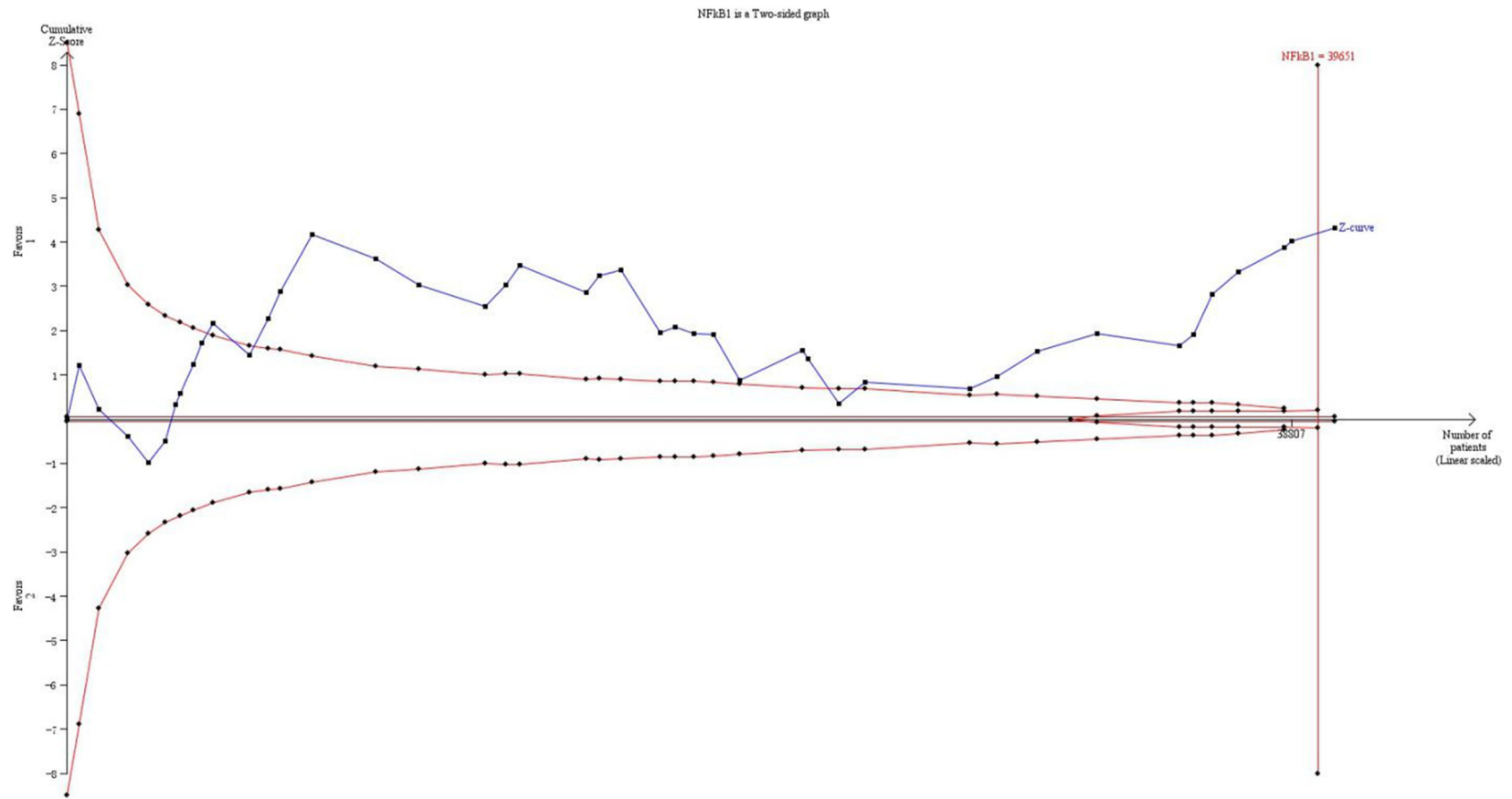


Figure 4. Trial sequential analysis with a type I error of 5% on NFκB1 promoter 94insdel ATTG polymorphism and cancer risk.

NFκB1 -94ins/del ATTG promoter polymorphism can increase the overall cancer risk. However, this result was contradictory with the meta-analysis performed by Zou et al [26]. Those contradictory results might be caused by the bias due to limited samples enrolled in meta-analyses.

After the reported study, numerous high-quality, large-sample case-control studies further evaluated the relationship between the NFκB1 promoter -94ins/del ATTG polymorphism and cancer risk. In this meta-analysis we enrolled 42 case-control studies with 16814 cases and 23367 controls. Our results indicated that the NFκB1 -94ins allele was a risk factor of cancer.

The result in ethnicity subgroup analyses indicated that the NFκB1 -94ins allele was a risk factor on cancer in Asian and Mixed population but had no effect on cancer in Caucasian population. This discrepancy may be caused by the different function of the -94ins/del polymorphism in different populations, which may result from interactions with non-genetic risk factors including diet, environment and lifestyle [83-86]. Our results indicated genotyping method will not affect the result that the NFκB1 -94ins allele was a risk factor on cancer. In SOC subgroup analysis, the result suggested that the -94ins allele was a risk factor on cancer in hospital-based studies in all five models, but not in population-based studies in dominant, recessive and allele model. This result suggested that more high-quality population-based studies with large samples should be enrolled in meta-analysis to reduce the bias. We provided the cancer type subgroup analysis and the result suggested that except colorectal cancer, multiple myeloma and esophageal squamous cell carcinoma (the NFκB -94ins allele has no association to cancer risk in all five models), NFκB -94ins allele was a risk factor on the rest types of cancer involved in our meta-analysis. This result suggested that the NFκB1 gene might function as a prominent factor in these cancers.

The TSA result showed statistical significance in favor of NFκB -94ins/del ATTG polymorphism increasing cancer risk. In addition, the number of samples has reached the low-bias heterogeneity adjusted information size (39651), which suggested the evidence of our meta-analysis is sufficient and the result is reliable and robust.

Limitations also inevitably existed in our meta-analysis like any other meta-analysis. First, we only enrolled the articles which studied on the association between the NFκB1 -94ins/del polymorphism and cancer risk from genetic perspective. Considering the complex mechanism of tumor occurrence, more studies focus on the interaction between gene and environment should be enrolled. Second, limited studies of some type of cancer were involved in the meta-analysis, which could increase the bias in subgroup analysis. On the other hand, many several strengths were shown in our meta-analysis. First, the number of articles, samples enrolled in meta-analysis was much larger than previous meta-analysis [81, 82, 86, 87]. Second, a more comprehensive subgroup analysis by cancer type was performed and the result suggested that the -94ins/del polymorphism may play a different role in different cancer types. Third, the sufficient evidence and robust result were proved by trial sequential analysis.

In conclusion, our meta-analysis and TSA result suggested that the association between NFκB -94ins/del polymorphism and cancer risk is statistically significant and the association might be ethnic-specific. The result of our study will provide clues and evidence for further therapeutic approaches target on interruption of the NFκB signaling pathway.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Newcastle-Ottawa Scale was performed to estimate the strength of the evidence of included studies

Author	Year	NOS score	Author	Year	NOS score
Lin [39]	2006	6	Kopp [65]	2013	8
Bu-1 [23]	2007	8	Li [19]	2013	7
Bu-2 [23]	2007	8	Suzairi [46]	2013	7
Lewander [15]	2007	7	Umar [48]	2013	7
Riemann [62]	2007	7	Gao [53]	2014	7
Lo [40]	2008	6	Hua [51]	2014	7
Barnik [41]	2009	6	Liu [67]	2014	6
Tang [63]	2009	7	Oltulu [54]	2014	8
Zhang [43]	2009	7	Wang [66]	2014	7
Zhou [42]	2009	7	Wang [68]	2014	8
Andersen [22]	2010	8	Zhang [52]	2014	7
Zhou [44]	2010	7	Chen [70]	2015	8
Fan [18]	2011	7	Cui [59]	2015	8
Lin [20]	2012	7	Han [60]	2015	7
Vangsted [45]	2012	8	Kopp [56]	2015	8
Arisawa [49]	2013	8	Li [58]	2015	7
Cai [17]	2013	7	Pallavi [55]	2015	7
Cheng [16]	2013	7	Wang [69]	2015	8
Ebrahim [64]	2013	8	Zhang [57]	2015	7
Huang [50]	2013	7	Escobar [71]	2016	7
Huo [47]	2013	7	Lu [61]	2016	8

NOS: Newcastle-Ottawa Scale.