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The genetic association between *EGF* A61G polymorphism (rs4444903) and risk of colorectal cancer

An update meta-analysis and trial sequential analysis

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Abstract

Background: Colorectal cancer was a complex disease with multiple causative factors including genetic and environmental factors, as well as the interaction of the 2 factors. Relationship between epidermal growth factor (*EGF*) A61G polymorphism and colorectal cancer risk has been widely investigated previously, whereas results derived from these studies were inconclusive and controversial. The aim of this study was to investigate the association between the *EGF* A61G polymorphism and colorectal cancer using a meta-analysis of existing literature.

Methods: Literature search was conducted from PubMed, EMBASE, China National Knowledge Infrastructure, Wanfang, and Cochrane library databases before July 2017. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of the association between *EGF* A61G and colorectal cancer.

Results: A total of 9 studies that involved 1448 cases and 1928 healthy controls and found allelic (OR = 1.18, $P = .04$) and recessive models (OR = 1.36, $P = .03$) of *EGF* A61G were significantly associated with the risk of colorectal cancer. Stratification analyses by ethnicity indicated that the *EGF* 61G significantly increased the risk of colorectal cancer in the Caucasian subgroup (OR = 1.24, $P = .02$), but not in Asian subgroup (OR = 1.12, $P = .08$). And the frequency of GG genotype of *EGF* A61G significantly increased in cases than that in healthy controls in both Caucasian (OR = 1.40, $P = .04$) and Asian subgroups (OR = 1.27, $P = .01$). Furthermore, the sample sources and genotyping methods seem to have no influence on the correction of *EGF* A61G and colorectal cancer susceptibility ($P > .05$).

Conclusion: The results indicate that *EGF* A61G might increase the risk of colorectal cancers.

Abbreviations: CIs = confidence intervals, CRC = colorectal cancer, *EGF* = epidermal growth factor, HB = hospital-based, NOS = Newcastle–Ottawa Scale, ORs = odds ratios, RFLP-PCR = restriction fragment length polymorphism-polymerase chain reaction, SNP = single nucleotide polymorphism, TSA = trial sequential analysis.

Keywords: colorectal cancer, epidermal growth factor (*EGF*), meta-analysis, rs4444903

1. Introduction

Colorectal cancer (CRC) is one of the major cause of morbidity, mortality, and death worldwide especially in Caucasian and Asian populations.[1–3] The information on mechanism of this cancer is not clear yet. Previous studies have indicated that lifestyle, personal exposures including dietary, and genetic factors might increase the susceptibility to CRC.[4] Hypothesis supported that genetic factors may be an important contributor to the pathology of colorectal cancer. Alterations in certain key genes have already been linked to increase the risk of CRC.

Recently, large amount of genes have been reported to be associated with the susceptibility of CRC in elevated studies.[5,6] One of the most important cancer-related gene should be the epidermal growth factor (*EGF*).[7] The *EGF* gene encodes epidermal growth factor, which performs as a key role in promoting survival, proliferation, and differentiation of epithelial cells by binding to its receptor (EGFR).[8] Shahbazi et al[9] firstl reported a functional variation involving an A-to-G mutation at position 61 (rs4444903 -61A > G) of 5'-untranslated region of the *EGF* gene was associated with increased EGF expression and risk of malignant melanoma of skin. Subsequently, genetic association between *EGF* A61G and susceptibility of various cancers such as breast cancer,[10] cervical cancer,[11] lung

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YZ and ZHC contributed equally to this work.

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cancer,[12] hepatocellular carcinoma,[13] gastric cancer,[14] esophageal cancer,[15] and colorectal cancer[16] were identified. Spindler et al firstly investigated association of *EGF* A61G and CRC in Denmark population and found no significant association.[17] Significant association between this polymorphism and risk of CRC was firstl identified by Wu et al in the German population,[18] while the positive result could not be replicated in most of other populations.[19,20] These discrepancies may be due to insufficient calculated power, different ethnicity, and limited sample sizes in individual studies.

In light of the inconclusive results of the previous studies and the insufficient statistical power of an individual study, we performed a meta-analysis by including the most recent and relevant articles to further evaluate the precise association of *EGF* +61A > G polymorphism and CRC risk.

2. Methods

2.1. Patient and public involvement

No patient and public involvement and ethical approval is necessary for the present meta-analysis.

2.2. Literature search strategy

This study was performed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[21] The PubMed, EMBASE, China National Knowledge Infrastructure, Wanfang, and Cochrane library databases were searched with no language restrictions, using the following terms: "Epidermal growth factor" or "*EGF*" and "rs4444903" or "61A>G" or "A61G" and "polymorphism" or "variant" or "gene mutation" or "single nucleotide polymorphism (SNP)" or "gene variation" and "colorectal cancer" or "colorectal neoplasms" or "colorectal carcinoma". In addition, the time period for literature searching was from the first available article until January 01, 2018. And all hits from the databases were screened by title first. Then, the abstracts of articles with titles fulfilling the study criteria were reviewed. Only full-text available articles were included; meeting or conference abstracts were excluded. Additional potentially relevant literature was identified by assessing the reference lists of eligible studies.

2.3. Inclusion/exclusion criteria

Inclusion criteria: studies were included if they fulfilled the following criteria:

(1) case–control and/or cohort studies;
(2) contained SNP genotype data; and
(3) adequate data for the calculation of odds ratios (ORs) and 95% confidence intervals (CIs).

Exclusion criteria: studies were excluded if they:

(1) not regarding the genetic association of EGF polymorphisms and risk of colorectal cancer;
(2) were duplicate publications;
(3) were case reports, letters, commentaries, meeting records, or review articles;
(4) insufficient published data for calculating an OR with 95% CI.

2.4. Data extraction and quality assessment

Data extraction from eligible articles was independently performed by ZH Chen and HG Jiang. The extracted data included first author, year of publication, ethnicity of the study population, number of cases and controls; gender ratio in case and control, ages in case and control, genotyping methods, sample source, Hardy–Weinberg equilibrium in control group, quality scores. Any disagreements were resolved by a consensus achieved by the third author Y Zhu.

BH Lu and HG Jiang accessed the quality of included studies independently as proposed by the Newcastle–Ottawa Scale (NOS).[22] A quality score was calculated from group selection, comparability, and assessment of outcome or exposure. The quality scores ranged from 0 to 10 (0 being the least and 10 being the highest). The methodological quality assessment of the included studies was assessed using the risk-of-bias tool of Review Manager software (version 5.3, Nordic Cochrane Centre, Denmark).[23] Any discrepancies in the assessment were resolved by the third author (Y Zhu).

2.5. Statistical analyses

The Stata software (version 12.0; Stata Corp LP, College Station, TX) and RevMan (version 5.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) were used in this meta-analysis. Crude OR and 95% CIs were calculated to test the strength of associations between the allelic, dominant, and recessive models of studied polymorphism and CRC susceptibility. The significance of the pooled OR was determined by Z test ($P < .05$ suggests a significant OR). A test of heterogeneity was conducted using Cochran's Q test and Higgins I-squared statistic. I^2 values of >50% indicate heterogeneity among studies. A random effect model was applied if heterogeneity was observed ($I^2 > 50\%$, $P < .05$). Otherwise the fixed effect model was used. Subgroup analysis by ethnicity, genotyping methods, and sample source was also carried out. Sensitivity analysis was performed to assess the effects of each individual study on pooled results. Publication bias was examined visually by the funnel plot, and statistically using the Begg and Egger tests. Power analysis was performed by STATA (https://www.stat.ubc.ca/). And, a trial sequential analysis (TSA) was carried out to estimate the sample size in meta-analysis with the TSA software (www.ctu.dk/tsa/). $P < .05$ was considered statistically significant.

3. Results

3.1. Characteristics of the eligible studies

The search process and study selection are presented in Figure 1. A total of 352 articles were identified through the literature search. Three hundred thirty four were excluded as duplicate, irrelevant studies or not original articles, and 18 selected. After full-text scanning, 9 studies were removed for not case–control design, cohort design, or not regarding to the association between *EGF* A61G and CRC risk. Finally, 9 case–control studies with 1448 cases and 1928 controls were included in the meta-analysis.[16–27] The information for the selected studies was presented in Table 1. And the NOS quality assessment of these included studies is provided in Table 1 and Table s1, http://links.lww.com/MD/C743. Only studies with NOS scores larger than 6 were selected in this meta-analysis.

3.2. Results of meta-analysis

Significant association was detected between allelic and recessive models of *EGF* A61G and the risk of CRC (allelic model: OR =

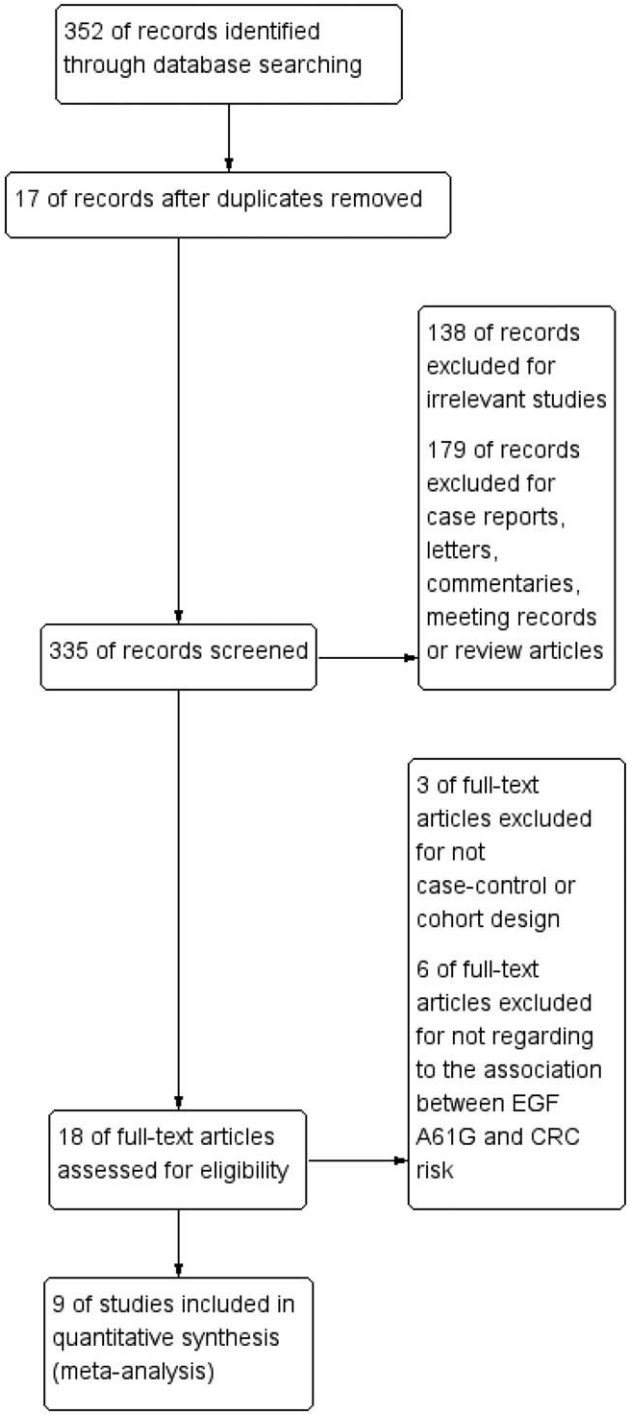


Figure 1. PRISMA flow chart of studies inclusion and exclusion. PRISMA = preferred reporting items for systematic reviews and meta-analyses.

1.18, 95% CI [1.01, 1.37], $P = .04$; recessive model: OR = 1.36, 95% CI [1.04, 1.79], $P = .03$). And no significant association was observed between *EGF* A61G and CRC in dominant model ($P > .05$). Stratification analysis by ethnicity showed that the frequency of *EGF* 61G allele significantly increased in cases than that in healthy controls in Caucasian (OR = 1.24, 95% CI [1.03, 1.49], $P = .02$), but not in Asian (OR = 1.12, 95% CI [0.99, 1.27], $P = .08$). However, the recessive model of *EGF* A61G was significantly increased in cases than that in healthy control in both Caucasian (OR = 1.40, 95% CI [1.01, 1.93], $P = .04$) and Asian (OR = 1.27, 95% CI [1.05, 1.53], $P = .01$). Furthermore, subgroup analysis stratified by sample sources (population-based and hospital-based [HB]) and genotyping methods (restriction fragment length polymorphism-polymerase chain reaction [RFLP-PCR] and TaqMan) showed no significant association between the *EGF* A61G and CRC susceptibility in allelic, dominant, and recessive models ($P > .05$) (Table 2 and Fig. 2).

3.3. Test of heterogeneity

Significant heterogeneity was observed in allelic and recessive models of *EGF* A61G both in total group (allelic model: $I^2 = 54$, $P = .03$; recessive model: $I^2 = 61$, $P = .008$), Caucasian subgroup (allelic model: $I^2 = 85$, $P = .001$; recessive model: $I^2 = 88$, $P = .0002$), HB subgroup (allelic model: $I^2 = 59$, $P = .02$; recessive model: $I^2 = 65$, $P = .005$), and RFLP-PCR subgroup (allelic model: $I^2 = 63$, $P = .01$; recessive model: $I^2 = 70$, $P = .003$). The heterogeneity in all these comparisons was contributed mainly by Wu et al[18]. The removal of this study from the meta-analysis gave 0% to 40% ($P > .05$) heterogeneity and showed that it had the highest effect on *EGF* A61G in allelic, dominant, and recessive models in CRC (Table 2).

3.4. Power analysis

It is expected that the limited sample size causing serious power-loss. Before making a conclusion on the heterogeneity, power calculations about the meta-analysis should be performed. The power analysis suggested that power of 91% was determined for rs4444903. As shown in Figure s1, http://links.lww.com/MD/C743, our meta-analysis collected 9 studies with 1448 cases. The required information size for this meta-analysis was 4312 with the indexes: type I error ($\alpha = 0.05$), type II error ($\beta = .2$). However, the results of TSA showed the EGF A61G contribute to the risk of CRC was reliable.

3.5. Sensitivity analysis and publication bias

Sensitivity analysis which excluded the influence of a single study on the overall risk estimate by excluding 1 study at a time was confirmed. The ORs were significantly altered by excluding Wu et al (Fig. 3). After omitted this study, significant association was only detected between the recessive model of *EGF* A61G and CRC in Asian population (OR = 1.27, 95% CI [1.05, 1.53], $P = .01$) (Table s2, http://links.lww.com/MD/C743). Sensitivity analysis based on these 8 studies showed that The ORs were not significantly altered in each genetic models (Fig. s2, http://links.lww.com/MD/C743).

Funnel plots and Egger test were performed to assess publication bias. The results suggested that there was no publication bias for the comparison of *EGF* A61G in allelic, dominant, and recessive models (Fig. 4).

4. Discussion

This meta-analysis demonstrated that the *EGF* 61G allele was significantly associated with CRC in the Caucasian subgroup. And the GG genotype of *EGF* A61G was significantly associated with CRC in both Caucasian and Asian subgroups. The sample source and genotyping methods didn't affected the significant association between *EGF* A61G and CRC in allelic, dominant and recessive models. Thus, the *EGF* A61G might be a risk factor for CRC susceptibility.

Table 1

The characters of individual studies.

Author	Year	Ethnicity	Case/control	M:F (case/control)	Age (case/control)	Genotyping	Stage 0–2	Stage 3 and 4	Grade 1	Grade 2	Grade 3	Sample sources	HWE	NOS scores
Chaleshi	2013	Iranian	220/220	118/93:102/127	61.02±12.09/41.99±14.35	PCR-RFLP	78	65	NA	NA	NA	HB	$P>.05$	9
Daraei	2011	Iranian	115/120	57/53:60/60	57.86±11.62/58.48±10.66	PCR-RFLP	NA	NA	NA	NA	NA	PB	$P>.05$	9
Lau	2014	Malaysian	130/212	NA	NA	TaqMan	NA	NA	NA	NA	NA	HB	$P>.05$	6
Lin	2012	Chinese	180/180	95/85:93/87	60.8±7.9/61.0±8.0	PCR-RFLP	140	40	NA	NA	NA	HB	$P>.05$	8
Mahmood	2013	Caucasian	173/303	104/69:166/137	66±12/52±13.8	PCR-RFLP	34	90	3	38	19	HB	$P>.05$	8
Spindler	2007	Denmark	81/232	NA	NA	TaqMan	NA	NA	NA	NA	NA	HB	$P>.05$	6
Wu	2009	German	157/117	100/57:74/43	NA	PCR-RFLP	86	62	2	128	13	HB	$P>.05$	7
Chen	2009	Chinese	174/227	85/89;110/117	NA	PCR-RFLP	NA	NA	NA	NA	NA	HB	$P>.05$	7
Yu	2011	Chinese	218/200	143/75:140/60	63.4±28.6/61.8±26.5	PCR-RFLP	NA	NA	32	143	43	HB	$P>.05$	8

EGF = epidermal growth factor, F = female, HB = hospital-based, HWE = Hardy–Weinberg equilibrium, M = male, NA = not available, NOS = Newcastle–Ottawa Scale, PB = population-based, RFLP-PCR = restriction fragment length polymorphism-polymerase chain reaction.

CRC is a multistep process, where genetic changes occur with mutations of several genes that accumulate with the progression from a normal epithelium to an adenoma to invasive cancer. The EGFR system is an important mediator in the tumor microenvironment that results in enhanced tumor growth.[28] EGF exerts effects on cell proliferation and differentiation by binding to the tyrosine kinase EGF receptor.[29] Both EGF and EGFR expression have been described to be significantly increased in patients with CRC compared with that in the normal individuals.[30] And the impact of *EGF* polymorphisms on cancers has been described. Recently, it has been shown that *EGF* A61G has a functional influence on the expression of *EGF* gene in CRC patients.[31] Subsequently, the genetic association between *EGF* A61G and CRC was also investigated by several studies with conflicting results.[16–27]

Notable, 8 of 9 included studies reported no significant association between *EGF* A61G and the risk of colorectal cancer.[16,17,19,20,24–27] While, positive result was detected between the allelic model of *EGF* A61G and CRC in our combined analysis, which was similar with the results of meta-analysis that conducted by Li et al[32] and Piao et al.[33] Notable, relatively small number of studies were included in Li et al[32] and Piao et al.[33] Our meta-analysis enrolled in 5 more studies with 1448 cases and 1928 controls and found that the significant association between the allelic model of *EGF* A61G and CRC can be only identified in the Caucasian subgroup when stratified by ethnicity, which may supply a new information about the influence of this polymorphism in the development of CRC in populations with different genetic background.

In addition, significant association was also observed between the recessive model (GG/AG + AA) of *EGF* A61G and CRC in both total group and subgroups stratified by ethnicity, which was partly similar with the results reported by Piao et al.[33] The G/G genotype was reported to lead to a higher production of EGF, and thus increase risk of colorectal cancer.[9] The mechanism that the GG genotype of *EGF* A61G increases the EGF production may due to the following reasons. First, G to A substitution might affect the DNA folding or processing of the mRNA transcript.[9] Second, this polymorphism might be closely linked to a functional polymorphism elsewhere in the gene.[34]

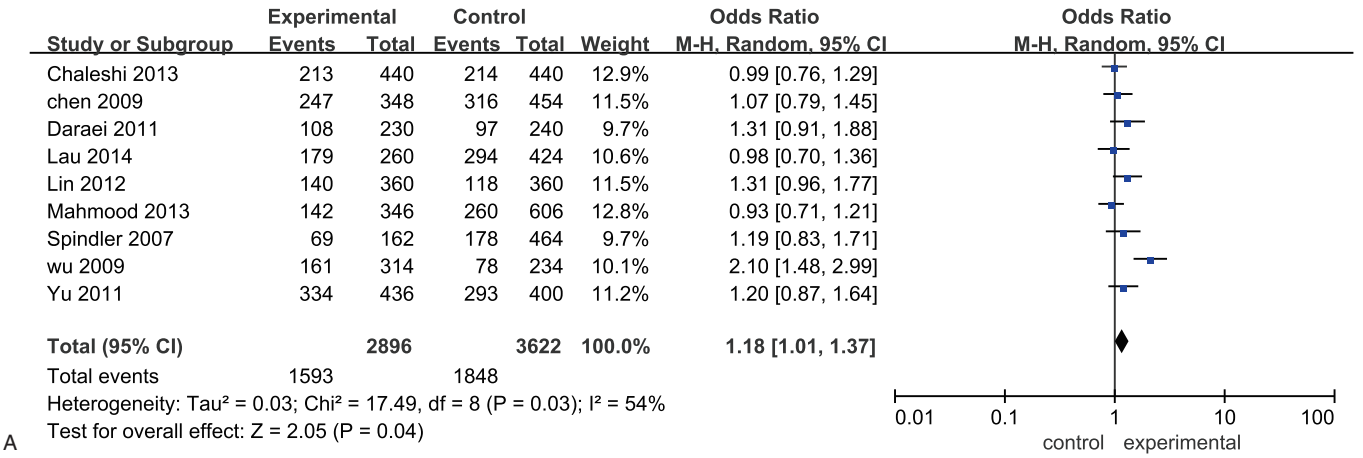
However, we failed to detect association between the dominant model (GG + AG/AA) of *EGF* A61G and CRC both in total group and subgroups analysis stratified by ethnicity, which was similar

Table 2

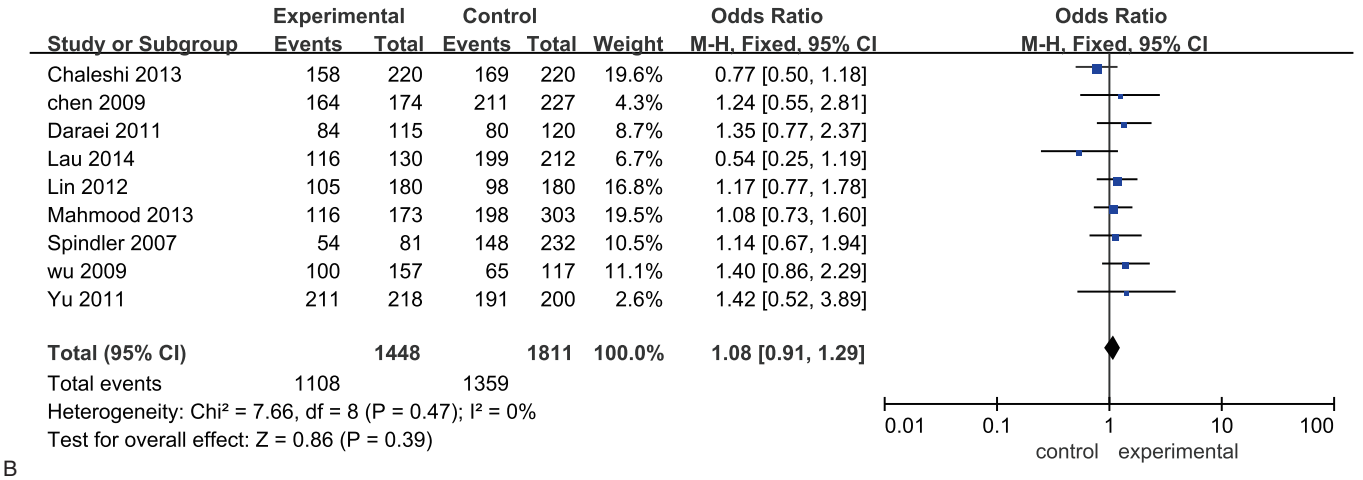
The main results of the association between EGF A61G and colorectal cancer.

SNPs (minor allele)	Genetic model	Subgroups	Number of studies	Numbers		Test of association		Model	Test of heterogeneity	
				Case	Control	OR [95% CI]	*P*-value		*P*-value	I^2 (%)
EGF A61G	Allelic model	Total	9	2896	3622	1.18 [1.01, 1.37]	.04	R	.03	54
		Asian	6	2074	2318	1.12 [0.99, 1.27]	.08	F	.64	0
		Caucasian	3	822	1304	1.24 [1.03, 1.49]	.02	R	.001	85
		HB	8	2666	3382	1.16 [0.98, 1.38]	.08	R	.02	59
		PB	1	230	240	1.31 [0.91, 1.88]	.15	NA	NA	NA
		RFLP-PCR	7	2474	2734	1.21 [1.00, 1.46]	.05	R	.01	63
		TaqMan	2	422	888	1.07 [0.84, 1.37]	.59	F	.43	0
	Dominant model	Total	9	1448	1811	1.08 [0.91, 1.29]	.39	F	.47	0
		Asian	6	1037	1159	1.01 [0.77, 1.32]	.94	F	.29	19
		Caucasian	3	411	652	1.18 [0.91, 1.54]	.22	F	.70	0
		HB	8	1333	1691	1.05 [0.88, 1.27]	.58	F	.43	0
		PB	1	115	120	1.35 [0.77, 2.37]	.29	NA	NA	NA
		RFLP-PCR	7	1237	1367	1.12 [0.92, 1.35]	.26	F	.60	0
		TaqMan	2	211	444	0.83 [0.41, 1.70]	.61	F	.13	57
	Recessive model	Total	9	1448	1811	1.36 [1.04, 1.79]	.03	R	.008	61
		Asian	6	1037	1159	1.27 [1.05, 1.53]	.01	F	.65	0
		Caucasian	3	411	652	1.40 [1.01, 1.93]	.04	R	.0002	88
		HB	8	1333	1691	1.35 [1.00, 1.81]	.05	R	.005	65
		PB	1	115	120	1.60 [0.81, 3.16]	.18	NA	NA	NA
		RFLP-PCR	7	1237	1367	1.40 [0.99, 1.98]	.06	R	.003	70
		TaqMan	2	211	444	1.26 [0.87, 1.82]	.22	F	.50	0

CI = confidence interval, EGF = epidermal growth factor, F = fixed model, HB = hospital-based, NA = not available, OR = odds ratio, PB = population-based, R = random model, RFLP-PCR = restriction fragment length polymorphism-polymerase chain reaction.



A



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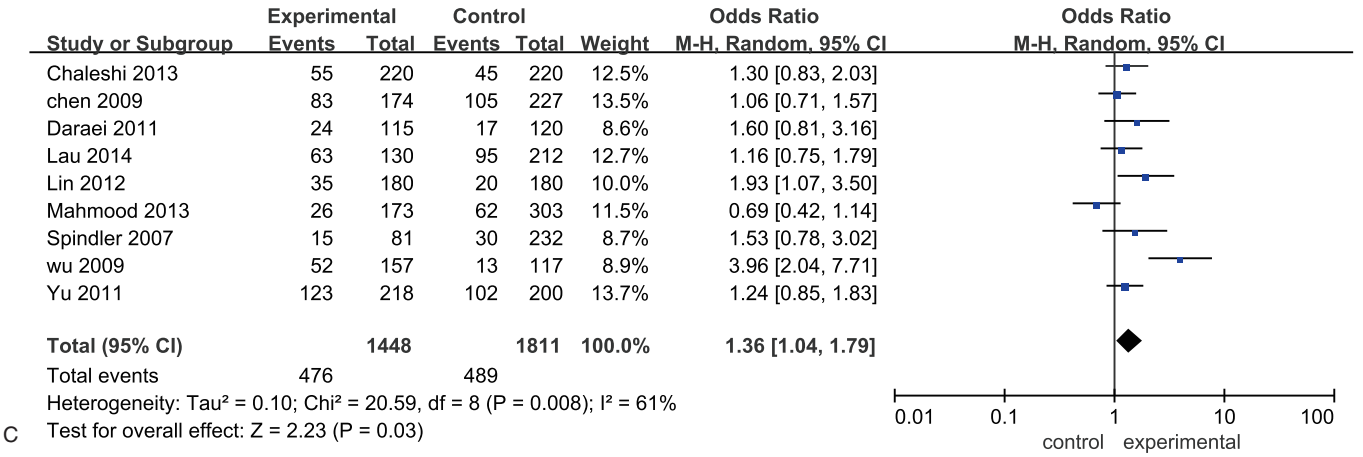


C

Figure 2. Forest plots of odds ratios for the association between EGF A61G and colorectal cancer A: allelic model; B: dominant model; C: recessive model. EGF = epidermal growth factor.

with the results reported by Li et al, but contrast to the results conducted by Piao et al. The inconsistent results in the previous meta-analysis may due to the following reasons. Firstl, the number of included studies in previous meta-analysis studies was relatively small. Although we included 6 more studies to investigate the association between the dominant model of *EGF* A61G and colorectal cancer, negative results were still obtained. Thus, more studies with larger number of cohorts and multiple ethnicity are still necessary. Second, differences in genetic and environmental background exist among different ethnicity. Third, different populations usually have different linkage disequilibrium patterns.

Furthermore, no significant association between the *EGF* A61G and the susceptibility of CRC was found in subgroup

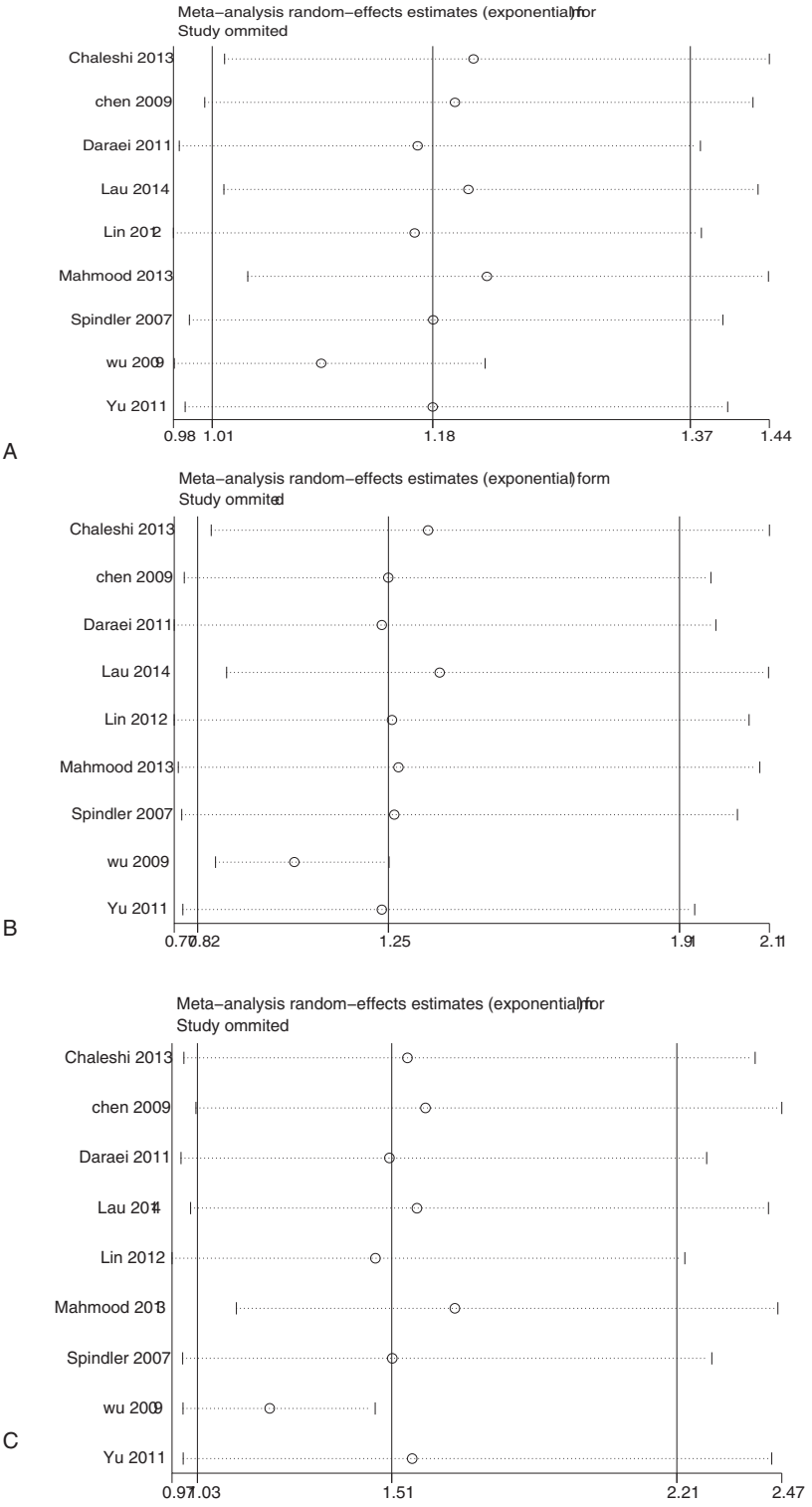
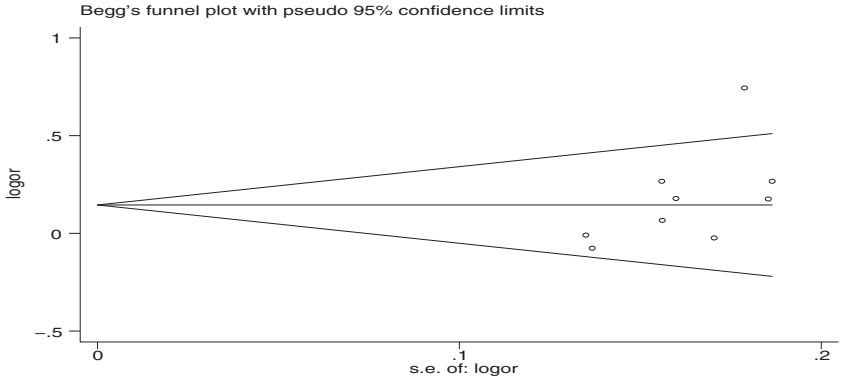


Figure 3. Sensitivity analyses between EGF A61G and colorectal cancer. A: allelic model; B: dominant model; C: recessive model. EGF = epidermal growth factor.

analysis stratified by both sample sources and genotyping methods, which was different from the results in Li et al.[32] This may indicate the sample sources and genotyping methods have no effect on the association between *EGF* A61G and colorectal cancer. And the small sample size included in study conducted by Li et al[32] might explain this difference.

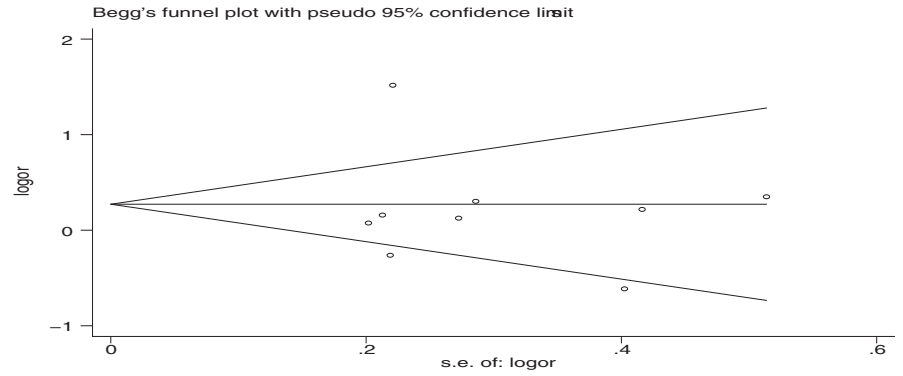
Despite including case–control studies, the results of the present meta-analysis should be interpreted carefully because of the following limitations. Firstl, limited number of studies and subjects included in this meta-analysis were relatively small. Only 9 eligible studies with 1448 case and 1928 control were included, especially for eligible studies included in subgroups were relatively insufficient. It was necessary to confirm these results

A



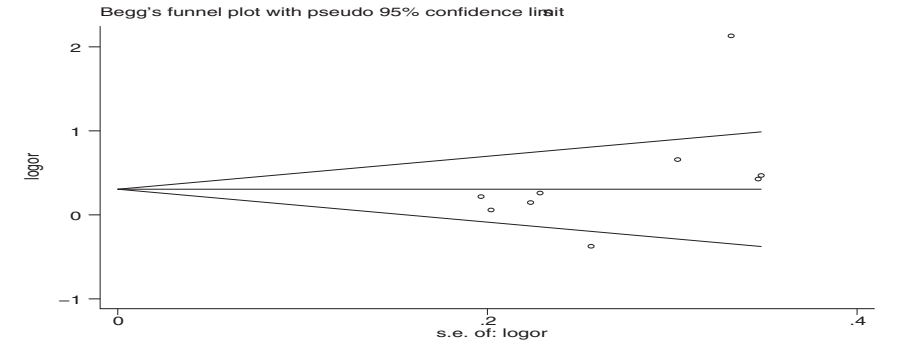
Std_Eff	Coef.	Std. Err.	t	P>|t|	[95%Conf. Interval]
slope	-1.049482	.5769757	-1.82	0.112	-2.413813~.3148489
bias	7.535832	3.615612	2.08	0.076	-1.013732~16.0854

B



Std_Eff	Coef.	Std. Err.	t	P>|t|	[95%Conf. Interval]
slope	.5936435	.7740124	0.77	0.468	-1.236605~2.423892
bias	-1.271297	2.934977	-0.43	0.678	-8.211414~5.66882

C



Std_Eff	Coef.	Std. Err.	t	P>|t|	[95%Conf. Interval]
slope	-1.073809	.8062324	-1.33	0.225	-2.980246~ .832628
bias	5.593232	3.195993	1.75	0.124	-1.96409~13.15055

Figure 4. Publication bias of literatures for allelic, dominant and recessive models of EGF A61G were tested by Begg funnel plot and Egger test. A: allelic model; B: dominant model; C: recessive model. EGF = epidermal growth factor.

by including larger number of case–control studies regarding with the correction of *EGF* A61G and the risk of colorectal cancer. Second, we included studies only in Caucasian and Asian populations. The results may be need further accessed in multiple ethnicity groups such as African. Third, CRC is a multi-factorial disease. Gene–gene/gene–environment interactions may play important roles in the pathology of colorectal cancer, but most studies lack information about gene–gene/gene–environment interactions.

5. Conclusion

The current meta-analysis suggests an increased risk of CRC for *EGF* G allele in the Caucasian subgroup and *EGF* GG genotype

in both Caucasian and Asian subgroups. To confirm these results, further study with larger sample size and multiple ethnicities is necessary.

Author contributions

Conceptualization: Yi Zhu.
Data curation: HongGang Jiang.
Funding acquisition: Yi Zhu.
Methodology: ZhiHeng Chen.
Software: HongGang Jiang.
Writing – original draft: Yi Zhu, BoHao Lu.
Writing – review and editing: Yi Zhu, ZhiHeng Chen, HongGang Jiang, BoHao Lu.

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