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Association between EGF +61 A>G Polymorphism and Gastric Cancer Risk: A Meta-analysis

Song-jie WU (吴松杰)†, Si-yu JIANG (蒋思宇)†, Jing WU (吴 静), Guang-lian XIONG (熊光练)#
Key Laboratory of Environment and Health, Ministry of Education & Department of Epidemiology and Biostatistics, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary: Previous studies suggested an association between the EGF +61 A>G polymorphism and susceptibility to gastric cancer, but the results have been inconsistent. To draw a more precise risk estimation of the association, we performed a meta-analysis of published studies. PubMed, EMBASE, Google Scholar and the Chinese Wanfang databases were systematically searched to identify relevant studies. There were 7 studies involving 1992 cases of gastric cancer and 3202 controls in this meta-analysis. Our study showed that, overall, the EGF +61 A>G polymorphism was significantly associated with the increased risk of gastric cancer in allele model (G *vs*. A: OR=1.18, 95% CI=1.00–1.39), dominant model (GG + GA *vs*. AA: OR=1.28, 95% CI=1.05–1.55), homozygous model (GG *vs*. AA: OR=1.31, 95% CI=1.06–1.63) and heterozygous model (GA *vs*. AA: OR=1.25, 95% CI=1.01–1.53). The stratified analysis by ethnicity revealed a significant association between EGF +61 A>G polymorphism and gastric cancer risks in Asians. This meta-analysis indicates that EGF +61 A>G polymorphism may increase the risk of gastric cancer, especially in Asians. Large-sized, well-designed studies involving different ethnic groups should be conducted to confirm this association.
Key words: EGF; gastric cancer; polymorphism; meta-analysis

Gastric cancer remains the fifth most common malignancy and the third leading cause of death due to cancer worldwide, with about 1 million new gastric cancer cases and around 700 000 deaths in 2012[1]. Previous studies have shown that multiple environmental and lifestyle factors, including *Helicobacter pylori* infection[2], smoking[3], alcohol consumption[4], a diet rich in red meat[5] and/or salt[6] may increase the risk of gastric cancer. However, not all of those who have been exposed to the risk factors develop gastric cancer, suggesting an individual susceptibility to the effects of carcinogens[7]. Host factors, including genetic polymorphisms, have been suggested as risk factors for the development of a variety of cancers, such as gastric cancer[8, 9].

EGF, located in chromosome 4q25-q27[10], contains 24 exons and 23 introns, and encodes a ligand for the EGF receptor (EGFR). As an endocrine growth factor, EGF can activate DNA synthesis and cellular proliferation and stimulate mitogenesis in epidermal tissue by binding to EGFR[11]. Previous studies have reported that the gene variations in EGF can lead to deregulation of the EGFR pathway and over-expression of EGF proteins, which are associated with gastric cancer and various malignancies[12]. A study has shown that higher levels of EGF in gastric cancer are associated with advanced tumor stage and a poor clinical outcome[13]. The EGF +61 A>G polymorphism is a commonly functional single-nucleotide polymorphism (SNP) in the 5' untranslated region of the EGF gene, regulating EGF and the effects on individuals' susceptibility to various carcinomas, including gastric cancer[14–16].

Several recent studies have examined the association between EGF +61 A>G polymorphism and gastric cancer risk[17–23], but have yielded mixed results. Some studies reported that patients carrying GG genotypes have a higher susceptibility to gastric cancer[17, 19, 22, 23], while the other studies did not[18, 20, 21]. In addition, 5 published articles tried to find this association by meta-analysis[24–28], but the results were inconsistent and they had the same limitation of the relatively small size. Hence, we conducted a meta-analysis involving 7 case-control studies covering 1992 cases and 3202 controls to provide a more precise risk estimation of the association between EGF +61 A>G polymorphism and gastric cancer risk.

1 MATERIALS AND METHODS

1.1 Literature Search

We conducted a comprehensive literature search in PubMed, EMBASE, Google Scholar and the Chinese Wanfang databases up to October 1, 2014 using the search terms "epidermal growth factor," "EGF," "polymorphism" and "gastric cancer". No language restriction was imposed. Additional studies were also identified by a manual search of the references of retrieved articles

Song-jie WU, E-mail: wsj923@126.com; Si-yu JIANG, E-mail: jiangsiy@hust.edu.cn
†The two authors contributed equally to this work.
#Corresponding author, E-mail: weixiong@mails.tjmu.edu.cn

and reviews.

1.2 Inclusion and Exclusion Criteria

Studies had to fulfill the following criteria to be included: (1) case-control studies focusing on the association between EGF +61 A>G polymorphism and gastric cancer risk; (2) genotype frequencies on EGF +61 A>G polymorphism in case and control groups sufficient for estimation of OR with 95% CI. For the studies containing comparable or overlapping data published by the same investigators, we only included studies with complete design and of larger sample size that had strong power. Studies without providing controls and genotype frequency or redundant publications were excluded.

1.3 Data Extraction and Quality Assessment

All the data were extracted independently by two investigators and disagreements were resolved by discussion and consensus obtained among all reviewers. Following information was extracted from the eligible literature: first author's last name, year of publication, country, ethnicity, source of controls, number of cases and controls, genotype distributions of cases and controls.

The quality of included studies was assessed by the confirmation of Hardy-Weinberg equilibrium (HWE) in controls and by using the Newcastle Ottawa Scale (NOS)[29]. The NOS has a maximum of nine 'stars' on items related to the selection of the study groups (four stars), the comparability of the groups (two stars) and the ascertainment of the outcome of interest (three stars). A study was awarded a maximum of one star for each item, with the exception of the item related to comparability, which was awarded two stars.

1.4 Statistical Analysis

HWE for the control group of each study was assessed by using goodness-of-fit test (χ^2 of Fisher's exact test).

Based on both fixed effects and random-effects models, a pooled odds ratio (OR) with 95% interval confidence (95% CI) was used to measure the strength of association between EGF +61 A>G polymorphism and gastric cancer risk. We examined the association for an allele model (G *vs*. A), a dominant model (GG+GA *vs*. AA), a recessive model (GG *vs*. GA+AA), a homozygous model (GG *vs*. AA) and a heterozygous model (GA *vs*. AA).

Heterogeneity among studies was evaluated by Cochran's Q test and then quantified by I^2 statistic[30, 31]. If P>0.1 with the result of heterogeneity test, ORs were pooled according to the fixed-effects model (Mantel-Haenszel model)[32]. Otherwise, ORs were pooled in accordance with the random-effects model (Der Simonian and Laird model)[33]. I^2 was used to qualify the variation in OR attributable to heterogeneity. We conducted stratified analyses in terms of ethnicity to identify the cause of potential heterogeneity.

Funnel plot and Egger's test were employed[34]. Funnel plot asymmetry was used to evaluate publication bias. All statistical tests were performed in this study by using the Metafor package (version 1.6) in R (version 15.3; http://www.r-project.org/) and all P values were two-sided with the significant level set at 0.05.

2 RESULTS

2.1 Study Characteristics and Quality Assessment

The study selection process is depicted in fig. 1. A comprehensive search identified 40 references, and 11 full-text publications were preliminarily identified for further evaluation. Against the exclusion criteria, 4 publications were excluded, including a meta-analysis[25], a study without control group[35], a study lacking adequate data[36] and a study that was not about EGF +61 A>G polymorphism[37]. As a result, a total of 7 studies[17–23] including 1992 gastric cancer cases and 3202 controls were finally included in our meta-analysis. Of all the eligible studies, 6 were conducted in Asians, and 1 Caucasians. All studies had a case-control design, with the controls of 3 studies from the general population, and the rest from hospitals. The genotype distribution of the controls in one study was inconsistent with HWE[18]. In terms of NOS scores were awarded 7 stars in 4 studies, 3 studies earned 8 stars. The main features of the studies are listed in table 1.

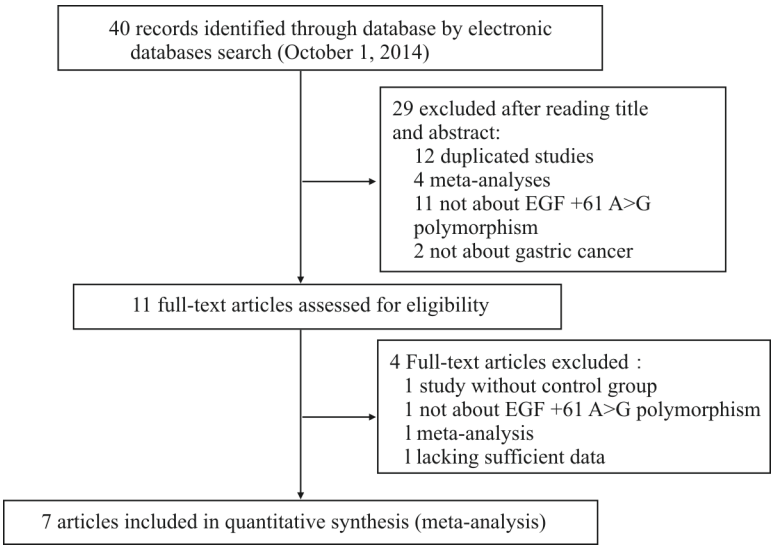

Fig. 1 Flow chart of the literature search

Table 1 Characteristics of the studies included in meta-analysis

Study	Year	Country	Ethnicity	Source of control	Sample size (case/control)	Cases			Controls			Study quality	HWE test	
						AA	AG	GG	AA	AG	GG		*P*	Test
Goto *et al*	2005	Japan	Asian	PB	202/454	14	88	100	47	188	215	8	0.537	Yes
Hamai *et al*	2005	Japan	Asian	HB	200/230	15	66	119	25	97	108	8	0.647	Yes
Jin *et al*	2007	China	Asian	PB	675/704	42	242	333	57	289	314	8	0.407	Yes
Araujo *et al*	2011	Portugal	Caucasian	PB	207/984	49	84	29	304	449	231	7	0.010	No
Yang *et al*	2012	China	Asian	HB	207/318	17	75	115	39	132	147	7	0.272	Yes
Lin *et al*	2012	China	Asian	HB	114/120	7	45	62	14	59	47	7	0.484	Yes
Zhan *et al*	2013	China	Asian	HB	387/392	32	166	177	37	142	204	7	0.099	Yes

PB: population-based; HB: hospital-based; HWE: Hardy-Weinberg equilibrium

2.2 Test of Heterogeneity

The association between EGF +61 A>G polymorphism and gastric cancer risk is presented in table 2. The heterogeneity of EGF +61 A>G polymorphism in 5 genetic models was analyzed for all 7 studies. Random effects model was used for allele model and recessive model in which significant heterogeneity was found (G *vs*. A: $P_{heterogeneity}$=0.009 for total population, and $P_{heterogeneity}$=0.028 for Asians; GG *vs*. GA+AA: $P_{heterogeneity}$=0.002 for total population and $P_{heterogeneity}$=0.007 for Asians). On the other hand, fixed effects model was empolyed in other genetic models in which heterogeneity was not found.

Table 2 Meta-analysis of the association between EGF +61 A>G polymorphism and gastrc cancer risk

Genetic models	*n*	Model for analysis	OR (95% CI)	*P* for OR	I^2	*P* for heterogeneity
Allelic model	7	REM	1.18 (1.00–1.39)	0.054	66.61	0.009
Asian	6	REM	1.23 (1.04–1.46)	0.016	61.48	0.028
Caucasian	1	–	0.91 (0.71–1.15)	0.410	–	–
Dominant model	7	FEM	1.28 (1.05–1.55)	0.015	0.00	0.742
Asian	6	FEM	1.39 (1.10–1.76)	0.005	0.00	0.897
Caucasian	1	–	1.03 (0.72–1.48)	0.869	–	–
Recessive model	7	REM	1.18 (0.91–1.52)	0.208	74.35	0.002
Asian	6	REM	1.26 (0.99–1.61)	0.064	69.76	0.007
Caucasian	1	–	0.71 (0.46–1.09)	0.118	–	–
Homozygous model	7	FEM	1.31 (1.06–1.63)	0.014	38.21	0.138
Asian	6	FEM	1.48 (1.17–1.89)	0.001	0.00	0.505
Caucasian	1	–	0.78 (0.48–1.27)	0.318	–	–
Heterozygous model	7	FEM	1.25 (1.01–1.53)	0.036	0.00	0.980
Asian	6	FEM	1.28 (1.01–1.64)	0.045	0.00	0.967
Caucasian	1	–	1.16 (0.79–1.70)	0.444	–	–

n: number of studies; OR: odds ratio; 95% CI: 95% confidence interval; REM: random-effects model; FEM: fix-effects model

2.3 Synthesis Results

Table 2 shows the summary ORs for EGF +61 A>G polymorphism and gastric cancer risk. Overall, significant association between EGF +61 A>G polymorphism and increased gastric cancer risk was observed in allele model (G *vs*. A: OR=1.18, 95% CI=1.00–1.39), dominant model (GG + GA *vs*. AA: OR=1.28, 95% CI=1.05–1.55), homozygous model (GG *vs*. AA: OR=1.31, 95% CI=1.06–1.63) and heterozygous model (GA *vs*. AA: OR=1.25, 95% CI=1.01–1.53), but no significant association was found in recessive model (GG *vs*. GA+AA: OR=1.18, 95% CI=0.91–1.52) (fig. 2).

The stratified analysis by ethnicity revealed significant associations in Asians in allele model (G *vs*. A: OR=1.23, 95% CI=1.04–1.46), dominant model (GG + GA *vs*. AA: OR=1.39, 95% CI=1.10–1.76), homozygous model (GG *vs*. AA: OR=1.48, 95% CI=1.17–1.89) and heterozygous model (GA *vs*. AA: OR=1.28, 95% CI=1.01–1.64), but not in recessive model (GG *vs*. GA+AA: OR=1.26, 95%, CI=0.99–1.61).

2.4 Publication Bias

Egger's funnel plot and Egger's linear regression test were performed to assess the publication bias of the included studies. The shape of funnel plots (fig. 3) did not exhibit conspicuous asymmetry and the *P* values of Egger's tests were greater than 0.05 in allele model (*P*=0.106), dominant model (*P*=0.092), recessive model (*P*=0.539) and heterozygous model (*P*=0.495), providing statistical evidence of the funnel plots' symmetry. However, the funnel plot did show some asymmetry, as subsequently corroborated by Egger's test in homozygous model (*P*=0.048).

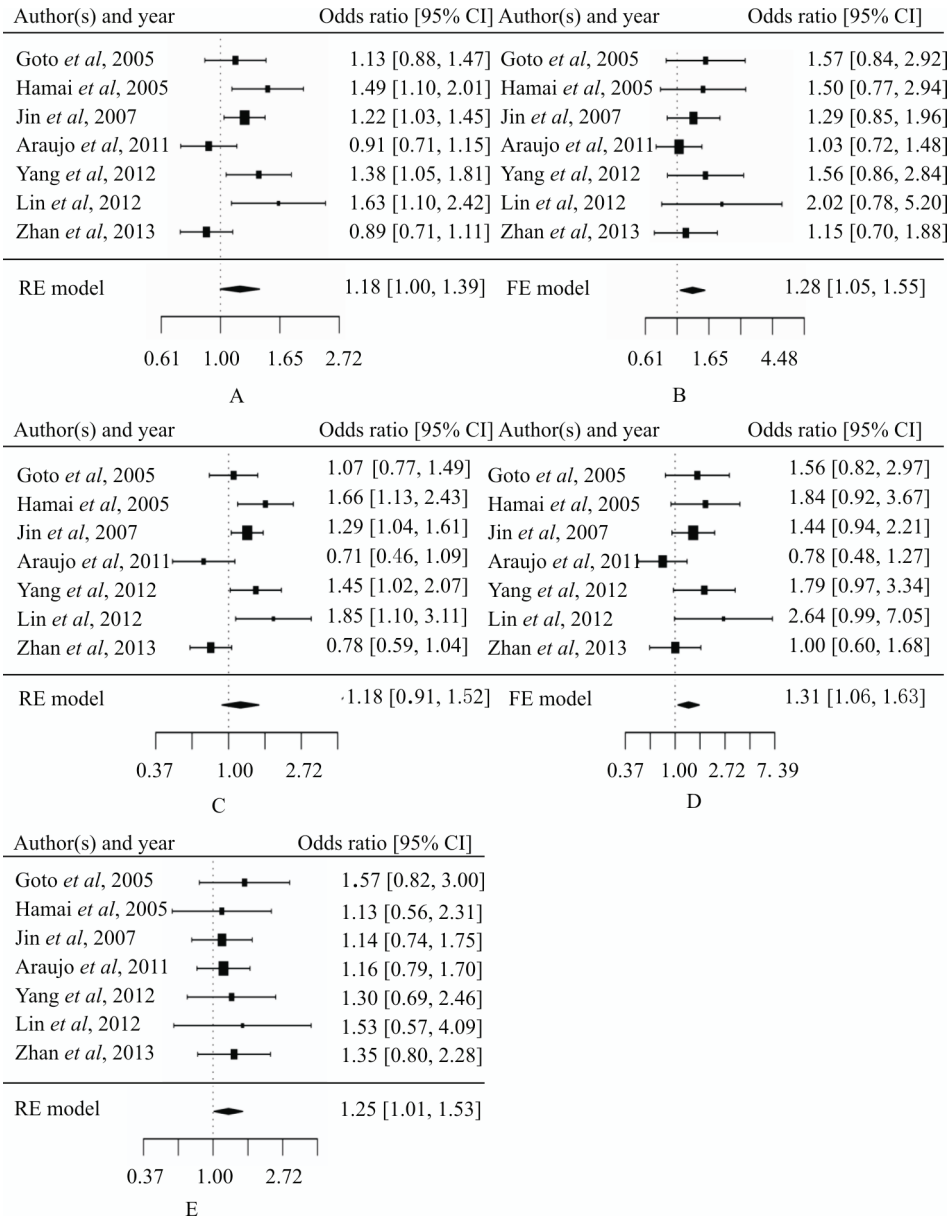

Fig. 2 The forest plots of OR with 95% CI for EGF +61 A>G polymorphism with gastric cancer
A: allele model; B: dominant model; C: recessive model; D: homozygous model; E: heterozygous model

3 DISCUSSION

Gastric cancer accounts for a considerable burden of morbidity and mortality worldwide[1]. Previous studies have suggested that genetic factors play an important role in gastric cancer susceptibility[38]. Strong biological evidence shows that EGF is involved in the development and progression of gastric cancer. An animal experiment[39] showed that EGF might modulate the growth of gastric cancer by stimulating the expression of EGF receptor protein in nude mice. Another animal experiment[40] exhibited that the incidence of the stomach tumors was higher in rats treated with EGF immediately after cessation of the N-methyl-N'-nitro-N-nitrosoguanidine treatment than in controls, suggesting that EGF might enhance the effect of EGF on stomach carcinogenesis in rats. Currently, while many studies showed the association between EGF +61 A>G polymorphism and gastric cancer risk, the results were inconsistent. The discrepancy among the researches might be ascribed to differences in country, ethnicity, study design, sample size *etc*. In this study, we performed a meta-analysis of 7 studies, covering 1992 cases of gastric cancer and 3202 controls, with an attempt to provide a more reliable and comprehensive result. The results of our meta-analysis indicated that EGF +61 A>G polymorphism correlates with increased gastric cancer risk, and the finding was in line with the results reported by a number of previous studies[17, 19, 22, 23, 36]. The plausible mechanism might be that EGF enhances gene transcription after binding to its high affinity cell surface receptor (EGFR)[41], stimulates the proliferation and differentiation of both normal and malignant cells, and eventually contributes to elevated tumor risk. Nevertheless, given that other potential confounding factors might influence the result, we further conducted a stratified analysis in terms of ethnicity. The

results showed a significant association between EGF +61 A>G polymorphism and gastric cancer risk in Asians but not in Caucasians, suggesting ethnic difference in the association between EGF +61 A>G polymorphism and gastric cancer risk. However, only one study was conducted in Caucasians and in the study the genotype distribution in controls was inconsistent with HWE. Therefore, more studies involving larger samples are warranted to more accurately estimate the association between EGF +61 A>G polymorphism and gastric cancer risk in Caucasians.

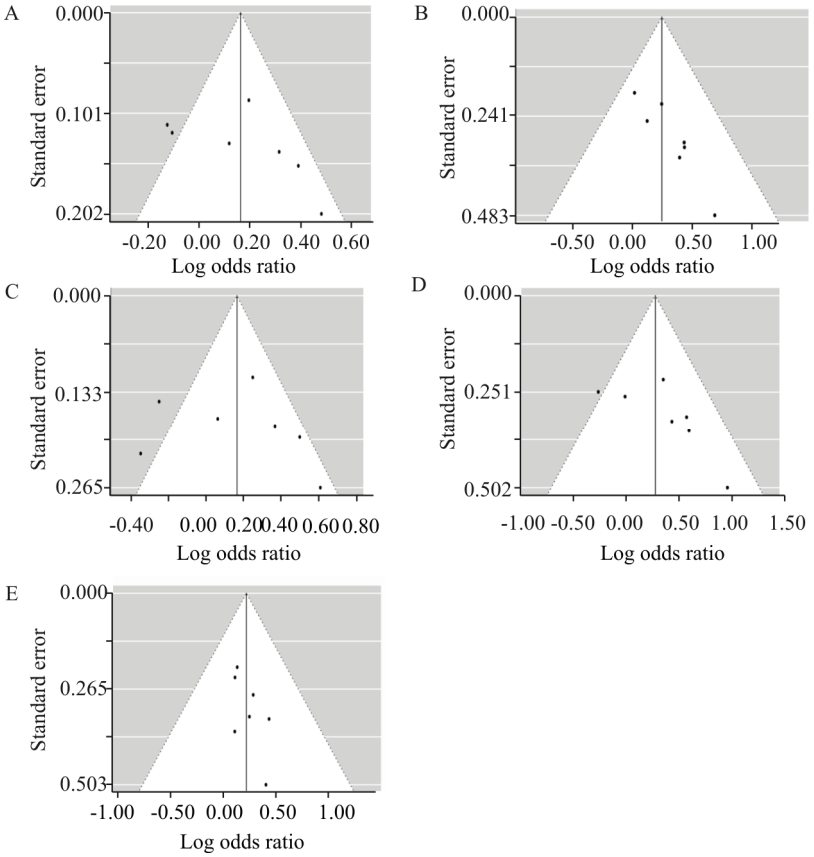

Fig. 3 The funnel plots of natural logarithm of OR against inverse standard error in each study
A: allele model; B: dominant model; C: recessive model; D: homozygous model; E: heterozygous model

Heterogeneity is an important issue in the interpretation of meta-analysis findings. Table 2 shows that there was obvious heterogeneity between allele model and recessive model. Even when we stratified them in terms of ethnicity, heterogeneity remained. One factor for this heterogeneity might be that the role of the polymorphism varies in different ethnicity.

Our meta-analysis had some limitations. First, significant publication bias was detected in homozygous model (GG *vs*. AA). Second, the number of included studies was not sufficient for a comprehensive analysis. In particular, the stratified analysis of a Caucasian population was based on only one study. Third, the results may be affected by additional confounding factors, such as the status of *Helicobacter pylori* infection, tumor status, gender or age, but most studies neither reported these baseline data nor aggregated them in any ways, rendering them ineligible for inclusion.

In summary, our meta-analysis indicates that EGF +61 A>G polymorphism might increase the risk of gastric cancer, especially in Asians. This results suggest that EGF +61 A>G polymorphism may play an important role in the development of gastric cancer. Large-sized, well-designed studies involving different ethnic groups should be conducted in future studies to further confirm the results of our meta-analysis. Moreover, the effect of gene-gene and gene-environment interactions must be examined.

Conflict of Interest Statement

The authors declared no potential conflicts of interest.

REFERENCES

1 Ferlay J, Soerjomataram I, Dikshit R, *et al*. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2014,136(5):E359-E386
2 Bornschein J, Malfertheiner P. *Helicobacter pylori* and gastric cancer. Dig Dis, 2014,32(3):249-264
3 González CA, Pera G, Agudo A, *et al*. Smoking and the risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer, 2003,107(4):629-634
4 Duell EJ, Travier N, Lujan-Barroso L, *et al*. Alcohol consumption and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Am J Clin Nutr, 2011,94(5):1266-1275
5 Zhu H, Yang X, Zhang C, *et al*. Red and processed meat intake is associated with higher gastric cancer risk: a

meta-analysis of epidemiological observational studies. PLoS One, 2013,8(8): e70955
6 D'Elia L, Galletti F, Strazzullo P. Dietary salt intake and risk of gastric cancer. Cancer Treat Res, 2014,159:83-95
7 Saeki N, Ono H, Sakamoto H, *et al*. Genetic factors related to gastric cancer susceptibility identified using a genome-wide association study. Cancer Sci, 2013,104(1):1-8
8 Dong LM, Potter JD, White E, *et al*. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. JAMA, 2008,299(20):2423-2436
9 McLean MH, El-Omar EM. Genetics of gastric cancer. Nat Rev Gastroenterol Hepatol, 2014,11(11):664-674
10 Zeng F, Harris RC. Epidermal growth factor, from gene organization to bedside. Semin Cell Dev Biol, 2014,28:2-11
11 Laurence DJ, Gusterson BA. The epidermal growth factor. A review of structural and functional relationships in the normal organism and in cancer cells. Tumour Biol, 1990,11(5):229-261
12 Carpenter G, Cohen S. Epidermal growth factor. J Biol Chem, 1990,265(14):7709-7712
13 Yamada A, Saito N, Kameoka S, *et al*. Clinical significance of epidermal growth factor (EGF) expression in gastric cancer. Hepatogastroenterology, 2007,54(76): 1049-1052
14 Costa BM, Ferreira P, Costa S, *et al*. Association between functional EGF +61 polymorphism and glioma risk. Clin Cancer Res, 2007,13(9):2621-2626
15 Lin L, Li G, Zhang Z, *et al*. Association of epidermal growth factor +61 A/G polymorphism in Chinese patients with colon cancer. Genet Test Mol Biomarkers, 2012,16(9):1142-1145
16 Abu Dayyeh BK, Yang M, Fuchs BC, *et al*. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology, 2011,141(1):141-149
17 Hamai Y, Matsumura S, Matsusaki K, *et al*. A single nucleotide polymorphism in the 5' untranslated region of the EGF gene is associated with occurrence and malignant progression of gastric cancer. Pathobiology, 2005, 72(3):133-138
18 Araújo AP, Costa BM, Pinto-Correia AL, *et al*. Association between EGF +61A/G polymorphism and gastric cancer in Caucasians. World J Gastroenterol, 2011, 17(4):488-492
19 Lin X. Wu G, Fu J. The relationship between EGF 61G/A Polymorphisms and gastric cancer risk. Med Front, 2012,(32):231-232
20 Zhan Z, Chen Y, Wu J, *et al*. Functional epidermal growth factor gene polymorphisms and risk of gastric cancer. Oncol Lett, 2013,5(2):631-636
21 Goto Y, Ando T, Goto H, *et al*. No association between EGF gene polymorphism and gastric cancer. Cancer Epidemiol Biomarkers Prev, 2005,14(10):2454-2456
22 Yang G, Rao L, Tian L, *et al*. An association between EGF and EGFR gene polymorphisms with gastric cancer in a Chinese Han population. Hepatogastroenterology, 2012,59(120):2668-2671
23 Jin G, Miao R, Deng Y, *et al*. Variant genotypes and haplotypes of the epidermal growth factor gene promoter are associated with a decreased risk of gastric cancer in a high-risk Chinese population. Cancer Sci, 2007,98(6):864-868
24 Piao Y, Liu Z, Ding Z, *et al*. EGF +61A>G polymorphism and gastrointestinal cancer risk: a HuGE review and meta-analysis. Gene, 2013,519(1):26-33
25 Wang J, Dong W, Guo X, *et al*. Association between polymorphisms of epidermal growth factor 61 and susceptibility of gastric cancer: A Meta-analysis. Chin J Cancer Prev Treat, 2014,21(2):150-153
26 Li TF, Ren KW, Liu PF. Meta-analysis of epidermal growth factor polymorphisms and cancer risk: involving 9,779 cases and 15,932 controls. DNA Cell Biol, 2012, 31(4):568-574
27 Zhang YM, Cao C, Liang K. Genetic polymorphism of epidermal growth factor 61A>G and and cancer risk: a meta-analysis. Cancer Epidemiol, 2010,34(2):150-156
28 Xu W, Li Y, Wang X, *et al*. Association between EGF promoter polymorphisms and cancer risk: a meta-analysis. Med Oncol, 2010,27(4):1389-1397
29 Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol, 2010,25(9): 603-605
30 Higgins JP, Thompson SG, Deeks JJ, *et al*. Measuring inconsistency in meta-analyses. BMJ, 2003,327(7414): 557-560
31 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med, 2002,21(11):1539-1558
32 Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst, 1959,22(4):719-748
33 Der Simonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials, 1986,7(3):177-188
34 Egger M, Davey Smith G, Schneider M, *et al*. Bias in meta-analysis detected by a simple, graphical test. BMJ, 1997,315(7109):629-634
35 Zhang J, Zhan Z, Wu J, *et al*. Relationship between EGF, TGFA, and EGFR gene polymorphisms and traditional Chinese medicine ZHENG in gastric cancer. Evid Based Complement Alternat Med, 2013,2013:1-13
36 Cacina C, Arikan S, Duzkoylu Y, *et al*. Investigation of EGF A61G gene variation and serum EGF level on gastric cancer susceptibility and clinicopathological parameters[abstract]. FEBS J, 2014,281:75-75
37 Kobayashi D, Nomoto S, Kodera Y, *et al*. Suppressor of cytokine signaling 4 detected as a novel gastric cancer suppressor gene using double combination array analysis. World J Surg, 2012,36(2): 362-372
38 Kang C, Song JJ, Lee J, *et al*. Epigenetics: an emerging player in gastric cancer. World J Gastroenterol, 2014, 20(21):6433-6447
39 Okuda T, Onda M, Tokunaga A, *et al*. Stimulatory effect of EGF and inhibitory effect of sialoadenectomy on growth of an EGF receptor-hyperproducinghuman gastric cancer xenograft in nude mice. Surg Today, 1994, 24(8):725-733
40 Yasui W, Takekura N, Kameda T, *et al*. Effect of epidermal growth factor on rat stomach carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine. Acta Pathol Jpn, 1990,40(3):165-171
41 Henson ES, Gibson SB. Surviving cell death through epidermal growth factor (EGF) signal transduction pathways: implications for cancer therapy. Cell Signal, 2006,18 (12):2089-2097

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