Turk J Gastroenterol *2017; 28: 272-82*

Gastrointestinal Tract

Role of TNF-α -308G/A gene polymorphism in gastric cancer risk: A case control study and meta-analysis

Li-Chuan Du, Ru Gao

Department of Gastroenterology, Beijing Chaoyang Hospital, Beijing, China

Cite this article as: Du LC, Gao R. Role of TNF-α -308G/A gene polymorphism in gastric cancer risk: a case control study and meta-analysis. Turk J Gastroenterol 2017; 28: 272-82.

ABSTRACT

Background/Aims: In the Chinese population, gastric cancer (GC) is ranked as the third most common type of cancer. Although the exact etiology of GC development is unclear, several factors, including genetic and environmental, have been identified as risk factors. Variations in cytokine genes and their receptors have been related to a higher risk of GC. A single nucleotide polymorphism in the promoter region of tumor necrosis factor-α (TNF-α) (-308G>A) has been associated with a higher risk of GC and in the present study we evaluated its possible association with GC in a Chinese cohort. In addition, we performed a meta-analysis to draw a firm conclusion about the association between TNF-α gene polymorphisms and GC.

Materials and Methods: We enrolled 400 Chinese GC patients and matched healthy controls hailing from similar geographical areas. The TNF-α -308G/A polymorphism was genotyped by allele-specific polymerase chain reaction (AS-PCR). For the meta-analysis, earlier published articles were searched and eligible studies were included.

Results: Prevalence of the heterozygous mutant (GA) and minor allele (A) were significantly higher in GC cases compared to healthy controls (GA: p<0.0001, odds ratio (OR)=4.90; A: p<0.0001, OR=2.84). A total of 36 eligible studies including the present report, encompassing of 8353 GC patients and 12099 controls, were analyzed for the meta-analysis. A significant association of the TNF-α polymorphism (-308G>A) with susceptibility to GC was only found in the Caucasian population (A vs G: p=0.001; AA vs GG: p=0.01; AG vs GG: p<0.0001; AA vs AG+GG: p=0.01; AA+AG vs p=0.003).

Conclusion: The results of the present case control study and meta-analysis showed that associations between TNF-a variants with susceptibility to GC development is population and ethnic specific.

Keywords: Gene polymorphism, TNF-α, gastric cancer, meta-analysis, association, Chinese

Original Article

INTRODUCTION

In China, gastric cancer (GC) ranks third as the most common form of cancer. In the year 2005, 400,000 new cases and 300,000 deaths due to GC in China have been reported (1). The age standardized incidence for men and women was 37.1 and 17.4, respectively, and the mortality rate was 32.7 for men and 15 for women in 2005 (1). The incidence of GC varies among different populations and ethnicity. Although the exact etiology of GC development is unclear, several factors such as host genetics and the environment are believed to play a major role in pathogenesis. The importance of diet including consuming fruit and vegetables, smoking habits, and alcohol consumption have been shown to modulate disease severity (2,3). Infection with *Helicobacter pylori* is also a major cause of non-cardia and chronic GC. Although a limited number of infected humans (<1%) develops GC, the contribution of *H. pylori* infection to GC cannot be ruled out because it enhances the development of chronic gastritis to GC through various clinical phenotypes (atrophic gastritis, intestinal metaplasia, and dysplasia) in a sequential manner.

Immune responses to *H. pylori* has been well investigated. Various cytokines produced in response to *H. pylori* infection are intended to clear the microbes. However,

Address for Correspondence: Ru Gao E-mail: rugao145@hotmail.com

Received: January 15, 2017 **Accepted:** March 24, 2017

 • Available online at www.turkjgastroenterol.org • DOI: 10.5152/tjg.2017.16741

a dual function of certain pro-inflammatory molecules, including tumor necrosis factor-α (TNF-α) have been demonstrated; optimum levels help in clearance of microbes and in contrast an excessive level is associated with chronic inflammation. A role of TNF-α in GC has been well characterized. Elevated TNF-α induces inflammation in gastric mucosa, one of the important steps toward GC development (4). Several *in vitro* studies have demonstrated the induction of TNF-α production by *H. pylori* and inhibition of gastric acid secretion reveling the importance of TNF-α in GC pathogenesis (5,6).

TNF-α is located on the small arm of chromosome 6. So far, 106 single nucleotide polymorphisms (SNPs) in *TNF-α* have been reported (https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=7124). However, certain gene polymorphisms in the regulatory region of *TNF-α*, which correlate with the plasma level of TNF-α, is point of interest for most researchers. Although several polymorphisms in the promoter region of *TNF-α* such as -238G>A, -308G>A, -857C>T, -863C>A, and -1031T>C have been shown to regulate TNF-α levels, the results are not consistent. An association of TNF-α -308G>A (rs1800629) with susceptibility/resistance to GC development has been widely investigated and yielded conflicting results. Studies including GC patients and controls from China (7-14), Brazil (15,16), Portugal (17,18), United States (19), Poland (20), South Korea (21-23), Honduras (24), Italy (25), Colombia (26), and Japan (27) have been associated with susceptibility to GC development. However, other studies including those from India (28), Brazilian (29,30), Romania (31), Spain (32,33), South Korea (34,35), China (36), Mexico (37), Germany (38) and Finland (39) failed to show a possible link between the *TNF-α* (-308G>A) polymorphism and GC. These observations highlight the necessity for a population-based investigation into the possible link between the *TNF-α* polymorphism (-308G>A) and GC. In the present study, we conducted a case control study followed by a meta-analysis to draw a firm conclusion on the role of this *TNF-α* polymorphism in GC.

MATERIALS AND METHODS

Patients and Controls

We enrolled 400 GC patients that were reported/referred to the Department Of Gastroenterology, Beijing Chaoyang Hospital, from 2012 to 2016. This study was approved by the ethics committee of Beijing Chaoyang Hospital, China (Approval No: TCX13461). All patients' diagnosis was confirmed endoscopically and histopathologically. A total of 400 healthy individuals from similar geographical areas without any history of gastric or any other form of cancer, gastritis, or gastric ulcers, were included as controls. Information about age, sex, smoking habits, and drinking habits were also collected from each participant. Approximately 5 mL of intravenous blood was collected from patients and controls. *H. pylori* infection status was screened by Enzyme Linked Immunosorbent Assay (ELISA) as instructed by the manufacturer. The protocol was approved by the Institutional Ethical Committee and written informed consent was obtained from all participants.

DNA Extraction and Genotyping of TNF-α (-308G>A) Polymorphism

DNA was extracted by using a QIAamp DNA Blood Mini Kit (QIAGEN, USA) according to the manufacturer's protocol. Extracted DNA was stored at -70 degree Celsius until used for genotyping. *TNF-α* -308G>A polymorphism was typed by AS-PCR as described previously (28,40). Around 20% of samples were chosen randomly and subjected to direct sequencing and those were found to be absolute concordant with the AS-PCR method.

Literature Search for Meta-Analysis

Two authors, LD and RG, independently searched various databases such as PubMed (Medline), EMBASE and Google Scholar with the following key words: 'Tumor Necrosis Factor' or *TNF-α* or TNF gene polymorphism and Gastric Cancer or GC (last updated on October 2016). Any discrepancy or disagreement about inclusion was resolved by group discussion. All extracted studies were investigated by their titles, abstracts, and we screened appropriate publications based on predetermined inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

The following inclusion-exclusion criteria were selected for the present study: a) all studies must be case-controls that investigated the relationship between *TNF-α* -308G>A polymorphism and GC; b) should include confirmed GC patients and appropriate controls; and d) must have reported genotype and allele frequency. Reports were excluded based upon the following crite-

Table 1. Distribution of TNF-α (-308G>A) polymorphism in gastric cancer patients and healthy controls

TNF-α -308G> A genotype and allele	HC (n=400)	GC (n=400)	p	OR (95% CI)
Genotype			ref	1
GG	326 (81.5)	204 (51)		
GA	60 (15)	184 (46)	<0.0001	4.90 (3.48 to 6.88)
AA	14 (3.5)	12 (3)	0.53	1.37 (0.62 to 3.02)
Allele				
G	712 (89)	592 (74)	ref	1
A	88 (11)	208 (26)	<0.0001	2.84 (2.16 to 3.73)

HC: healthy controls; GC: gastric cancer patients; OR: odds ratio; CI: confidence interval; TNF-α: tumor necrosis factor-α

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Table 2. Main characteristics of all studies included in the meta-analysis

First authors and year	Country	Ethnicity	Control	Cases	Type	Association
Present study	China	Asian	400	400	ASP	Yes
Bhayal et al. 2013 (28)	India	Asian	229	114	ARMS-PCR	No
Xu et al. 2016 (13)	China	Asian	319	296	RFLP-PCR	Yes
de Oliveira et al. 2015 (29)	Brazil	American	240	204	RFLP-PCR	No
Zabaglia et al. 2015 (16)	Brazil	American	40	24	RFLP-PCR	Yes
Yu et al. 2014 (14)	China	Asian	300	360	PCR	Yes
Hong et al. (test) 2013 (8)	China	Asian	750	834	TaqMan	Yes
Hong et al. (validation) 2013 (8)	China	Asian	936	1060	TaqMan	Yes
Burada et al. 2012 (31)	Romania	Caucassian	242	105	TaqMan	No
Canedo et al. 2008 (17)	Portugal	Caucassian	713	508	TaqMan	Yes
Crusius et al. 2008 (32)	Spain	Caucassian	1125	236	Real-time PCR	No
El-Omar et al. 2003 (19)	United States	Caucassian	210	314	TaqMan	Yes
Guo et al. 2005 (7)	China	Asian	437	264	RFLP-PCR	Yes
Jang et al. 2001 (34)	South Korea	Asian	92	52	RFLP-PCR	No
Fei et al. 2004 (36)	China	Asian	164	56	PCR	No
Garcia-Gonzalez et al. 2007 (33)	Spain	Caucassian	404	404	TaqMan	No
Garza-Gonzalez et al. 2005 (37)	Mexico	Caucassian	215	63	RFLP-PCR	No
Glas et al. 2004 (38)	Germany	Caucassian	145	88	RFLP-PCR	No
Hou et al. 2007 (20)	Poland	Caucassian	428	305	TaqMan	Yes
Kamangar et al. 2006 (39)	Finland	Caucassian	208	112	TaqMan	No
Kim et al. 2006 (21)	South Korea	Asian	461	237	RFLP-PCR	Yes
Lee et al. 2004 (22)	South Korea	Asian	261	341	PCR	Yes
Lee et al. 2005 (35)	South Korea	Asian	120	122	RFLP-PCR	No
Li et al. 2005 (9)	China	Asian	264	59	RFLP-PCR	Yes
Lu et al. 2005 (10)	China	Asian	300	250	DHPLC-PCR	Yes
Machado et al. 2003 (18)	Portugal	Caucassian	304	287	SSCP-PCR	Yes
Melo et al. 2009 (15)	Brazil	Caucassian	100	30	RFLP-PCR	Yes
Morgan et al. 2006 (24)	Honduras	Caucassian	161	168	TaqMan	Yes
Perri et al. 2005 (25)	Italy	Caucassian	362	184	RFLP-PCR	Yes
Rocha et al. 2005 (30)	Brazil	Caucassian	535	161	RFLP-PCR	No
Sugimoto et al. 2007 (27)	Japan	Asian	172	105	RFLP-PCR	Yes
Torres et al. 2004 (26)	Colombia	Caucassian	66	44	PCR	Yes
Wu et al. 2002 (12)	China	Asian	220	150	Direct Sequencing	Yes
Wu et al. 2004 (11)	China	Asian	210	204	Direct Sequencing	Yes
Yang et al. 2009 (23)	South Korea	Asian	322	83	SNaPshot	Yes
Zambon et al. 2005 (52)	Italy	Caucassian	644	129	TaqMan	Yes

PCR: polymerase chain reaction; ASP: allele specific PCR; RFLP: restriction fragment length polymorphism

ria: a) duplicated or overlapping publication, b) study involving only GC cases and devoid of controls, c) without genotype or allele frequency data, d) a review, abstract, or case report.

Data Extraction

Data from each eligible study were extracted such as publication year, first author name, sampling area, ethnicity, source of

Table 3. Genotypic distribution of the TNF-308G/A gene polymorphism included in the meta-analysis

	Controls				Cases				HWE
Authors and year (ref)	**GG**	**GA**	**AA**	**MAF**	**GG**	**GA**	**AA**	**MAF**	**p**
Present Study	326	60	14	0.22	204	184	12	0.26	0.000
Bhayal et al. 2013 (28)	76	128	25	0.388	32	76	6	0.385	0.007
de Oliveira et al. 2015 (29)	167	69	4	0.160	138	63	3	0.169	0.296
Yu et al. 2014 (14)	251	38	11	0.1	325	6	29	0.088	0.000
Hong et al. (test) 2013 (8)	589	154	7	0.112	690	139	5	0.089	0.376
Hong et al. (validation) 2013 2013 (8)	746	179	11	0.107	895	156	9	0.082	0.943
Xu et al. 2016 (13)	237	50	32	0.178	142	66	88	0.408	0.000
Zabaglia et al. 2015 (16)	33	4	3	0.125	17	4	3	0.208	0.000
Burada et al. 2012 (31)	196	44	2	0.099	78	26	1	0.133	0.784
Canedo et al. 2008 (17)	544	169	0	0.118	330	178	0	0.175	0.000
Crusius et al. 2008 (32)	820	274	31	0.149	170	64	2	0.144	0.165
El-Omar et al. 2003 (19)	152	52	6	0.152	201	87	26	0.221	0.548
Gou et al. 2005 (7)	391	40	6	0.059	240	20	4	0.053	0.000
Jang et al. 2001 (34)	85	7	0	0.038	46	4	2	0.076	0.704
Fei et al. 2004 (36)	143	20	1	0.067	53	3	0	0.026	0.743
Garcia-Gonzalez et al. 2007 (33)	320	77	7	0.112	309	84	11	0.131	0.350
Garza-Gonzalez et al. 2005 (37)	1	35	179	0.913	0	8	55	0.936	0.607
Glas et al. 2004 (38)	105	36	4	0.151	66	19	3	0.142	0.669
Hou et al. 2007 (20)	304	109	15	0.162	186	98	21	0.229	0.186
Kamangar et al. 2006 (39)	154	52	2	0.134	86	23	3	0.129	0.292
Kim et al. 2006 (21)	400	59	2	0.068	199	34	4	0.088	0.911
Lee et al. 2004 (22)	218	42	1	0.084	297	43	1	0.065	0.493
Lee et al. 2005 (35)	103	17	0	0.070	112	10	0	0.040	0.403
Li et al. 2005 (9)	228	34	2	0.071	55	4	0	0.033	0.559
Lu et al. 2005 (10)	274	24	2	0.046	214	36	0	0.072	0.080
Machado et al. 2003 (18)	231	69	4	0.126	179	105	3	0.193	0.649
Melo et al. 2009 (15)	86	13	1	0.075	24	5	1	0.116	0.528
Morgan et al. 2006 (24)	149	12	0	0.037	151	17	0	0.050	0.623
Perri et al. 2005 (25)	290	65	7	0.109	152	30	2	0.092	0.145
Rocha et al. 2005 (30)	399	123	13	0.139	120	37	4	0.139	0.343
Sugimoto et al. 2007 (27)	169	3	0	0.008	101	4	0	0.019	0.908
Torres et al. 2004 (26)	56	10	0	0.075	41	3	0	0.034	0.505
Wu et al. 2002 (12)	180	27	13	0.120	114	27	9	0.15	0.000
Wu et al. 2004 (11)	171	26	13	0.123	163	29	12	0.129	0.000
Yang et al. 2009 (23)	288	34	0	0.052	75	8	0	0.048	0.317
Zambon et al. 2005 (52)	496	138	10	0.122	95	31	3	0.143	0.909

HWE: Hardy Weinberg Equilibrium; MAF: minor allele frequency

samples, number of cases and controls, genotype frequencies, and reported associations. Disagreements or discrepancies were resolved by discussion.

Statistical Analysis

The genotype and allele frequency was calculated by direct counting and their distributions among cases and controls were

analyzed by Fisher's exact test. P<0.05 was defined as statistically significant. For meta-analysis, Comprehensive meta-analysis (CMA) V.2 was employed for calculation of pooled odds ratios (ORs) and 95% confidence interval (CIs). Heterogeneity among included studies for meta-analysis was analyzed by the Q-test and I^2 statistics. I^2 values range from 0% to 100%, where a value of 0% indicates no significant observed heterogeneity and larger values indicate an increasing degree of heterogeneity. Based on the heterogeneity results, a random or fixed-effects model was employed for derivation of pooled odds ratio, p value, and 95% CI. Publication bias was investigated by Egger's regression analysis and construction of funnel plots.

RESULTS

Baseline Characteristics of Patients and Controls

Out of 400 GC patients, 70% (n=284) were men and 30% (n=116) were women. Since the present study was a matched case-control study, we enrolled a similar number of health men and women as controls. The mean age of GC patients and healthy controls were 56.3 and 54.5 years, respectively. Smoking and drinking alcohol habits of both patients and controls were comparable (data not shown). *H. pylori* infection was more prevalent in GC cases compared to controls.

Association of -308G>A Polymorphism with Gastric Cancer

TNF-α -308G>A polymorphism was genotyped by AS-PCR. To explore any relationship between the promoter -308G>A polymorphism and GC, allele and genotype distributions were compared among patients and healthy controls. As shown in Table 1, heterozygous mutant (GA) and minor alleles (A) were more prevalent in GC cases compared to healthy subjects (GA: p<0.0001, OR= 4.90, 95% CI= 3.48 to 6.88; A: p<0.0001, OR=2.84, 95% CI=2.16 to 3.73) indicating a possible association between the *TNF-α* -308G>A variant and GC susceptibility.

Studies Included in the Meta-Analysis

A meta-analysis is a powerful tool that pools similar studies to draw a firm conclusion. In the primary search with various online tools, we obtained 238 articles and after detailed evaluation of the titles, abstracts, removal of duplicates, and careful reading of the full text of the articles, 35 eligible articles were screened for the meta-analysis. Furthermore, data from the present study was also included leading to a total of 36 research publications, including 8353 confirmed GC patients and 12099 control subjects. The major characteristics of the selected studies are shown in Table 2. Other relevant extracted data such as genotype and minor allele frequency and Hardy-Weinberg equilibrium (HWE) probability values of control genotypes are shown Table 3. Out of 36 studies included for the present meta-analysis, the genotype distribution in eight studies deviated from HWE.

Sensitivity Analysis

Sensitivity analysis was performed by eliminating each individual study to investigate their effect on the pooled result. The results of the sensitivity analysis revealed that none of the included studies disproportionately influenced the results of the meta-analysis (data not shown).

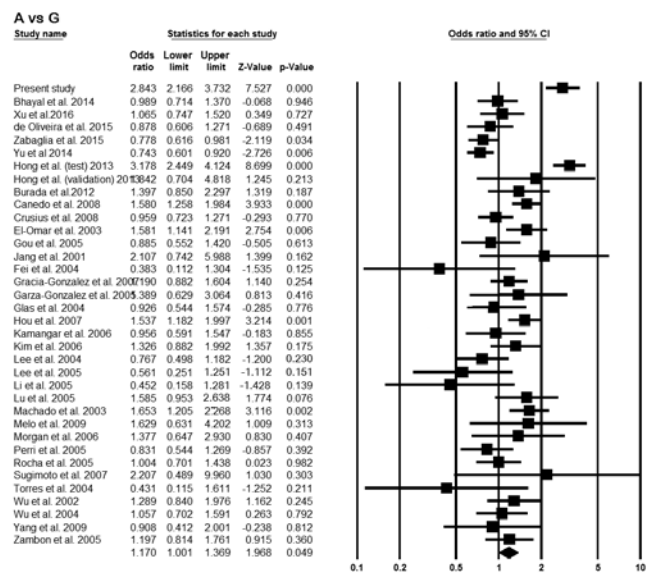
Results of the Meta-Analysis

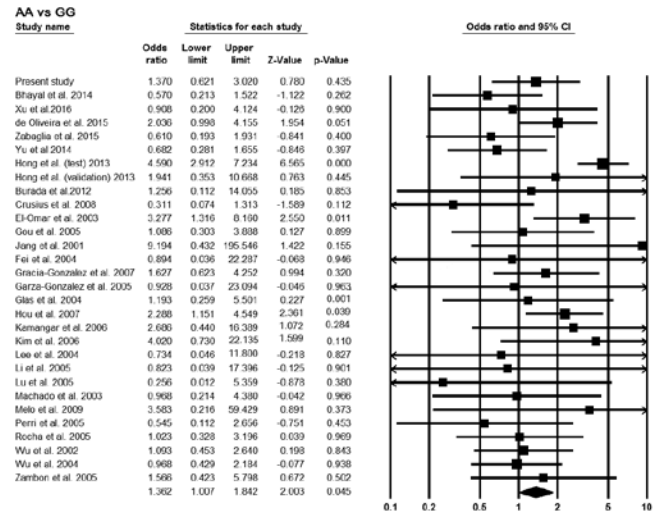
A total of 36 case-control studies including 12099 controls and 8353 confirmed GC cases were included in this meta-analysis. The Q test and I^2 statistics revealed heterogeneity among the included studies and thus a random-effects model was employed for allele and genotype comparison (Table 4). As shown in Figure 1, the allele (A vs G) and genotype comparison (AA vs GG) model showed a statistical significant role of the *TNF-α* -308G>A polymorphism in susceptibility to GC (A vs. G: p=0.04; AA vs. GG: p=0.04). However, other genetic comparison models failed to show a possible association (AG vs. GG: p=0.24; AA+AG vs. GG: p=0.09; AA vs. GG+AG: p=0.08).

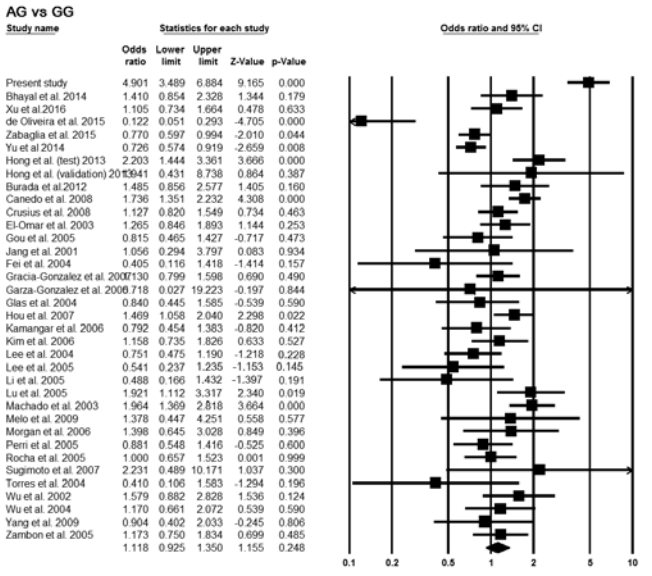
Analysis based on ethnic group has been advised for meta-analysis; thus, in the present study we grouped all published literature including the present report into two broad groups based on ethnicity. a) Asian and b) Caucasian. A total of 18 case-control studies were from Asian ethnic backgrounds comprising 5957 controls and 4987 GC cases. Egger's regression analysis showed an absence of publication bias and all comparison models revealed heterogeneity among the included studies; thus, we employed a random-effects model for construction of a forest plot (Table 5). As shown in Figure 2, meta-analysis failed to show any possible association of the *TNF-α* -308G>A polymorphism with GC development in Asian ethnic groups (A

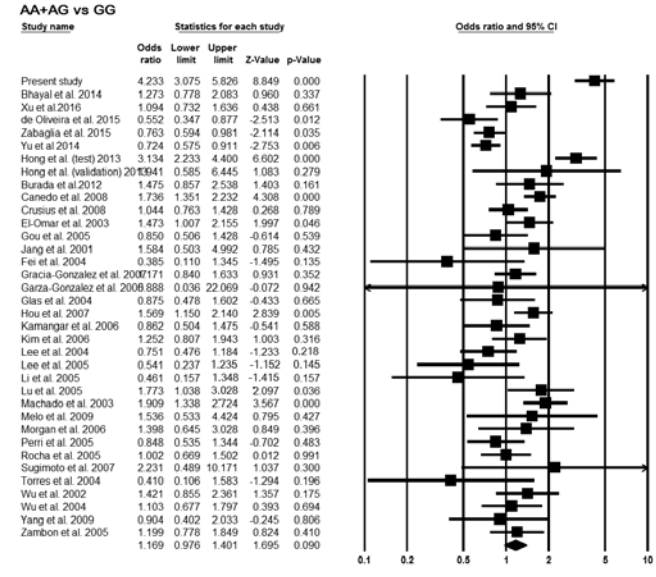
Table 4. Statistics to test publication bias and heterogeneity in the meta-analysis

	Egger's regression analysis			Heterogeneity analysis			
Comparisons	**Intercept**	**95% Confidence Interval**	**p**	**Q**	**$P_{heterogeneity}$**	**I^2 (%)**	**Model used for meta-analysis**
A vs G	-0.75	-2.54 to 1.04	0.40	176.88	0.000	80.21	Random
AA vs GG	-1.17	-2.14 to -0.20	0.01	51.21	0.007	43.37	Random
AG vs GG	-0.77	-2.47 to 0.92	0.35	176.07	0.000	80.12	Random
AA+AG vs GG	-0.59	-2.38 to 1.19	0.50	180.95	0.000	80.65	Random
AA vs AG+GG	-1.05	-2.06 to -0.04	0.04	50.21	0.009	42.25	Random









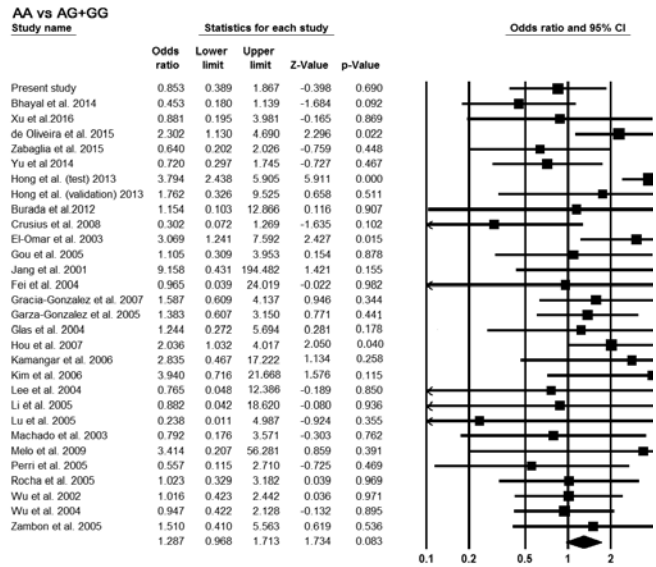


Figure 1. Forest plots of GC risk associated with the TNF-α −308G>A polymorphism (Overall). A total of 36 studies were included in the present meta-analysis. A random-effects model was used for calculation of a combined effect for all studies. The square and horizontal line represent the odds ratio and 95% confidence interval of each study. The square height denotes the weight of the study. The filled diamond represents the combined odds ratio and 95% confidence interval

GC: gastric cancer

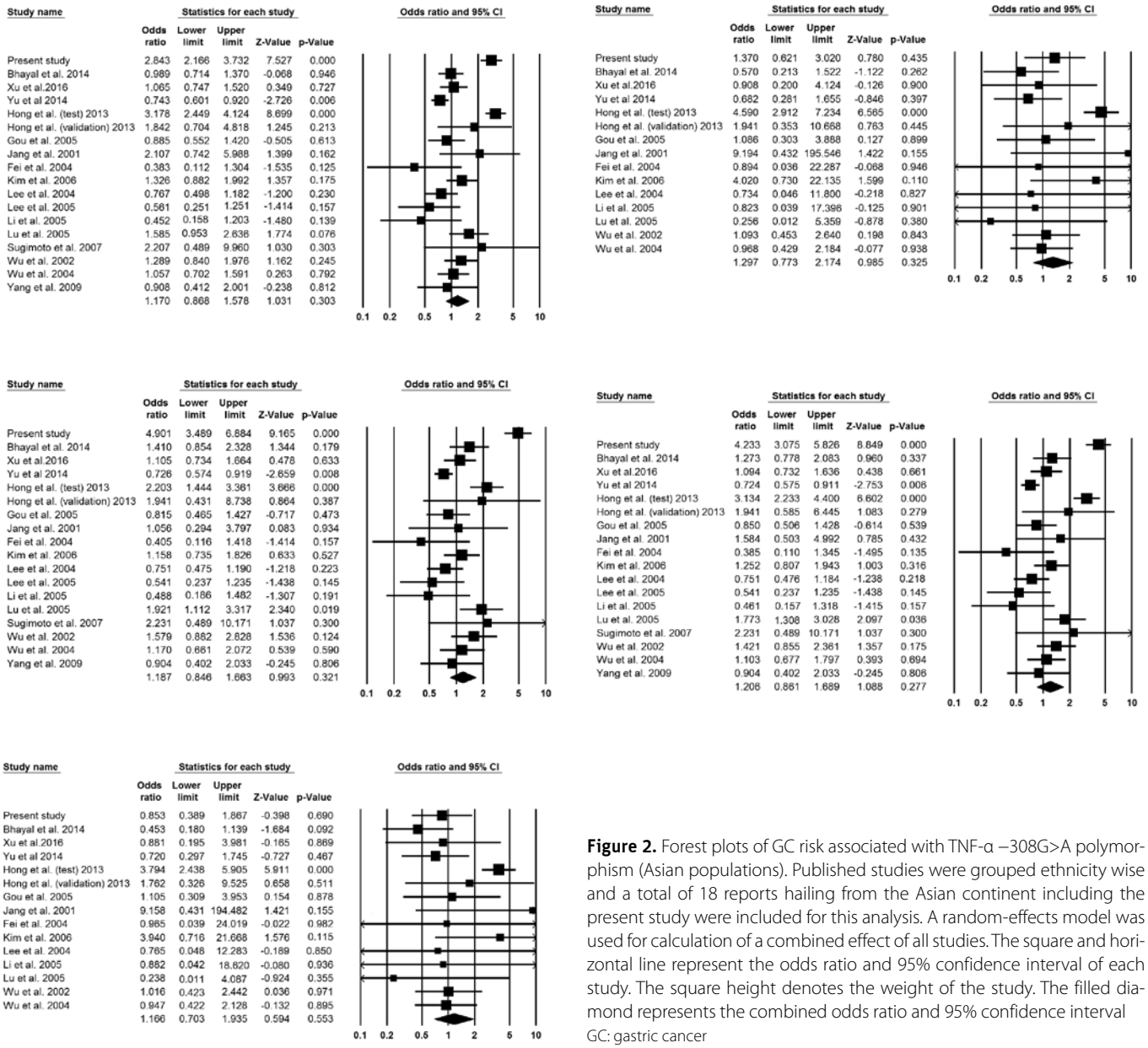


Figure 2. Forest plots of GC risk associated with TNF-α −308G>A polymorphism (Asian populations). Published studies were grouped ethnicity wise and a total of 18 reports hailing from the Asian continent including the present study were included for this analysis. A random-effects model was used for calculation of a combined effect of all studies. The square and horizontal line represent the odds ratio and 95% confidence interval of each study. The square height denotes the weight of the study. The filled diamond represents the combined odds ratio and 95% confidence interval
GC: gastric cancer

Table 5. Statistics to test publication bias and heterogeneity in meta-analysis (Asian)

	Egger's regression analysis			Heterogeneity analysis			
Comparisons	**Intercept**	**95% Confidence Interval**	**p**	**Q**	**$P_{heterogeneity}$**	**I^2 (%)**	**Model used for meta-analysis**
A vs G	-1.04	-4.08 to 1.98	0.47	131.65	0.000	87.08	Random
AA vs GG	-1.24	-2.83 to 0.34	0.11	35.73	0.001	60.82	Random
AG vs GG	-0.29	-3.20 to 2.62	0.83	111.60	0.000	84.76	Random
AA+AG vs GG	-0.64	-3.76 to 2.46	0.66	127.23	0.000	86.63	Random
AA vs AG+GG	-1.06	-2.66 to 0.54	0.17	35.16	0.001	60.19	Random

vs. G: p=0.30; AA vs. GG: p=0.32; AG vs. GG: p=0.32; AA+AG vs. GG: p=0.27; AA vs. GG+AG: p=0.55).

Sixteen studies comprising 5862 controls and 3138 GC patients were from a Caucasian background. Egger's regression analysis revealed no publication bias in the studies considered for meta-analysis; however, two genetic models showed (A vs. G and AA+AG vs. GG) heterogeneity among the included studies (Table 6). Based on the Q statistics and I^2 value, we used a random-effects model for calculation of an OR and 95% CI. As

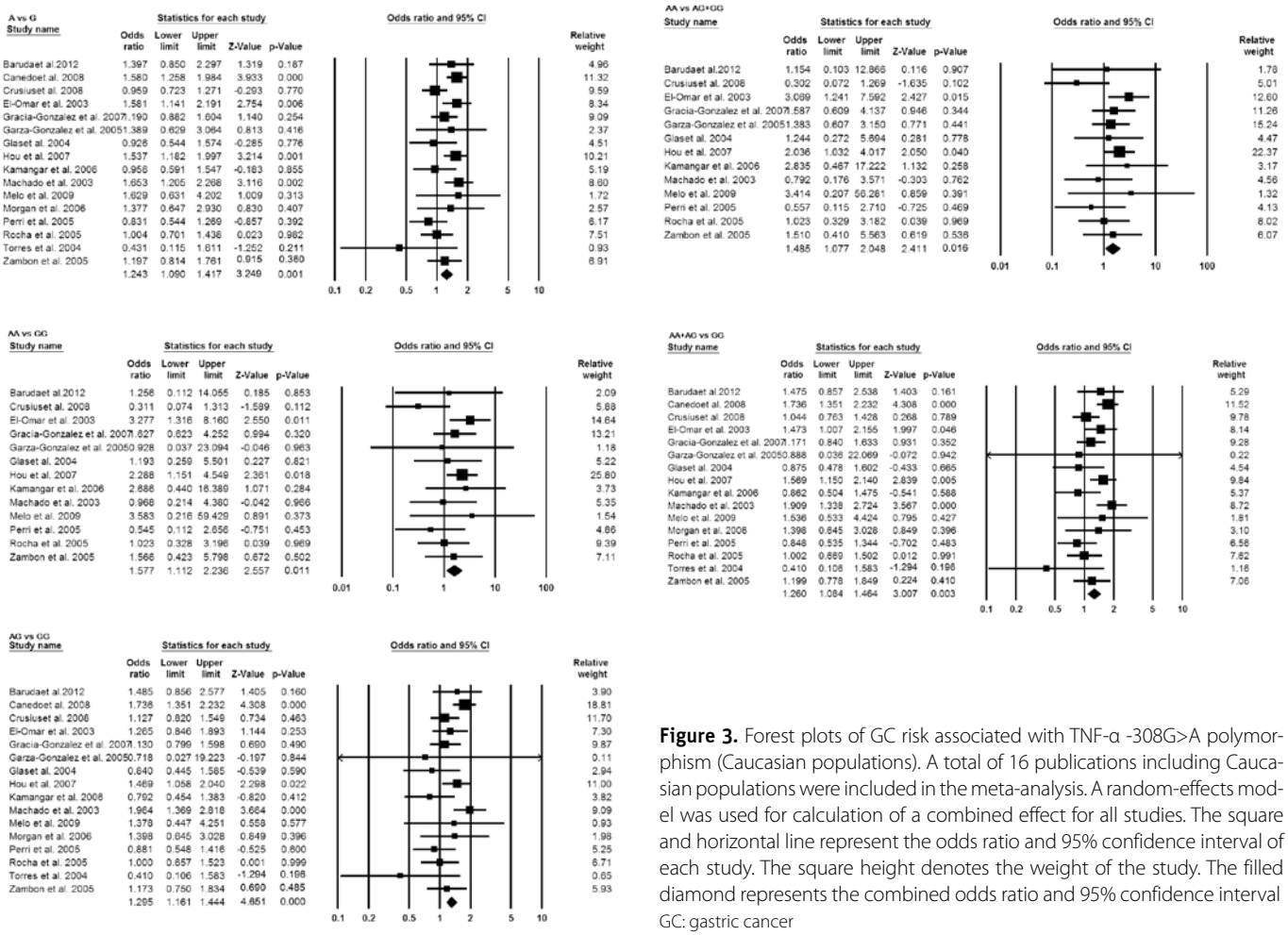


Figure 3. Forest plots of GC risk associated with TNF-α -308G>A polymorphism (Caucasian populations). A total of 16 publications including Caucasian populations were included in the meta-analysis. A random-effects model was used for calculation of a combined effect for all studies. The square and horizontal line represent the odds ratio and 95% confidence interval of each study. The square height denotes the weight of the study. The filled diamond represents the combined odds ratio and 95% confidence interval
GC: gastric cancer

Table 6. Statistics to test publication bias and heterogeneity in the meta-analysis (Caucasians)

	Egger's regression analysis			Heterogeneity analysis			
Comparisons	**Intercept**	**95% Confidence interval**	**p**	**Q**	**$P_{heterogeneity}$**	**I^2 (%)**	**Model used for meta-analysis**
A vs G	-1.19	-2.94 to 0.54	0.16	25.31	0.04	40.74	Random
AA vs GG	-0.97	-2.48 to 0.53	0.18	12.09	0.43	0.78	Fixed
AG vs GG	-1.43	-2.93 to 0.05	0.05	24.40	0.06	38.74	Fixed
AA+AG vs GG	-1.30	-2.90 to 0.29	0.10	25.77	0.04	41.80	Random
AA vs AG+GG	-0.89	-2.52 to 0.72	0.24	11.55	0.48	0.00	Fixed

shown in Figure 3, the *TNF-α* -308G>A polymorphism was significantly associated with GC in all genetic comparison models (A vs. G: p=0.001; AA vs. GG: p=0.01; AG vs. GG: p=0.00; AA+AG vs. GG: p=0.003; AA vs. GG+AG: p=0.01).

DISCUSSION

In the present investigation, a common polymorphism (-308G>A) in *TNF-α* was genotyped in a Chinese cohort and its association with development of GC was investigated. In addition, we searched the previous published literature on for the association of this polymorphism with GC susceptibility and performed a meta-analysis, including data from the present study. The results of the hospital-based case-control study revealed an association between heterozygous mutants and minor allele with a susceptibility to GC. Furthermore, the meta-analysis showed a link between TNF-α (-308G>A) variants with GC predisposition.

Several reports on the distribution of the *TNF-α* (-308G>A) genotype in healthy controls have been reported for Chinese populations. In the present study, controls were recruited from Beijing and genotyping data found 15% had heterozygous mutations and 3.5% had homozygous mutations. This observation is comparable with previous reports including healthy

controls from Hubei province (9,36), Henan province (13), Taiwan (11,12) and Nanjing (8). However, other reports from Beijing (10) and Hubei province (7) showed a lower prevalence of heterozygous mutations (8-9%). Interestingly, the distribution of *TNF-α* (-308G>A) genotypes deviated from HWE in all earlier reports including the present study in a Chinese population (7-13,36). Deviation of a genotype distribution from HWE has been attributed to genotyping error, population stratification, or selection pressure. The present study and earlier reports enrolled controls from an ethnic group and employed robust genotyping methods (7-13,36). Deviation from HWE is possibly due to prevalence of various infectious diseases in Chinese populations, which applies selection pressure on the human genome (41).

Various studies have been conducted in different populations of China to identify a possible association of *TNF-α* -308G>A with the development of GC (7-13,36). Most of the studies showed an association of variants with susceptibility to GC. However, a study including patients and controls from Hubei province failed to demonstrate such an association. Consistent with earlier observations, we observed a significant association of heterozygotes and minor allele with GC susceptibility.

The exact mechanism whereby *TNF-α* -308G>A variants are predisposed to GC is unclear. The minor allele for *TNF-α* (-308G>A) polymorphism increases binding of transcription factors and elevated mRNA production compared to the major allele (G) (42,43). *In vitro* stimulation of peripheral blood mononuclear cells (PBMC) derived from heterozygous subjects (GA) with lipopolysaccharide displayed higher levels of TNF-α than those of wild type individuals (GG) (44). This minor allele is possibly linked with a higher transcription rate and that may induce a higher rate of inflammation in subjects harboring the minor allele (45). Furthermore, higher levels of TNF-α inhibit secretion of gastric acid and that may lead to spreading of *H. pylori* to the corpus and subsequently development of GC (6).

Meta-analysis is a powerful investigative method that combines similar studies to draw a firm conclusion. Since the association of the *TNF-α* (-308G>A) polymorphism with a predisposition to GC is unclear, a meta-analysis was performed combining earlier reports with the data of the present study. Results of the meta-analysis revealed a significant association of the *TNF-α* (-308G>A) polymorphism with GC susceptibility. Overall, the analysis showed subjects with the minor allele (A) or homozygous mutation (AA) had a 1.17 and 1.36 fold higher chance of development of GC, respectively. These observations are consistent with earlier meta-analyses (46,47). In addition, we observed an association between *TNF-α* polymorphisms with GC susceptibility in Caucasians but not in Asian ethnic groups, indicating a race-specific link between *TNF-α* -308G>A and GC susceptibility. Several reports have shown ethnic-specific genetic associations of variants with diseases and it has been advised to investigate genetic associations being aware of ethnicity.

Consistent with this, a recent study showed an association for a *RAD51* 135G>C substitution with susceptibility to breast cancer in Caucasians but not in East-Asians (48). Earlier meta-analyses by two different group also reported similar observations in the year 2014 (46,49). In addition, several reports including only Caucasian ethnic group for analysis demonstrated a possible association with GC susceptibility, further strengthening our observations (50-52). However, the very first meta-analysis including only 15 studies had shown an opposite association, i.e., *TNF-α* -308G>A variants were linked with GC susceptibility in Asians but not in Caucasians (47). Our present meta-analysis has several advantages over previous reports. The most recent meta-analyses were reported in the year 2014 (46,49,51). In the present study, five recent case-control studies investigating the role of *TNF-α* -308G>A in GC susceptibility were included, leading to an analysis including a much larger number of cases (n=8353) and controls (n=12099) (13,14,16,28,29).

There are several discrepancies between the results of the present case-control study and the results of the meta-analysis. First, the present case-control study revealed a significant association of heterozygous mutants (GA) with susceptibility to GC but the combined meta-analysis in the Caucasian population showed susceptibility of homozygous mutants to GC development. One possible explanation could be a lower prevalence of homozygous mutants in the studied population (3-3.5%). Because the meta-analysis combined several similar studies from different populations, the total number of studied subjects increased and possibly attained significant power to show an association, if any. Second, heterozygous mutants were associated with susceptibility to GC in the Chinese population but the meta-analysis failed to show any such link between the TNF-α genotype and GC in the Asian population. Because the Asian population included in the meta-analysis consist of various studies reported from China (n=11), India (n=1), North Korea (n=3), South Korea (n=1), and Japan (n=1), these diverse sample origins may be the reason for the discordant observations between the case-control study and the meta-analysis. Interestingly, out of 11 Chinese studies enrolled in the present meta-analysis, only one report failed to demonstrate an association between the *TNF-α* -308G>A polymorphism and GC.

In conclusion, *TNF-α* heterozygous and minor alleles are associated with susceptibility to GC in the studied Chinese population. However, a combined meta-analysis and studies on Asian subjects failed to demonstrate a possible association of the *TNF-α* allele with GC development. Interestingly, meta-analysis

of the *TNF-α* (-08G>A) polymorphism in Caucasians revealed a significant association with GC. We conclude that the *TNF-α* (-308G>A) polymorphism is linked to a GC predisposition in a population-specific manner. In future, studies from different populations including larger samples size are essential to establish a role of TNF-α in GC.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Beijing Chaoyang Hospital, China (Decision No: TCX13461).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - L.D., R.G.; Design - L.D., R.G.; Supervision - R.G.; Resource - L.D., R.G.; Materials - L.D., R.D.; Data Collection and/or Processing - L.D., R.G.; Literature Search - L.D., R.G.; Writing - L.D., R.G.; Critical Reviews - L.D., R.G.

Acknowledgements: Authors would like to thank patients and controls participated in the present study.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by Beijing Chaoyang Hospital (Project No: YX132267).

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