Tumour necrosis factor-α(-308) polymorphism and the risk of gastric cancer: A meta- analysis and trial sequential analysis

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Abstract

Background: Numerous studies have reported that polymorphisms in the tumour necrosis factor-alpha (TNF-a-308) gene are implicated in susceptibility to gastric cancer. However, individual genetic association studies that assessed the relationship between TNF-a- 308 and the risk of gastric cancer showed inconclusive results. The objective of this study was to synthesis evidence on the association between polymorphisms in the TNF-a-308 and gastric cancer risk. Methods: This is a meta-analysis of genetic association studies. We searched relevant case-control studies, assessing TNF- 308 polymorphisms and gastric cancer in health-related electronic databases. The methodological quality of included studies was assessed by the Newcastle-Ottawa quality assessment scale. The strength of association was calculated as odds ratios (ORs) with its 95% confidence intervals (CIs). Pooled ORs and 95 % CIs were estimated using random-effect model or fixed effect model, based on between-study heterogeneity. We analysed the strength of association under dominant, recessive, additive and allele models. Multiple subgroup analyses including ethnic groups, HWE status, study quality were done for robustness of the estimates. Publication bias was detected by inspection of funnel plot asymmetry. Results: A total of 33 studies, comprising 7695 patients and 12327 controls were identified. Based on the studies that met HWE, significant association was found between this polymorphisms and gastric cancer risk under dominant model (OR 1.2, 95%CI 1.1-1.3, I 2 :37%), recessive model OR 1.27, 95%CI 1.0-1.62, I 2 :0%) and additive model (OR 1.31, 95%CI 1.08-1.32, I 2 :0%). The TSA plot indicated the analyses was with the required information size. There was no publication bias. In the subgroup analysis by ethnicity, the ethnic groups and the quality of studies had impact on the estimates. Conclusions: The findings suggest that TNF-α-308 gene polymorphism plays an important role as host genetic factor predisposing to gastric carcinogenesis, and it would be useful for a screening marker.

Background

Gastric cancer (GC) is globally the fifth most common cancer with the highest prevalence in both sexes in Asia (74.5%) and the third leading cause of cancer death In 2018 alone, the estimated number of deaths attributed to GC was 782,685. On gender-specific, GC is the fourth most common types of cancer occurred in men and the seventh most common cancer in women [1]. As such, a greater understanding of the risk factors that play a role in the early and late stages of gastric carcinogenesis is important to strengthen and improve the preventive and therapeutic interventions [2].

Epidemiologic studies showed that GC is a multifactorial in aetiology [3, 4], in which dietary factors and *Helicobacter pylori* infection may contributed to the development of GC [3]. Of note is that a high prevalence of these risk factors do not always correspond to a high incidence of GC, suggesting that other susceptible factors such as genetic variations and environmental differences may play a role in the gastric carcinogenesis [5]. For instance, *H. pylori* infection is observed in more than half of the world's population, however there is only 1-2% of the infected population will develop GC in their lifetime [6].

Gastric neoplasms are composed not only with cancer cells but also other "non-cancer" compartments (including immune cells), which are the major players in GC disease progression and aggressiveness [7]. Tumour necrotic factor (TNF) is a pro-inflammatory cytokine, which is produced mainly by the immune cells such as macrophages, dendritic cells, lymphocytes and mast cells [8]. The tumour microenvironment, which is mainly composed of inflammatory cells, is a crucial player in the neoplastic process, fostering proliferation, survival and migration [9]. Thus, TNF-α may through inflammation, act as a tumour promoter. Individual studies reported the significant relationship between TNF- α -308 (rs 1800629) and the risk of GC [10, 11]. However, other studies reported differently [12, 13, 14]. On the whole, the objective of this study was to summarize the evidence of association between TNF-α-308 and the risk of GC.

Methods

Study search

The search of relevant studies was performed in the health-related databases of PubMed, Ovid Medline, google scholar and web of science, using the terms ("gastric cancer" OR "gastric carcinoma" OR "stomach cancer" OR gastric adenocarcinoma AND "tumour necrosis factor- alpha-308" OR "rs1800629" OR "TNF-α-308 G>A" OR "TNF-α-307 G>A"). The search strategy in PubMed database is provided (Additional File 1: Table S1).

The search was limited to the publications in English until June 2019. Moreover, we searched manually the references of included studies and relevant systematic reviews for any additional studies.

Inclusion criteria

Human studies that assessed GC, irrespective of location or histological type were included, if they (i) assessed TNF-α-308 G>A (rs 1800629) or TNF-α-307 G>A; (ii) were case-control design (retrospective or nested case-control) with an outcome of GC risk; (iii) provided the genotype frequency in cases and controls, (iv) mentioned sufficient data to compute odds ratio (OR) and its 95% confidence interval (CI) as the outcome measure. GC is as defined in the primary studies.

Studies which did not meet the inclusion criteria were excluded. Studies done on family or sibling-pairs were also excluded.

Data extraction

One investigator screened the titles and abstracts and selected the relevant full-text articles, following the inclusion criteria. Two investigators extracted the data from each study independently, by using a piloted data extraction form. Information collected included: first author, publication year, country, study setting, the number of cases/controls, ethnicity (Asian or Caucasians), method of genotyping and genotype/allele frequencies in cases/controls. If an allele frequency was zero in both case and control, we added 1 to that allele, following the Laplace approximation [15]. Any discrepancy between the two investigators were resolved by consensus.

Assessment of the methodology quality

The two investigators independently evaluated the methodological quality of studies, using the Newcastle-Ottawa Scale (NOS) [16]. The assessment is based on the three factors such as 'selection of the study groups' (4 points), 'comparability of the groups' (2 points) and 'ascertainment of the exposure' (3 points). The total score for each study spanned from 0 (the worst) to 9 (the best). We categorised study quality as good (≥7), moderate (≥5) and poor (≤4), according to scores achieved. Any discrepancy between the two investigators was resolved by consensus.

Statistical analysis

We assessed an evidence of HWE in the control populations in the included studies using the goodness-of-fit test and p>0.05 was considered to indicate consistency with HWE [17]. As described elsewhere [18], the strength of the association between TNF-α-308 G>A and the risk of GC in each study was estimated using OR and its 95% CI. Between- study heterogeneity was determined with the I^2 test. The I^2 test values indicate the percentage of total variation across studies attributed to the heterogeneity rather than chance. I^2 values >50% is regarded as a substantial heterogeneity [19]. For pooling of the estimates, the summary ORs and its 95% CIs were calculated with the random-effect model (The Der Simonian and Laird method) in the presence of statistical heterogeneity of the studies. Otherwise, we used the fixed-effect model. We calculated the summary ORs and its 95% CIs in four genetic models: the allelic contrast model (A vs G), the dominant model (AA+GA vs GG), the recessive model (AA vs GA+GG), and the additive model (AA vs GG). In order to investigate the source of heterogeneity, several subgroup analyses were conducted under the dominant model such as the ethnicity, study quality. Moreover, a subset analysis was done on the gene frequency distribution by location or histological type of GC. As a sensitivity analysis, we reassessed the relationship between TNF- a 308 and the risk of GC in all 4 genetic models, after removal of studies that deviated from HWE The publication bias was assessed by visual inspection of funnel plots under dominant model [20, 21].

Trial sequential analysis (TSA)

To estimate the required information size, we performed trial sequential analysis (TSA) [22]. It is classified as 'firm evidence of effect' or 'potentially spurious evidence of effect', which depends on whether the cumulative Z-curve cross the monitoring boundaries or not [23]. Meta-analysis was done with RevMan 5.3 (The Cochrane collaboration, Copenhagen) and *metan* command in Stata 14 (Stata Corp, Txt). TSA plot was done with TSA software (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen).

Results

Study search results

Figure 1 illustrates a four-phase study selection process. The initial search yielded a total of 1322 records. After removing the duplicates and screening of abstracts, 43 full-text articles that were potentially eligible were retrieved. We included a final of 33 studies (with

7695 cases and 12327 controls) in this review [2 ,5, 10-14, 24-49]. Summary of the 10 excluded studies were provided (Additional File 2: Table S2).

Study characteristics

Table 1 shows the characteristics of the studies identified. Of 33 studies included, slightly more than half (54.5%, 18/33) were done in the Asian region. The most frequent 5 studies were conducted in China, South Korea or Brazil. Figure 2 shows geographical distribution of the studies included. The years of publication were from 2001 to 2017. The participants were adults with male predominance in all 33 studies. In total, 82% of the studies were consistency with HWE in genotype distribution of the controls.

Regarding the methodology, the majority (78.8%) were categorized as high (14 studies) or moderate quality (12 studies) and the remaining 7 studies were of low quality. Of total 33 studies, 8 studies (24%) were gastric adenocarcinoma stage. By anatomical locations or histological types of GC, only 5 studies (7 datasets) consistently provided data that could make pooling of analysis (2,10,13,35,45). Twelve studies (36.4%) used TaqMan method for genotyping. In total, 24 studies (72.7%) had provided information that GC cases were infected with *H. pylori* infection, albeit with variation in distribution. For instance, all cases (100%) were infected with *H. pylori* in one study [33], while this was only 46% in another study [2] (Table 1). The remaining 9 studies did not measure *H. pylori* status of the participants or not reported the status explicitly.

Quantitative estimates

The genotype frequencies in individual studies are presented in Table 2. Overall, there were significant associations between TNF-α-308 G>A and the GC risk under the allelic model (OR,1.22;95% CI,1.04-1.44, I^2:78%) and the dominant model (OR,1.25;95%CI:1.08–1.45, I^2:69%), but not under the additive model (OR,1.22;95%CI:0.99-1.52, I^2:0%) or the recessive model (OR,1.8, 95% CI; 0.96–1.46, I^2:2%). On stratification, only the Caucasian population showed susceptible to the GC risk under the allelic model (OR,1.27;95% CI,1.02-1.59, I^2:80%) and the dominant model (OR:1.25, 95% CI; 1.1-1.43, I^2:39%) (Figure 3 A, B, C, D).

Subgroup analyses

TNF-α-308 G>A was with an increased risk of GC only with the high quality studies (Additional File 3: Figure S1). Based on a subset of 5 studies (with 7 datasets), there were no significant differences in distribution of gene frequency between the sites of GC (cardia vs Non-cardia) (OR: 0.84, 95%CI: 0.58-1.23) or histological types (diffuse vs intestinal) (OR: 0.71, 95%CI: 0.49-1.03) (Additional File 4: Figure S2)

By stratification on *H. pylori* infection status, overall TNF-α-308 G>A had a 25% increased risk of GC in those infected cases compared to non-infected cases (OR,1.25, 95% CI; 1.08-1.45); however, this association was with a substantial heterogeneity (I^2:69%) (Additional File 5: Figure S3).

The pooled analysis on the 27 studies that were consistent with HWE also showed that TNF-α-308 G>A polymorphism was significantly associated with an increased risk of GC under the dominant model in overall analysis (OR,1.2;95%CI:1.1–1.3, I^2:37%), regardless of ethnic groups. Moreover, this association showed a decreased statistical heterogeneity (i.e. I^2 values from 69% to 37%). In the recessive model, there was a significant association in overall analysis (OR,1.27;95%CI:1.0–1.62) in the absence of statistical heterogeneity (I^2 .0%), but not in any particular ethnic group. This pattern was not found under the allelic model, which changed from a significant association into no significant association (OR,1.17,95% CI,0.98-1.39, I^2:75%). Under the additive model, there was a significant association in overall analysis (OR,1.31;95%CI:1.08–1.32, I^2:0%), but not in any particular ethnic group) (Table 3). A funnel plot showed no evidence of publication bias (Additional File 6: Figure S4).

TSA plot

We performed TSA of the dominant model with the use of an overall type I error of 5% and type II error of 20%. The included total participants in this meta-analysis reached the required information size (for an expected RRR 26%). Briefly, a TSA monitoring boundary

crossed with *Z* curve, which confirms the presence of robust evidence (Figure 4). In such case further studies are not needed to provide sufficient information.

Discussion

The current study provides evidence on the relationship between TNF-α-308 G>A and the risk of GC, comprising 7695 cases and 12327 controls from 33 studies. The major observations are as follows;

1. Based on 27 studies that met HWE, TNF-α-308 G>A SNP was significantly associated with the GC risk under the dominant, recessive and addictive models.
2. On stratification, HWE status of the controls, the ethnicity or study quality had an impact on the effect estimates.
3. The TSA plot revealed that the required information size for evidence of effect was sufficient. Any future studies in this field will less likely to change the direction of estimates.

Our findings were comparable with earlier reviews, in which the significant association was limited to the Caucasians [50, 51] in the dominate models [11, 51]. The association was statistically significant only for the Caucasians, indicating a dominance of racial specific factors. This difference may be explained partly due to variations in the frequency of the A allele between the different ethnic groups that could contribute to the diverse results. Moreover, it might also be related to difference in environmental factors such as smoking and diet between these two major ethnic groups. This was indirectly supported by an individual study in Poland, in which 72% of the GC cases were smokers (ex-smokers or current smokers) [39]. Due to paucity of data, we were not able to perform subgroup analysis with the smoking status of participants in the studies identified. The effects of inflammatory polymorphisms might have been masked by smoking [35]. Moreover, there was no significant differences of the gene frequencies between the anatomical sites or the histological types of GC. A silico analysis of TNF expression, using the bioinformatics web GEPLA had reported that the expression of TNF in GC tissue was higher than in normal stomach ($p < 0.01$) [52]. A published meta-analyses [50] focused on this polymorphism in the risk of GC reported that *H. pylori* infected cases had higher risk of developing GC. This was also observed in the present review.

The current findings suggest that *H. pylori* infection had contributed to a 30% increased risk of association between TNF-α-308 G>A and the GC. This could be explained in the light of immune-biological plausibility. *H. pylori* infection activates the synthesis of cytokines in the lining of the stomach. The most commonly studied inflammatory-related genes in gastric diseases include TNF-α, among others. During the early stage of *H. pylori* colonization, the expression of TNF-a is up-regulated and this activates the transcription of various pro-inflammatory cytokines and chemokines, leading to the amplification of inflammatory cascade against the infection [53]. TNF-a has been shown to inhibit the gastric acid secretion which is important in inducing cell apoptosis and promoting epithelial cell damage [54]. The cellular immune response to *H. pylori* infection is a main factor which contribute to the damage in gastric mucosa. Studies reported that the hypochlorhydric milieu plays a role in promoting the growth of non–*H. pylori* bacteria, which may cause the damage to mucosal wall and/or the production of carcinogenic N-nitroso compounds [55]. Moreover, *H. pylori* infection activates the cytokines production in the lining of the stomach including inflammatory-related genes such as TNF- a in the present analysis.

Our findings of overall or sensitivity analyses had some similarities to earlier reviews, in which the significant associations were also found with the dominant, allelic, additive models and recessive models, despite variations in number of studies [11, 50, 51]. Although there are more recent studies in this analysis, the results, in general, retained the evidence of association. Moreover, we introduced TSA for confirmation of the estimates to assess a required information size. TSA plots indicated that there was sufficient information to provide conclusive results.

Public health Implications

The difference in association between the ethnic groups observed in the current analysis has implications. Studies had reported that the regulation of tumor immunity factors at the genetic and gene expression level may be different in the Asian and non-Asian GC populations, and this can affect the region-specific effects on therapy outcome and prognosis [56].

Limitations

We acknowledge the study limitations. Only 36% of the studies in this review used TaqMan SNP genotyping assays, which is the preferred technology due to its high throughput and is highly accurate [57] compared to other methods. Hence, accuracy of genotype frequency is of concern as some studies included in this analysis were small studies with small sample sizes. Hence, there might likely have type II statistical error. However, meta-analysis is a retrospective pooling of published studies, and type II errors are less likely than in individual studies.

There might be other confounding factors that were not included in our subgroup analyses. For example, infections with carcinogenic potentials (e.g. EBV) or smoking and alcohol drinking of the participants were not addressed due to limited data. This bias was likely to be pronounced as the calculations used unadjusted assessment of ORs. Moreover, it is likely to miss relevant studies that are available in non-English or non-indexed databases. Furthermore, there might be interactions of TNF-α and other genes such as interleukins (gen-gen interaction/synergism) or other potential confounding factors such as nutritional status and life style of the patients that might have significant roles in the GC risk. Due to limited number of studies, we could not perform pooled analysis with these potential confounding factors. Hence, findings in this meta-analysis should be interpreted with cautions in relation to these factors.

Nevertheless, there are strengths in our present meta-analysis study. More than half of the included studies were carried out in the Asia region, in which the GC was more prevalent. This implied that there was geographical representativeness. Moreover, the majority of GC patients in the primary studies identified for the present analysis were males. This also implied a gender representativeness in terms of higher prevalence of GC among males globally. The vast majority of included studies had evidence of HWE. Numerous studies had highlighted the issue of deviations from HWE in genetic association studies such as genotyping error, population admixture/substructure, among others [58-60]. Furthermore, for robustness of the findings, we have attempted several subgroup analyses. Additionally, there are some strengths in this meta-analysis compared with published reviews in this field [11, 50, 51, 58-60]. To be comprehensive, we have attempted the TSA technique, which is useful to adjust random-error risk. An add-on TSA approach to this field will highlight to researchers about the optimal sample size to make judgement of the effect estimates. This will help the researchers and policy makers to consider on the need of future similar studies. This will further help to save the resources for more important future studies, rather than repeating a study that has been already confirmed.

Conclusions

The current findings suggest that *TNF-α-308* gene polymorphism plays an important role as host genetic factor predisposing to gastric carcinogenesis, and it would be useful for a screening marker. As the relationship of GC risk is ethnic specific, the consideration as a biomarker should be tailored to specific population group. To substantiate this, studies from the Asian regions, using more reliable genotyping technique are recommended.

Abbreviations

CI - Confidence interval

EBV - Epstein–Barr virus

GC - Gastric cancer

GEPIA- Gene expression profiling and interactive analyses

H. pylori- Helicobacter pylori

HWE - Hardy–Weinberg Equilibrium

NOS - Newcastle-Ottawa Scale

OR - Odds ratio

RevMan- Review Manager

SNP - Single nucleotide polymorphism

TNF - Tumour necrotic factor

TSA - Trial sequential analysis

Declarations

Ethics approval (and consent to participate)

The need for approval was waived as this study solely used published human data.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JWM Conceptualized; JWM, KS, WST, NHH, CN, TTW participated in the design; WST, NHH, CN, TTW data acquisition; CN, NHH, WST performed data analysis, data interpretation. CN drafted the manuscript; JWM, WST, NHH substantively contributed during the writing progress. All authors read and approved the final manuscript

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Tables

Table 1 Characteristics of the studies included in the meta-analysis

	Author, year	Ref no.	Country	Ethnicity	Cases/ Controls	Age group	Males	Type of cancer	Smokers	Study design	Genotyping method	HP +ve	HWE, *p* value	NOS criteria
1	Jang, 2001	12	South Korea	Asian	52/92	adult	NA	GAC	NA	CC	Nested PCR	NA	0.704	5
2	Wu, 2002	24	Taiwan	Asian	120/220 (others: EBV)	adult	NA	GC	NA	CC	PCR, dir seq	NA	0.000	5
3	El–Omar, 2003	2	USA	Caucasian	314/210	adult	87% 77%	GC-C GC-NC	Yes (80%)	CC	TaqMan	46%	0.548	8
4	Machado, 2003	10	Portugal	Caucasian	287/306	adult	58.3%	GC	NA	CC	PCR	61.3%	0.649	5
5	Fei, 2004	25	China	Asian	56/164		76.8%	GAC	NA	CC	PCR	NA	0.743	4
6	Glas, 2004	13	Germany	Caucasian	88/145	adult	46.6%	GC	NA	CC	PCR	88%	0.669	5
7	Lee, 2004	26	South Korea	Asian	341/261	adult	58.4%	GC	NA	CC	PCR	NA	0.493	7
8	Wu, 2004	27	Taiwan	Asian	204/210	adult	61.8%	GAC	NA	CC	PCR, dir seq	80.4%	0.000	5
9	Garcıa-Gonzalez, 2005	28	Spain	Caucasian	63/215	adult	64.1%	GC	NA	CC	PCR	49.2%	0.607	5
10	Lee, 2005	29	South Korea	Asian	122/120	adult	59%	GC	NA	CC	PCR	81%	0.403	5
11	Li, 2005	30	China	Asian	59/264	adult	66.1%	GC-NC	NA	CC	PCR-RFLP	93.2%	0.559	5
12	Lu, 2005	31	China	Asian	250/300	adult	73.2%	GC	Yes (57.2%)	CC	PCR	70.4%	0.559	8
13	Perri, 2005	32	Italy	Caucasian	184/366	adult	59.8%	GAC	NA	CC	PCR	77.1	0.145	4
14	Rocha, 2005	33	Brazil	Caucasian	166/536	adult	69.9%	GC-NC	NA	CC	PCR-RFLP	100%	0.345	7
15	Zambo,	34	Italy	Caucasian	129/ 792	adult	60.5%	NC	NA	CC	Taqman	84%	0.909	4

	2005				(benign gastro-duodenal diseases)									
16	Kamangar, 2006	35	Finland	Caucasian	112/208	adult	100%	GC	Yes (100%)	CC	Taqman	91% (NC); 68% (C)	0.292	5
17	Kim, 2006	36	South Korea	Asian	237/474	adult	62.9	GC	NA	CC	Taqman and PCR-RFLP	86.5%	0.911	5
18	Morgan, 2006	37	Honduras	Caucasian	170/162	adult	69%	GC	NA	CC	TaqMan	80%	0.623	7
19	Garcıa-Gonzalez, 2007	38	Spain	Caucasian	404/404	adult	65.8%	GC	Yes	CC	TaqMan	70.3%	0.35	8
20	Hou, 2007	39	Poland	Caucasian	305/427	adult	66.2%	GC	Yes (71.1%)	CC	TaqMan_	NA	0.186	8
21	Sugimoto, 2007	40	Japan	Asian	105/172	adult	80.9%	GC	NA	CC	PCR-RFLP	100%	0.908	4
22	Canedo, 2008	41	Portugal	Caucasian	508/713	adult	58.3%	GC	NA	CC	Taqman	NA	0.000	4
23	Crusiu, 2008	42	Europe	Caucasian	248/770	adults	NC (47%) C (74%)	GC-C GC-NC	Yes (data not shown)	Nested CC	RT-PCR	NA	0.165	8
24	Melo, 2009	43	Brazil	Caucasian	30/100	adults	63%	GAC	NA	CC	PCR-RFLP	97%	0.528	7
25	Yang, 2009	44	South Korea	Asian	84/336	adult	70%	GC	Yes (55%)	CC	PCR	86%	0.317	8
26	Whiteman, 2010	14	Australia	Caucasian	307/1355	adult	88%	GAC	Yes (76%)	CC	PCR	25%	0.979	7
27	Burada, 2012	45	Romania	Caucasian	105/242	adult	62.9%	GAC	NA	CC	TaqMan	100% (Only HP +ve)	0.784	8
28	Santos, 2012	46	Brazil	Caucasian	64/202	adult	NA	GC	No	CC	PCR	100%	0.67	3
29	Bhayal,	47	India	Asian	114/229	adult	68.4%	GC	Smoker	CC	PCR	75.44%	0.007	5

	2013								(42.1%)					
30	Hong, 2013	5	China	Asian	1686/1894	adult	73%	GC-C GC-NC	NA	CC	TaqMan	NA	0.376	8
31	Oliveira, 2015	48	Brazil	Caucasian	207/240	adult	77.8%	GC	Yes (data not shown)	CC	SYBR Green& Taqman	NA	0.296	5
32	Zabaglia, 2015	49	Brazil	Caucasian	24/40	adult	45.5%	GC	NA	CC	Taqman	87.5%	0.000	3
33	Du, 2017	11	China	Asian	400/400	adult	70%	GC	Yes (data not shown)	CC, MA	allele-specific PCR	NA	0.000	7

Ref no.: Reference number; CC: case-control design; dir seq: direct sequencing HP +ve: *H. pylori* positive; GAC: gastric adenocarcinoma; GC: gastric cancer in general, not specified; NC: non-cardia cancer; C: cardia; MA: meta- analysis; HWE: Hardy–Weinberg Equilibrium; NOS criteria: 0-9 score; RT-PCR: Real-time PCR; NA: not available/not reported/not shown

Table 2: Distribution of gene frequencies in the studies

Study	Year	Ref. No.	Cases			Controls		
			G/G	G/A	A/A	G/G	G/A	A/A
Jang	2001	12	46	4	2	85	7	0
Wu	2002	24	96	17	7	180	27	13
El–Omar	2003	2	201	87	26	152	52	6
Machado	2003	10	179	105	3	231	69	4
Fei	2004	25	53	3	0	143	20	1
Glas	2004	13	66	19	3	105	36	4
Lee	2004	26	218	43	1	297	42	1
Wu	2004	27	163	29	12	171	26	13
Garcıa-Gonzalez	2005	28	0	8	55	1	35	179
Lee	2005	29	112	10	0	103	17	0
Li	2005	30	55	4	0	228	34	2
Lu	2005	31	214	36	0	274	24	2
Perri	2005	32	71	14	1	118	24	4
Rocha	2005	33	120	37	4	399	123	13
Zambo	2005	34	95	31	3	496	138	10
Kamangar	2006	35	86	23	3	154	52	2
Kim	2006	36	199	34	4	400	59	2
Morgan	2006	37	151	17	0	149	12	0
Garcıa-Gonzalez	2007	38	309	84	11	320	77	7
Hou	2007	39	186	98	21	304	109	15
Sugimoto	2007	40	101	4	0	169	3	0
Canedo	2008	41	330	178	0	544	169	0
Crusiu	2008	42	170	64	2	820	274	31
Melo	2009	43	24	5	1	86	13	1
Yang	2009	44	75	8	0	288	34	0

Whiteman	2010	14	196	93	6	842	403	48
Burada	2010	45	78	26	1	196	44	2
Santos	2012	46	44	20	0	22	4	0
Bhayal	2013	47	32	76	6	76	128	25
Hong	2013	5	1335	333	18	1585	295	14
Oliveira	2015	48	138	66	3	167	69	4
Zabaglia	2015	49	17	4	3	33	4	3
Du	2017	11	204	184	12	326	60	14

Table 3. Sensitivity analysis of studies with Hardy-Weinberg Equilibrium

Description	Number of studies included	Overall risk OR (95%CI)	Subgroup analysis
dominant model	27 Asians (10), Caucasians (17)	**1.2 (1.1–1.3);** I^2:37%	Asians, 1.20 [1.05, 1.38] Caucasians: 1.20 [1.08, 1.32]
recessive model	Do	**1.27 (1.0–1.62);** *I^2:0%*	Asians, 1.50 [0.88, 2.55] Caucasians: 1.22 [0.93, 1.60]
additive model	Do	**1.31 (1.08–1.32);** *I^2:0%*	Asians, 1.53 [0.91, 2.56] Caucasians: 1.25 [0.94, 1.67]
allelic contrast model	Do	**1.17 (0.98-1.39);** I^2:75%.	Asians: 1.06 [0.81, 1.39] Caucasians: 1.24 [0.97, 1.58]

Significant association is indicated in bold.

Figures

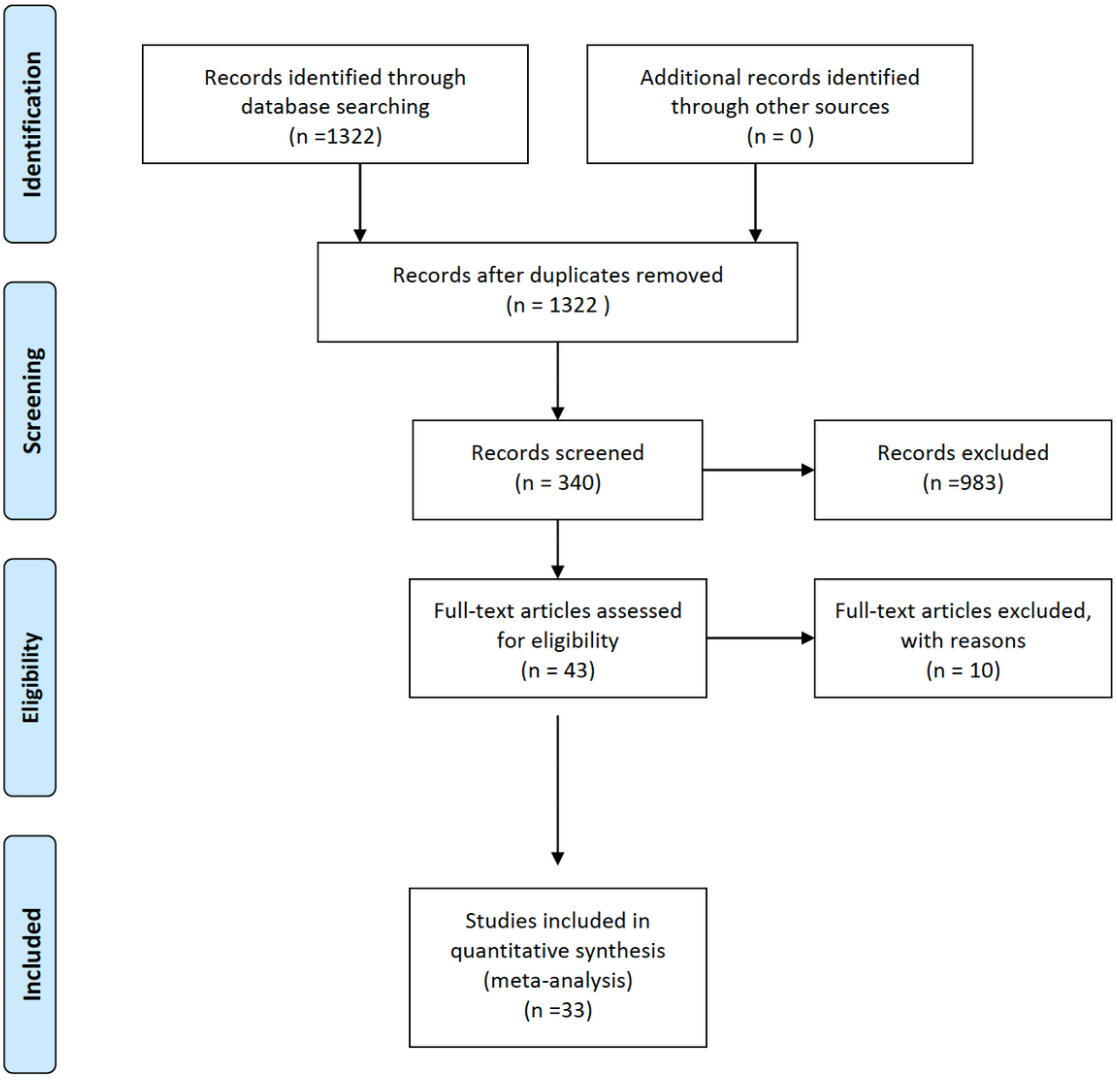

Figure 1

Study selection process

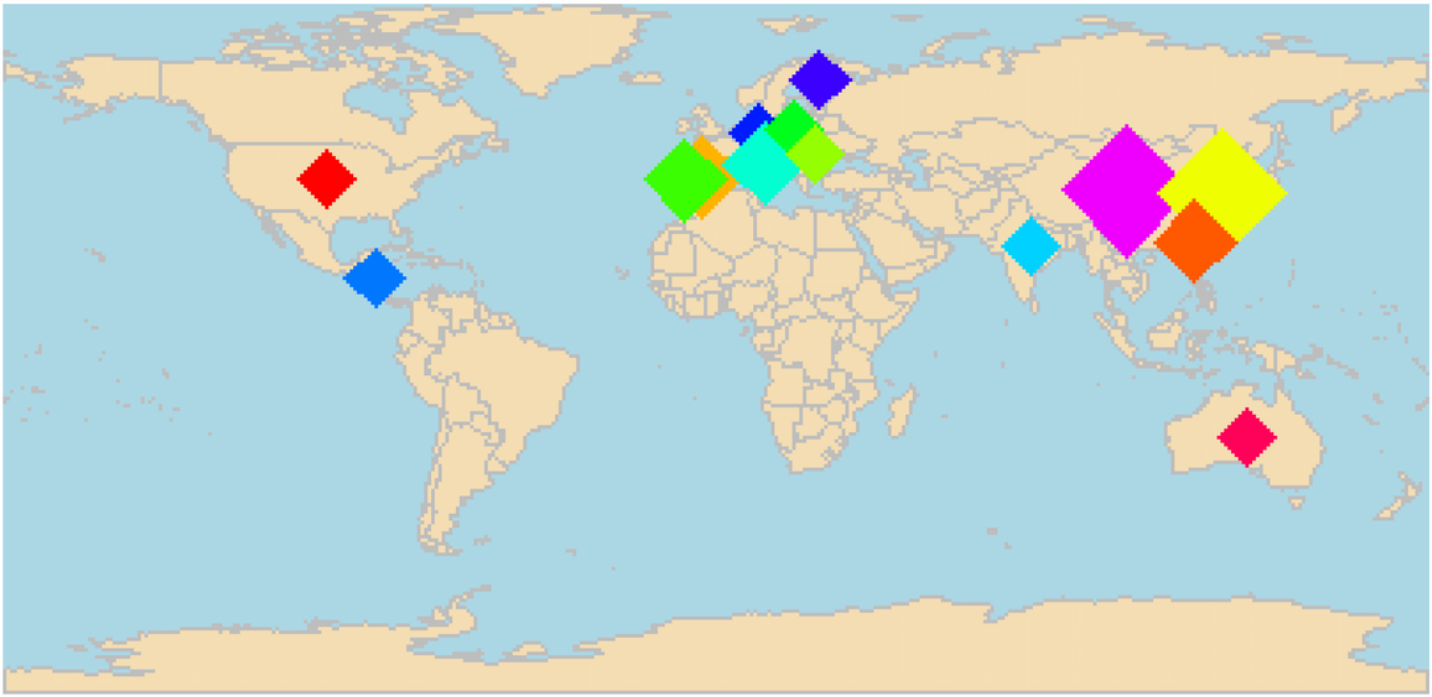

Figure 2

Geographical distribution of included studies

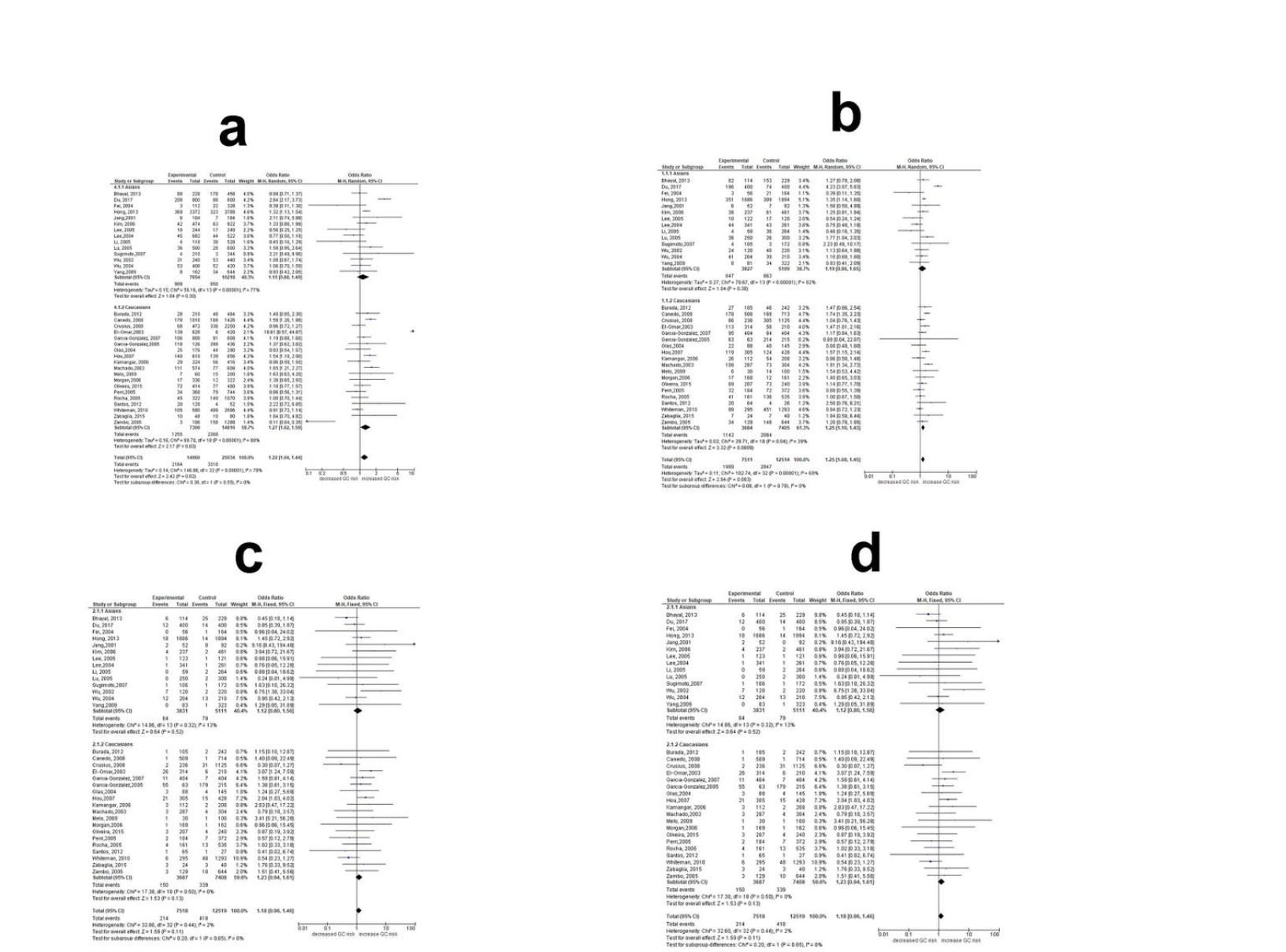

Figure 3

Forest plot of gastric cancer risk associated with TNF-α 308 G/A polymorphism by ethnic group 3A: Allelic model 3B: Dominant model 3C: Additive model 3D: Recessive model

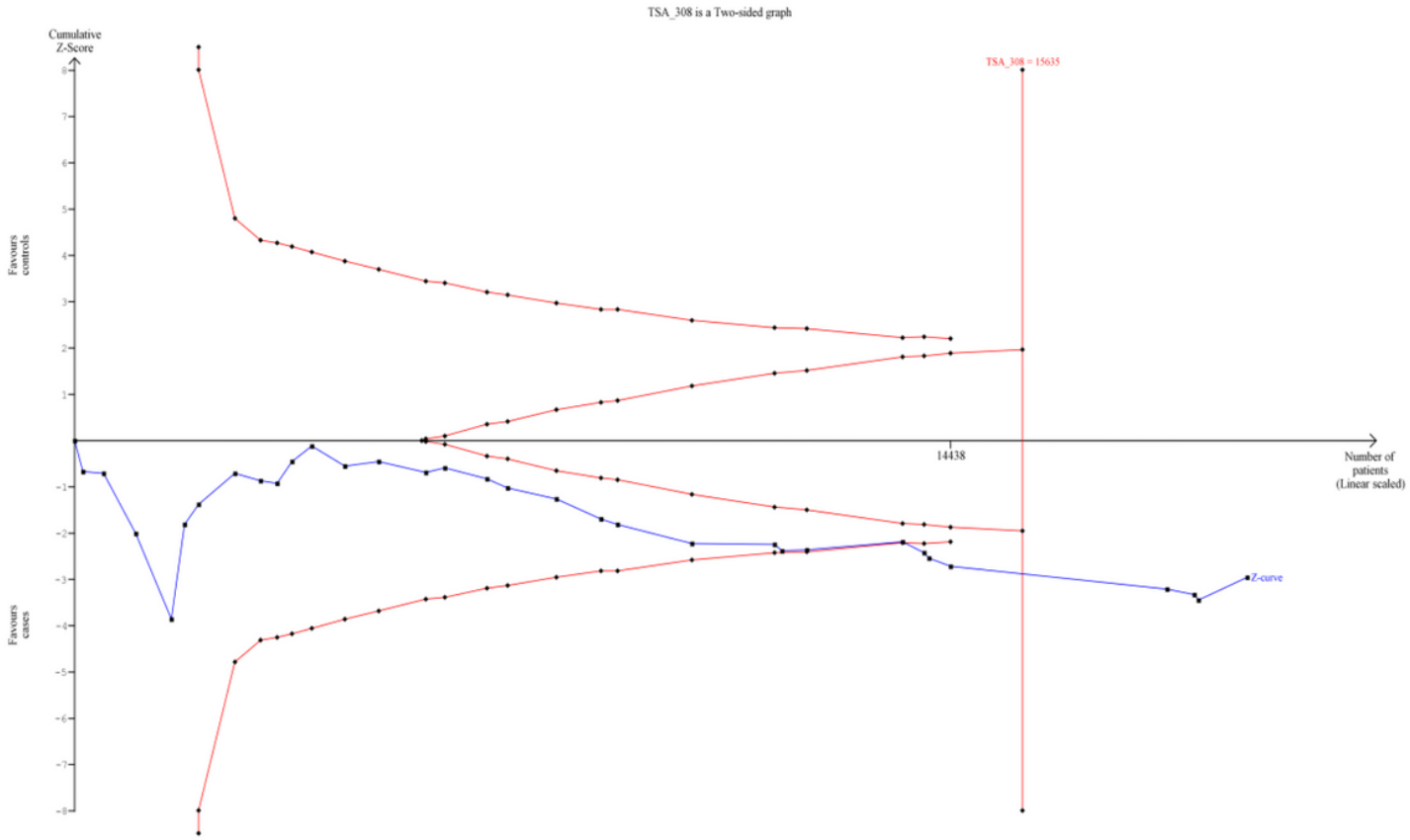

Figure 4

Trial sequential monitoring plot of TNF-α-308 in gastric cancer risk under dominant model

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