Polymorphisms of Tumour necrosis factor-α-308 (rs 1800629) and gastric cancer susceptibility: A meta-analysis of associations studies with trial sequential analysis

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Systematic Review

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Introduction

Gastric cancer (ICD10: C16) 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, the estimated number of deaths attributed to gastric cancer was 782,685 [1]. As such, a greater understanding of the risk factors that play a role in gastric carcinogenesis can improve preventive and therapeutic interventions [2].

Epidemiologic studies showed that gastric cancer is multifactorial in aetiology [3, 4], in which dietary factors and Helicobacter pylori infection may contribute to its development [3]. Of note is that a high prevalence of these risk factors do not always correspond to a high incidence of gastric cancer, suggesting other susceptible factors such as genetic variations and environmental differences may be involved in gastric carcinogenesis [5]. For instance, it has appeared that only 1-2% of the infected will develop gastric cancer in their lifetime, albeit with more than half of the world's population being infected with H. pylori infection [6].

Gastric neoplasms are composed of cancer cells and other “non-cancer” compartments (including immune cells), which are the major players in gastric cancer disease progression and aggressiveness [7]. Tumour necrosis 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, composed mainly of inflammatory cells, is a crucial player in the neoplastic process, fostering proliferation, survival and migration [9]. Thus, TNF-α may act as a tumour promoter through inflammation. Moreover, TNF-α can induce the transcription of a wide range of other proinflammatory cytokines and chemokines, amplifying the inflammatory cascade against the infection [2]. Individual studies reported the significant relationship between TNF-α-308 (rs 1800629) and the risk of gastric cancer [10, 11]. However, other studies reported differently [12-14]. The published reviews in this field [15-16], did not report the required information size leading to concerns over confidence in estimates. We have also found more individual studies on this. Taken together, the objective of this study was to summarize the evidence of association between TNF-α-308 and the risk of gastric cancer by meta-analysis of data from eligible studies.

Materials And Methods

This is a meta-analysis of genetic association studies (GAS), following the Plos One checklist (S1 Table).

Study search

We searched relevant studies in the health-related databases of PubMed, Ovid Medline, Embase, Google scholar and Web of science. To maximize search scope, we used a simple term combination strategy: (“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 (S2 Table). The search was limited to the publications in English until November 2020. We also manually searched the references of included studies and relevant systematic reviews to capture any additional studies.

Inclusion criteria

Human studies that assessed gastric cancer, irrespective of site or histological type were included, if they met all the following criteria:

1. The study investigated TNF-α-308 G>A (rs 1800629) or TNF-α-307 G>A;
2. The study was a case-control design (retrospective or nested case-control);
3. The study recruited healthy people as the controls;
4. The study assessed histopathologically confirmed gastric cancer as an outcome;
5. The study reported the genotype frequency in cases and controls;
6. The study provided sufficient data to compute odds ratio (OR) and its 95% confidence interval (CI) as the outcome measurement.

Gastric cancer 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. Studies on gastric adenoma are not considered.

**Data extraction**

One investigator (WST) screened the titles and abstracts and selected the relevant full-text articles, following the inclusion criteria. Two investigators extracted the data (NHH, WST) from each study individually, by using a pretested data extraction sheet. 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 [17]. Any discrepancy between the two investigators was discussed with the third investigator (CN) to reach consensus.

**Assessment of the methodology quality**

The two investigators (WST, CN) independently evaluated the methodological quality of studies, using the Newcastle-Ottawa Scale (NOS) [18]. The assessment is based on the three domains such as ‘selection of the study groups’ (4 points), ‘comparability of the groups’ (2 points) and ‘ascertainment of the exposure’ (3 points). A total score for each study can vary from 0 (the worst) to 9 (the best). A score achieved ≥7, 6-5 or ≤4 is regarded as good, moderate or low quality study, respectively. Any discrepancy between the two investigators was resolved by consensus.

**Statistical analysis**

We assessed Hardy-Weinberg equilibrium (HWE) in the control populations of the included studies using the goodness-of-fit test and p>0.05 was considered to indicate consistency with HWE [19]. As described elsewhere [20], the strength of the association between TNF-α-308 G>A and the risk of gastric cancer in each study was estimated using OR and its 95% CI. Between-study heterogeneity was determined with the Χ² test, which indicates the percentage of total variation across studies attributed to the heterogeneity rather than chance. Χ² values >50% is regarded as substantial heterogeneity [21]. For pooling of the estimates, the summary ORs and its 95% CIs were calculated with the random-effects model (The Der Simonian and Laird method) in the presence of substantial heterogeneity. 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, subgroup analyses were employed under the dominant model for ethnicity, H. pylori infection status and study quality. Due to inconsistent reporting, we were unable to analyse by location or histological type of gastric cancer. For sensitivity analysis, we reassessed the relationship between TNF-α-308 and the risk of gastric cancer in all four genetic models only with studies in conformity of HWE. The publication bias was assessed by visual inspection of funnel plots under dominant model [22, 23].

**Trial sequential analysis**

To estimate the required information size, we performed trial sequential analysis (TSA) [24]. It is classified as ‘firm evidence of effect’ or ‘potentially spurious evidence of effect’, depending 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 *rworldmap* package in *R* version 3.6.1 (The *R* Foundation). TSA plot was done with TSA software (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen).

**Results**

**Study search**

Fig 1 illustrates a four-phase study selection process. The initial search yielded a total of 1335 records. After removing the duplicates and screening of abstracts, 45 potentially eligible full-text articles that were retrieved. We included a final of 35 studies (with 11353 cases and 12827 controls) in this review [2, 5, 10-14, 25-52]. Summary of the 10 excluded studies were provided (S3 Table).

**Study characteristics**

Table 1 shows the characteristics of 35 studies identified. Of these, slightly more than half (54.3%, 19/35) were from the Asian region. The years of publication spanned from 2001 to 2017. The participants were adults with male predominance in all these studies. Eighty percent of the studies were consistent with HWE in genotype distribution of the controls.

The most frequent five studies were performed in China, South Korea or Brazil. Fig 2 shows geographical distribution of the studies included.

Of 35 studies, less than half (40%, 14/35) were categorized as high quality in methodology (14 studies). Eight studies (22.8%) were gastric adenocarcinomas. Only five studies provided (2, 10, 13, 34, 44) anatomical location or histological type of gastric cancers. Twelve studies (34%) used TaqMan
method for genotyping. The majority of studies (24/35, 68.6%) included *H. pylori* infected gastric cancer cases, albeit with variation in distribution. For instance, all cases (100%) were infected with *H. pylori* in one study [32], while this was only 46% in another study [2] (Table 1).

**Effect estimations**

The genotype frequencies in individual studies are presented in Table 2. Overall, there were significant associations between TNF-α-308 G>A and the gastric cancer risk under the allele model (OR,1.17, 95% CI,1.0-1.38, $I^2$80%) and the dominant model (OR,1.2, 95% CI,1.03–1.4, $I^2$70%), but not under the recessive model (OR,1.1, 95% CI, 0.9–1.35, $I^2$16%) or the additive model (OR,1.25, 95% CI,1.01-1.54, $I^2$0%). On stratification, only the Caucasian population showed susceptible to the gastric cancer risk under the allelic model (OR, 1.25, 95% CI,1.0-1.55, $I^2$80%) and the dominant model (OR,1.24, 95% CI, 1.08-1.42 $I^2$40%) (Fig 3A, Fig 3B, Fig 3C and Fig 3D).

**Subgroup analyses**

Under dominant model, TNF-α-308 G>A has significantly associated with an increased risk of gastric cancer with the high or moderate quality studies, but not with poor quality studies (S1 Fig). Based on 24 studies in which patients were infected with *H. pylori*, a significant association was observed between TNF-α-308 G>A and gastric cancer risk (OR, 1.17, 95% CI, 1.05-1.29). On further stratification, this was only with the Caucasian group (OR, 1.17, 95% CI, 1.04-1.32) (Table 3).

A sensitivity analysis based on 28 studies that were consistent with HWE, showed TNF-α-308 G>A polymorphism was significantly associated with an increased risk of gastric cancer under the dominant model in overall analysis (OR, 1.19, 95% CI,1.1–1.29, $I^2$37%), regardless of ethnic groups. Moreover, this association showed a decreased statistical heterogeneity (i.e. $I^2$ values from 69% to 37%) (Table 3, S2 Fig). In the allele and recessive model, there was no significant association in overall analysis even in the absence of statistical heterogeneity ($I^2$0%). Under the additive model, there was a significant association in overall analysis (OR, 1.31,95% CI,1.02–1.69, $I^2$0%) but not in any particular ethnic group (Table 3). A funnel plot showed no evidence of publication bias (S3 Fig).

**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, confirms the presence of robust evidence (Fig 4). In such case further studies are not needed to provide sufficient information.

**Discussion**

**Summary information**

The present study provides evidence on the relationship between TNF-α-308 G>A and the risk of gastric cancer, comprising 11353 cases and 12827 controls from 35 individual studies. The major observations are as follows;

1. Based on 28 studies that met HWE, TNF-α-308 G>A SNP was significantly associated with the gastric cancer risk under the dominant and additive models.
2. On stratification, the HWE status of the controls, ethnicity, *pylori* infection status 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 change the direction of estimates.

The association was statistically significant only for the Caucasians in overall allele model, 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 gastric cancer cases were smokers (ex-smokers or current smokers) [39] as the effects of inflammatory polymorphisms might have been masked by smoking [35]. Due to paucity of data, we were not able to perform subgroup analysis with the smoking status of participants included in the studies identified. A published meta-analyses [53] reported that *H. pylori* infected cases had higher risk of developing gastric cancer. This was also observed in the present review. This could be explained based on immune-biological plausibility. *H. pylori* infection activates the cytokines production in the lining of the stomach including inflammatory-related genes such as TNF-α in the present analysis.

Our findings were comparable with earlier reviews, in which the significant association was limited to the Caucasians [53, 54] in the dominant models [11, 54]. Although there are more studies in this analysis, the results in general, retained the evidence of association. The most commonly studied inflammatory-related genes in gastric diseases include TNF-α, among others. TNF-α has been shown to inhibit the gastric acid secretion which is important in inducing cell apoptosis and promoting epithelial cell damage [57].
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 gastric cancer populations, and this can affect the region-specific effects on therapy outcome and prognosis [58].

Study 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 high accurate [59] compared to other methods. Hence, accuracy of genotype frequency is a concern. Some studies included were with small sample sizes. Hence, there might be type II statistical error. Meta-analysis is a retrospective pooling of published studies, and type II errors are, therefore, 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. The effects of inflammatory polymorphisms might have been masked by smoking [35]. 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 (gene-gene interaction/synergism) or other potential confounding factors such as nutritional status and life style of the patients that might have significant roles in the gastric cancer 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 caution 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 gastric cancer was more prevalent. Moreover, the majority of gastric cancer patients in the primary studies identified were males. The current analysis reflected geographical and gender representativeness. 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 [60-62]. Furthermore, for robustness of the findings, we have attempted several subgroup analyses. There are strengths in this meta-analysis compared with published reviews in this field [11, 53, 54, 60-63]. To be comprehensive, we have attempted the TSA technique, which is useful to adjust random-error risk. 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. An add-on TSA approach to this field will highlight to researchers the optimal sample size to make judgement of the effect estimates. This will help the researchers and policy makers to determine the need for future similar studies, which can save limited resources.

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 could be useful as a screening marker. As the relationship of gastric cancer risk is ethnic specific, the consideration as a biomarker should be tailored to the specific population group. To substantiate this, studies only from the Asian regions, using more reliable genotyping technique are recommended.

Declarations

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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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| Author, Year     | Ref no. | Country | Ethnicity     | Cases/Controls | Age group | Males | Type of cancer | Smokers | Study design | Genotyping method | HP +ve | HWE, p value |
|------------------|---------|---------|---------------|----------------|-----------|-------|----------------|---------|--------------|--------------------|--------|--------------|
| El-Omar, 2003    | 2       | USA     | Caucasian     | 314/210        | adult     | 87%   | GC-C GC-NC    | Yes (80%) | CC           | TaqMan             | 46%    | 0.548        |
| Hong, 2013       | 5       | China   | Asian         | 1686/1894      | adult     | 73%   | GC-C GC-NC    | NA      | CC           | TaqMan             | NA     | 0.376        |
| Machado, 2003    | 10      | Portugal| Caucasian     | 287/306        | adult     | 58.3% | GC             | NA      | CC           | PCR                | 61.3%  | 0.649        |
| Du, 2017         | 11      | China   | Asian         | 400/400        | adult     | 70%   | GC             | Yes     | CC, MA       | allele-specific PCR | NA     | 0.000        |
| Jang, 2001       | 12      | South Korea | Asian     | 52/92          | adult     | NA    | GAC            | NA      | CC           | Nested PCR         | NA     | 0.704        |
| Las, 2004        | 13      | Germany | Caucasian     | 88/145         | adult     | 46.6% | GC             | NA      | CC           | PCR                | 88%    | 0.669        |
| Whiteman, 2010   | 14      | Australia| Caucasian    | 307/1355       | adult     | 88%   | GAC            | 76%     | CC           | PCR                | 25%    | 0.979        |
| Wu, 2002         | 25      | Taiwan  | Asian         | 120/220        | adult     | NA    | GC             | NA      | CC           | PCR, dir seq       | NA     | 0.000        |
| Fei, 2004        | 26      | China   | Asian         | 56/164         | adult     | 76.8% | GAC            | NA      | CC           | PCR                | NA     | 0.743        |
| Lee, 2004        | 27      | South Korea | Asian     | 341/261        | adult     | 58.4% | GC             | NA      | CC           | PCR                | NA     | 0.493        |
| Wu, 2004         | 28      | Taiwan  | Asian         | 204/210        | adult     | 61.8% | GAC            | NA      | CC           | PCR, dir seq       | 80.4%  | 0.000        |
| Garcia-Gonzalez, 2005 | 29 | Spain   | Caucasian     | 63/215         | adult     | 64.1% | GC             | NA      | CC           | PCR                | 49.2%  | 0.607        |
| Lee, 2005        | 30      | South Korea | Asian     | 122/120        | adult     | 59%   | GC             | NA      | CC           | PCR                | 81%    | 0.403        |
| Li, 2005         | 31      | China   | Asian         | 59/264         | adult     | 66.1% | GC-NC          | NA      | CC           | PCR-RFLP           | 93.2%  | 0.559        |
| Lu, 2005         | 32      | China   | Asian         | 250/300        | adult     | 73.2% | GC             | 57.2%   | CC           | PCR                | 70.4%  | 0.559        |
| Perri, 2005      | 33      | Italy   | Caucasian     | 184/366        | adult     | 59.8% | GAC            | NA      | CC           | PCR                | 77.1   | 0.145        |
| Rocha, 2005      | 34      | Brazil  | Caucasian     | 166/536        | adult     | 69.9% | GC-NC          | NA      | CC           | PCR-RFLP           | 100%   | 0.345        |
| Zambo, 2005      | 35      | Italy   | Caucasian     | 129/792 (benign gastrointestinal diseases) | adult | 60.5% | GC-NC          | NA      | CC           | Taqman             | 84%    | 0.909        |
| Kamangar, 2006   | 36      | Finland | Caucasian     | 112/208        | adult     | 100%  | GC             | 100%    | CC           | Taqman             | 91%    | 0.292        |
| Kim, 2006        | 37      | South Korea | Asian     | 237/474        | adult     | 62.9% | GC             | NA      | CC           | TaqMan and PCR-RFLP | 86.5%  | 0.911        |
| Morgan, 2006     | 38      | Honduras | Caucasian     | 170/162        | adult     | 69%   | GC             | NA      | CC           | TaqMan             | 80%    | 0.623        |
| Garcia-Gonzalez, 2007 | 39 | Spain   | Caucasian     | 404/404        | adult     | 65.8% | GC             | Yes     | CC           | TaqMan             | 70.3%  | 0.35         |
| Hou, 2007        | 40      | Poland  | Caucasian     | 305/427        | adult     | 66.2% | GC             | Yes (71.1%) | CC          | TaqMan             | NA     | 0.186        |
| Sugimoto, 2007   | 41      | Japan   | Asian         | 105/172        | adult     | 80.9% | GC             | NA      | CC           | PCR-RFLP           | 100%   | 0.908        |
Table 2: Distribution of gene frequencies in the studies

| Study          | Year | Ref. No. | Cases       | Controls     |
|----------------|------|----------|-------------|--------------|
| Canedo, 2008   | 42   | 508/713  | adults      |              |
| Crusiu, 2008   | 43   | Europe¹  | Caucasian   |              |
| Melo, 2009     | 44   | Brazil   | Caucasian   |              |
| Yang, 2009     | 45   | South Korea | Asian      |              |
| Burada, 2012   | 46   | Romania  | Caucasian   |              |
| Santos, 2012   | 47   | Brazil   | Caucasian   |              |
| Oliveira, 2015 | 48   | Brazil   | Caucasian   |              |
| Bhayal, 2013   | 49   | India    | Asian       |              |
| Yu, 2014       | 50   | China    | Asian       |              |
| Stubijar, 2015 | 51   | Slovenia | Caucasian   |              |
| Zabaglia, 2015 | 52   | Brazil   | Caucasian   |              |

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; Europe: Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden and the UK; 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
| Genetic model                        | Number of studies included | OR (95%CI) | Overall |      |      |
|-------------------------------------|---------------------------|------------|---------|------|------|
| Studies consistent with HWE         |                           |            |         |      |      |
| Allele                              | 28                        | 1.11 [0.97, 1.27] R | 1.06 [0.81, 1.39] R | 1.12 [0.95, 1.33] R |
| Dominant                            |                           | 1.19 [1.10, 1.29] F | 1.20 [1.05, 1.38] F | 1.19 [1.07, 1.31] F |
| Recessive                           |                           | 1.24 [0.98, 1.56] F | 1.50 [0.88, 2.55] F | 1.18 [0.90, 1.53] F |
| Additive                            |                           | 1.31 [1.02, 1.69] F | 1.53 [0.91, 2.56] F | 1.25 [0.94, 1.67] F |
| Studies based on *H. Pylori* infection status | 24                        | 1.17 [1.05, 1.29] F | 1.15 [0.93, 1.42] F | 1.17 [1.04, 1.32] F |

HWE: Hardy-Weinberg equilibrium; F: Fixed-effect model; R: random-effects model; Significant association is in bold;

**Figures**

![Figure 2](image_url)
Geographical distribution of included studies. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 3

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

Footnote: The circle and horizontal lines correspond to the study-specific OR and 95% CI. The diamond represents the summary OR and 95% CI.
Figure 4

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