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Evaluation of Tumor Necrosis Factor-Alpha Gene (−308 G/A, −238 G/A and −857 C/T) Polymorphisms and the Risk of Gastric Cancer in Eastern Indian Population

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Abstract: Introduction: Gastric cancer (GC) is one of the leading causes of cancer-related decimations worldwide. The gastric infection at both the stomach and duodenum with *Helicobacter pylori* causes inflammation by the tumor necrosis factor-alpha (TNF-α). The aim of the study was to associate and evaluate the three TNF-α gene polymorphisms at positions −308 G/A, −238 G/A, and −857 C/T with the risk of GC. Methods: A total of 156 individuals (consecutively diagnosed 95 GC patients and 61 controls) above the age of 18 years were enrolled in the study. Healthy individuals with normal upper gastrointestinal endoscopy (UGIE) irrespective of their family history of GC or peptic ulcer were included as controls. The cited three TNF-α gene polymorphisms were evaluated using polymerase chain reaction-restriction fragment length polymorphism (RFLP). Results: There was no significant difference in the distribution of gene polymorphisms as genetic factors, TNF-α−308 GA/AA (22.1% vs. 14.8%, $p = 0.2$), TNF-α−238 GA/AA (21% vs. 19.6%, $p = 0.8$), and TNF-α−857 CT/TT (8.4% vs. 11.5%, $p = 0.5$), between GC cases and healthy controls. A subgroup analysis of *H. pylori*-positive patients showed that there was no significant difference in the distribution of GA/AA polymorphisms in TNF-α−308 (15(45.5%) vs. 3(23%); $p = 0.17$) and −238 (12(36.3%) vs. 2(15.4%); $p = 0.17$), and the distribution of TT/CT −857 CT/TT (13(39.4%) vs. 2(15.4%); $p = 0.13$), among the GC cases and controls. Conclusion: The statistical comparisons of GA/AA vs. GG genotypes at −308 (with OR = 1.6, 95% CI: 0.6–3.8), −238 (OR = 1.09, 95% CI: 0.4–2.4) and TT/CT vs. CC genotypes at −857 (OR = 0.7, 95% CI: 0.2–2.1) did not suggest any association of TNF-α with GC in the population herein. Hence, the TNF-α (−308 G/A, −238 G/A and −857 C/T) may not be the associating factor for GC incidence determined by the PCR–RFLP method.

Keywords: gastric cancer; *Helicobacter pylori*; tumor necrosis factor-alpha; restriction fragment length polymorphism

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1. Introduction

Gastric cancer (GC) is a malignancy of high aggressiveness that poses as the fourth leading cause of cancer-related decimations worldwide [1,2]. The incidence of GC has a huge geographical variability with some high incidences in Central and South America, Eastern Europe, East Asia and low incidences in Australia, Southern Asia, North and East Africa, and North America [3,4]. In 2020, the incidence of GC was highest in Eastern Asia (22.4 per 100,000 people), which was followed by Central and Eastern Europe (11.3 per 100,000 people) [5]

India has some low risk with the wide regional variation in the GC incidence rates. Moreover, there are different risk factors for GC such as infection from the anaerobic bacterium, *Helicobacter pylori*, atrophic gastritis, adenomas including other factors, diet, smoking and alcohol abuse, etc. [6]. Apart from these, genetic factors such as single nucleotide polymorphisms, chromosomal aberrations, and epigenetic alterations also play a major role in increasing the susceptibility to GC. Almost all *H. pylori*-positive patients had chronic gastritis, and only 1–2% cases developed GC, which may vary from different geographical regions across the world [7]. The effect of *H. pylori* on the oncogenesis is attributed to two main mechanisms: an indirect inflammatory reaction on the gastric mucosa and a direct epigenetic modification on gastric epithelial cells. *H. pylori* infection induces inflammation in a gastric microenvironment, leading to the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6. The polymorphisms in the TNF-α gene promoter region had been identified to play a vital role in the development of different cancers such as prostate, lung, oral and cervical cancer episodes [8–10]. The expression level of TNF-α was affected by polymorphisms in its promoter region, and the previous studies recorded the polymorphisms at −238 (rs361525), −308 (rs1800629), 857 (rs1799724) and 1031 (rs1799964) positions, which may be associated with GC. However, the studies on the association of TNF-α polymorphisms and the risk of GC in the South Asian population have conflicting results [11–16]. In view of the inconclusive results and a limited literature regarding the risk of GC in relation to TNF-α from India, we aimed to study the association of TNF-α−308 G/A, −238 G/A, and T857C polymorphisms in GC in a cohort of individuals from Eastern India.

2. Materials and Methods

2.1. Ethics Statement

After obtaining informed consents from the participants, vials of 2 mL of venous blood were collected for DNA extractions and the associated polymerase chain reaction (PCR) reactions for the testing of TNF gene polymorphism. The study was approved by the institutional ethics committee with approval, DMR/IMS-SU/SOA/160129.

2.2. Patient Enrolment and Sample Collection

Consecutive patients diagnosed endoscopically and histopathologically above the age of 18 years from March 2018 to May 2021 (for 38 months) were included. Participants with the normal upper gastrointestinal endoscopy (UGIE), irrespective of the individual family history of occurrence of GC and peptic ulcer were included as control cases. The demographic details, laboratory parameters, endoscopy impression, and rapid urease test (RUT) results for the infection of *H. pylori* and biopsy findings were recorded in a structured proforma. The RUT test was performed using '*H-P* Test' kits (Lenus Medicare & Research, OPC Private Ltd., Kolkata, India). The participants were followed up for noting the survival status at every 3 months for a year. The cause and date of death, if any, were recorded, and duration was calculated from the time of enrolment as a patient.

2.3. DNA Extraction and PCR-RFLP

DNA was isolated from peripheral heparinized whole blood samples, using the salting-out method [17], and DNA were stored at −80 °C until further processing. Fragment analysis was performed by PCR-restriction fragment length polymorphism (PCR-RFLP) using the standard procedure [18]. The primers were designed with the help of the Primer 3 software (NCBI). The designed primers were TNF-α−308 G/A, TNF-α−238 G/A and TNF-α−857 C/T. The PCR thermal conditions were standardized to obtain the best annealing temperature, and the commercially procured restriction enzymes (Thermo Scientific) were used for the digestion of obtained PCR products as summarized (Table 1). After digestion, the PCR products were separated with 2% agarose gel electrophoresis and stained with ethidium bromide, which was followed by the band visualization under gel doc. The cut bands for gene number −308 G/A showing two fragments of 87 and 20 base pairs (bp)

represent homozygous GG, while a partial cut resulting in 3 cut fragments of 107, 87 and 20 bp represents heterozygous GA, and an uncut, non-digested fragment visualized at 107 bp represents AA. The digestion pattern at the location −238 G/A was 132 and 20bp had represented GA, while the uncut band of 152bp GG was the homozygous wild variant and the digestion pattern at the location −857 CT, the cut bands were 108 and 25bp had represented homozygous mutation CC, while the uncut band 133bp had represented wild variant (TT), respectively (Table 1).

Table 1. PCR primers and conditions for TNF gene polymorphisms.

Gene Name	Nucleotides/Sequences	Product Size (in bp)	Annealing Temp (in °C)	Digestive Enzyme	Allele Phenotype (in bp)
TNF-α−308 G/A	Forward: 5′-TCCTCCCTGCTCCGATTCCG-3′ Reverse: 5′-AGGCAATAGGTTTTGAGGGCCAT-5′	107	58 °C	*Nco I*	A: 107; G: 87 + 20
TNF-α−238 G/A	Forward: 5′-AGAAGACCCCCCTCGGAACC-5′ Reverse: 5′-ATCTGGAGGAAGCGGTAGTG-3′	152	60 °C	*Msp I*	A: 152; G: 132 + 20
TNF-α-T−857C	Forward: 5′-AAGTCGAGTATGGGGACCCCCCGTTAA-3′ Reverse: 5′-CCCCAGTGTGTGGCCATATCTTCTT-3′	133	58 °C	*Hinc II*	C: 108 + 25; T: 133

2.4. Statistical Analysis

The statistical analysis was performed by SPSS (Statistical Package for Social Sciences) software version 20.0 (Armonk, NY, USA; IBM Corp). The normality of the data was ascertained using the Shapiro–Wilk test. Thus, with the 'normal distribution' of the presented data, the frequencies of findings of gene polymorphisms and demographic features were calculated and stated as percentages. The comparison of categorical variables between GC cases and controls were carried out by Fisher's exact test, while the Kaplan–Meier analysis was applied for survival functions. The ORs were assessed by the Z-test, in which a two-sided *p*-value was generated to indicated the statistically significant difference. All the tests were two tailed, and the *p*-value of < 0.05 was considered as 'statistically significant'.

3. Results

A total of 156 individuals with 95 GC cases and 61 controls were included, and their baseline characteristics were summarized (Table 2). The parameters such as age, gender, BMI, socioeconomic status, dietary habits, smoking, and alcohol abuse were compared between the groups. Indeed, 34.7% (numbers, 33) among GC cases and 21.3% (numbers, 13) were positive among controls for *H. pylori*; and the difference was not statistically significant for *H. pylori* infection ($p = 0.07$). Among the 95 patients with GC, 24.2% (numbers, 23) had diffuse type and 75.8% (numbers, 72) had the intestinal type of GC.

The distributions of TNF-α between GC cases and controls are summarized in Table 3. There was no significant difference in the distribution of the three TNF-α gene polymorphisms between GC cases and controls. Comparison of GA/AA vs. GG genotypes at locations 308 and −238, and TT/CT vs. CC genotypes at the location −857 were not digested to be associated with GC in this cohort. In other words, no mutation was observed at location 857.

Table 2. Distribution of selected demographic variables and risk factors of both the cases and control groups.

	Cases (N_1 = 95)	**Controls (N_0 = 61)**	***p*-Value**
Age (Mean ± SD)	57 ± 12.4	57.3 ± 13.3	0.86
Gender (Males, %, n)	52 (54.7%)	35 (57.3%)	0.74
BMI	20.4 ± 3.4	20.6 ± 3.5	0.61
Dietary habits	**Cases (N_1 = 95)**	**Controls (N_0 = 61)**	***p*-Value**
Rice based	90 (94.7%)	57 (93.4%)	0.73
Wheat based	39 (41.0%)	18 (29.5%)	0.14
Spicy food	58 (61.0%)	29 (47.5%)	0.09
Alcohol	19 (20.0%)	14 (22.9%)	0.66
Smoking	27 (28.4%)	10 (16.3%)	0.08
Socioeconomic status			
Low	48 (50.5%)	36 (59%)	0.45
Middle	46 (48.4%)	25 (41%)	
Higher	1 (1.1%)	0	
H. pylori positive	33 (34.7%)	13 (21.3%)	0.07

N_1 = Total number of samples in case group. N_0 = Total number of samples in control group. Column 2 = Individual frequency distribution of different factors for case group. % = Fraction distribution of individual factors for the case group. Column 3 = Individual frequency distribution of different factors for control group and % = Fraction distribution of individual factors for the control group. A two-sided χ^2 test; the tabulated χ^2 value was 156 with df = 154 (as sample size was 156); the tabulated values of all individual criteria in the SPSS demi table were less the tabulated value, for df = 156, the p value = 0.05 for the comparison between cases and control data using χ^2 tests ± χ^2 test; $\chi^2 = \sum (O_i - E_i)^2/E_i$. χ^2 = Chi square. O_i = observed value. E_i = Expected value ± p-value < 0.05 is significance. For dietary factors such as rice based, wheat based, spicy food and consumption of alcohol, the p-values > 0.05; hence, we accepted the null hypothesis. Moreover, socioeconomic status of the p-values > 0.05, again retaining the null hypothesis.

Table 3. Comparison of TNF-α gene polymorphisms in cases and controls.

Polymorphisms	**Cases (n = 95)**	**Controls (n = 61)**	**Odds Ratio (95% CI)**	***p*-Value**
TNF-α−308 G/A				
GG	74 (77.9%)	52 (85.2%)	Ref.	
GA	12 (12.6%)	6 (9.8%)	1.4 (0.5–8.1)	0.2
AA	9 (9.5%)	3 (5%)	2.1 (0.5–3.9)	0.5
GA/AA	21 (22.1%)	9 (14.8%)	1.6 (0.6–3.8)	0.2
TNF-α−238 G/A	**Cases (n = 95)**	**Controls (n = 61)**	**Odds Ratio (95% CI)**	***p*-Value**
GG	75 (78.9%)	49 (80.3%)	Ref.	
GA	13 (13.7%)	7 (11.5%)	1.2 (0.4–3.2)	0.7
AA	7 (7.4%)	5 (8.2%)	0.9 (0.2–3.1)	0.8
GA/AA	20 (21.1%)	12 (19.7%)	1.09 (0.4–2.4)	0.8
TNF-α-T−857C	**Cases (n = 95)**	**Controls (n = 61)**	**Odds Ratio (95% CI)**	***p*-Value**
CC	71 (74.7%)	54 (88.5%)	Ref.	
CT	16 (16.8%)	4 (6.5%)	3.0 (0.9–9.6)	0.06
TT	8 (8.4%)	3 (5%)	2.0 (0.5–8)	0.3
CT/TT	24 (25.2%)	7 (11.5%)	0.7 (0.2–2.1)	0.5
H. pylori positive subjects	Cases (n = 33)	Controls (n = 13)		
TNF-α−308				
GG	18 (54.5%)	10 (77%)	Ref.	
GA/AA	15 (45.5%)	3 (23%)	2.7 (0.6–11.9)	0.17
TNF-α−238				
GG	21 (63.6%)	11 (84.6%)	Ref.	
GA/AA	12 (36.4%)	2 (15.4%)	3.1 (0.5–16.6)	0.17
TNF-α−857				
CC	20 (60.6%)	11 (84.6%)	Ref.	
CT/TT	13 (39.4%)	2 (15.4%)	3.5 (0.6–18.8)	0.13

OR and 95% CI for log-additive model for demographic and each allele estimated by unconditional logistic regression analysis. OR > 1 Associated with disease (null hypothesis was rejected). OR < 1 Protective. OR = 1 No association. If the 95% confidence interval for an OR includes 1, it means the results are not statistically significant. AOR: Adjusted odds ratio. AOR is a conditional odds ratio. p-value significant ≤ 0.05. The degrees of freedom for the chi-square df = $(r - 1)$ $(c - 1)$. when chi-square test statistic is greater than the critical value, the null hypothesis was rejected.

$$Odds\ ratio(OR) = \frac{Odds\ of\ being\ exposed - case}{Odds\ of\ being\ exposed - control}$$

A subgroup analysis of *H. pylori*-positive patients was performed. There was no significant difference in the distribution of GA/AA polymorphisms in TNF-α−308 (15 individuals (45.5%) vs. 3 individuals (23%) at $p = 0.16$) and at the location 238, (12 individuals (36.3%) vs. 2 individuals (15.4%) at $p = 0.16$) and −857 CT/TT (13 individuals (39.4%) vs. 2 individuals (15.4%) at $p = 0.11$), among *H. pylori* positive cases and controls.

Among the GC patients, a significantly higher percentage of *H. pylori* positives (numbers, 33) had GA/AA polymorphisms at the location 308 (45.5% vs. 9.7% at $p < 0.001$) and at the location 238 (36.4% vs. 12.9% at $p = 0.008$). However, at the location 857 CT/TT variants (9.1% vs. 8.1% at $p = 0.86$), no significant difference was observed when compared to *H. pylori*-negative patients (numbers, 62). However, the association of *H. pylori* positivity with any of the polymorphisms was not established in the one-year survival outcomes (Figure 1).

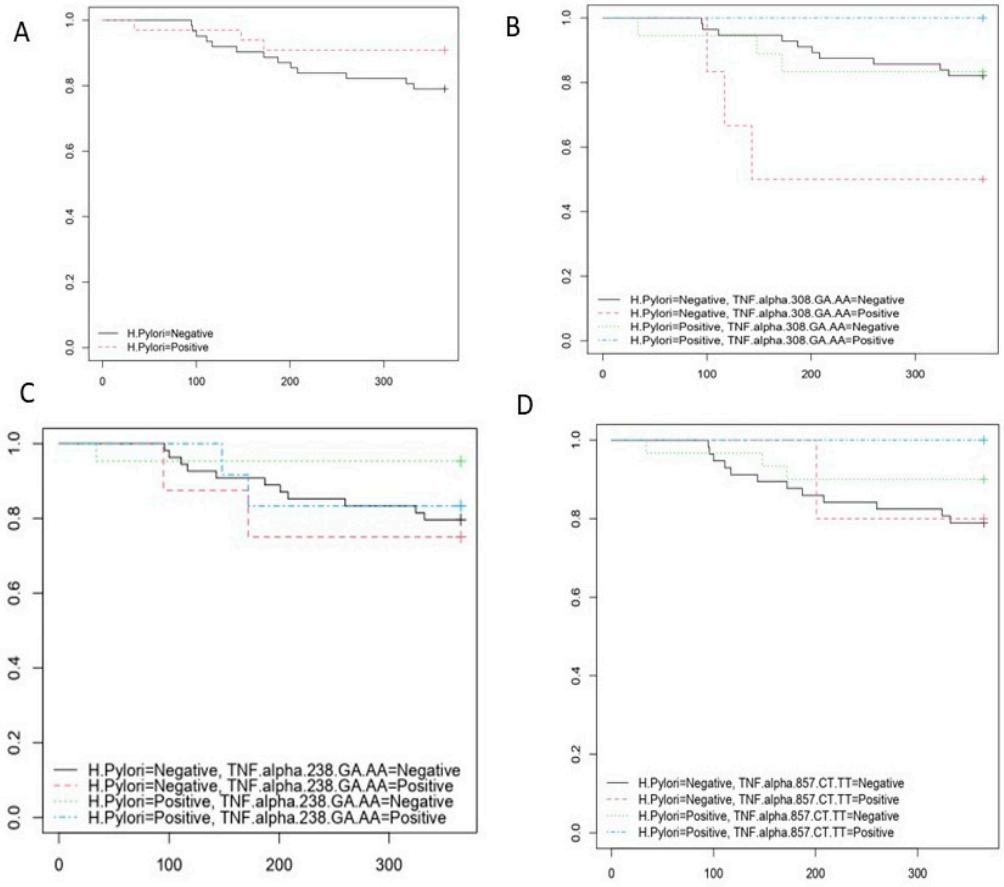

Figure 1. Kaplan–Meier plots (**A**–**D**) showing the one-year survival outcomes among different groups of patients with gastric cancer. Time in days on *x*-axis and survival probability on *y*-axis. On log rank test, no significant difference was observed between the compared groups ($p > 0.05$).

4. Discussion

TNF-α is an important cytokine mediating the inflammatory process and is also identified as a tumor promoter in the local tumor microenvironment [19]. It was observed from animal studies that TNF-α knock-down mice are resistant to chemical-induced tumorigenesis of skin and colon [20,21]. The NF-α mutations are found to be associated with advanced cancers and a poor prognosis. The mechanism of TNF-α and its receptor signaling can promote the development of GC by inducing NADPH oxidase organizer 1 and G protein subunit alpha 14 in tumor cells [19].

The TNF is a cytokine with pleiotropic effects on a variety of cell types. It has been identified as a key regulator of inflammatory responses and was implicated in the pathogenesis of several inflammatory and autoimmune diseases [22]. The TNF-α plays a major role in infection control; the release of TNF by macrophages appears critical for the formation and maintenance of granulomas and for defending intracellular organisms against invasion as well. TNF was stated to be involved in leukocyte trafficking and the clearance of immune complexes (IC) also [23]. Moreover, the endometriosis was caused by the combination of genetic and environmental factors; nevertheless, several genes and SNPs in physiopathology and development had been identified recently [24].

TNF-α was found to be not only involved in cell transformation and proliferation but also in tumor metastasis. Another study indicated that TNF-α induces the upregulation of CXCR4 expression in cancer cells, which was involved in the metastasis of GC [25].

Researchers from different parts of the world identified the association between TNF-α polymorphisms and the risk of GC. In a meta-analysis including 5054 subjects [26], TNF-α−857 C/T polymorphisms-TT/CT was associated with risk of GC (compared to CC, OR = 1.21, 95% CI: 1.07–1.38). Another similar study had reported an association of 857 C/T polymorphism with GC risk (TT + CT versus CC: OR = 1.16, 95% CI: 1.02–1.32) [27]. In a meta-analysis with 30 studies involving 7009 cases and 12,119 controls, a significant association was reported between the TNF-α−308 AA + GA, and the risk of GC compared to GG, OR = 1.20, 95% CI: 1.07–1.34, p = 0.001 was significant and suggested that the TaqMan method was the preferred genotyping method in DNA polymorphism studies [28]. The authors of this study reported that TNF-α−308 G/A polymorphism was correlated with GC risk in Caucasians but not in East Asians and other ethnic groups. Meanwhile, another study [29] reported that TNF-α 308 polymorphisms had a significant association with GC in normal population (GA/AA vs. GG; OR = 1.17, 95% CI: 1.10–1.23), but on analysis stratified by ethnicity, TNF-α 238 displayed an association with GC risk in eastern populations (GA/AA vs. GG, OR = 1.24, 95% CI: 1.02–1.50) but not in Western populations (GA/AA vs. GG, OR = 0.96, 95% CI: 0.79–1.18). In contrast, the association of NF-α−238 was not associated with the risk of GC in the present study at Eastern India. A recent metanalysis including 14 studies comprising a total of 2999 cases and 4685 controls revealed that no association exists between TNF-α−238 G/A polymorphism and GC occurrence. However, on a subgroup analysis based on ethnicity, a Chinese population with TNF-α−238 AA/GA had modestly increased risk of GC compared to those with GG (OR = 1.61, 95% CI: 1.27–2.03) [11]. In contrast, other case control studies from different East Asian and Latin American countries had revealed conflicting results in the association of GC with different TNF-α gene polymorphisms [13,15,30–32].

The result from the present study, when compared with a couple of studies from India, suggested that there was no combined significant difference between the combined genotype GA/AA and the GG genotype in TNF-α at the location 238 (p = 50.092; OR value was 53.20; 95% CI value was, 0.75–13.72) and TNF-α−308 G/A polymorphisms with GC risk, respectively [16,33], corroborating this study. The TNF-α−308 GA genotype had significant association, whereas homozygous genotype AA did not show association with GC risk [34]. In a meta-analysis, there was an overall statistically significant increased cancer risk associated with TNF-α at the location 308 G/G and A/A genotypes. The subgroup analyses had showed significant results for genotype G/G in Asians, whereas no such significant results were found for Caucasians and Hispanics [35], corroborating this study.

Furthermore, the results of the present study also corroborated with the previous study from the Asian population, which had explored that TNF-α−308 gene polymorphism might be significantly associated with the risk of gastric and hepatocellular carcinomas but not colorectal, pancreatic, or esophageal cancer in the Asian population [36]. The findings of another study had suggested that TNF−238G polymorphism of the TNF-α gene might be closely associated with susceptibility to *H. pylori* infected GC in Asian patients. This might be due to the high cytokine production by the TNF-α 238G allele, as suggested [37].

The present study obtained no association between TNF-α−308 (GA/AA vs. GG, OR = 1.6, 95% CI: 0.6–3.8), TNF-α−238 (GA/AA vs. GG, OR = 1.09, 95% CI: 0.4–2.4) and TNF-α−857 (CT/TT vs. CC, OR = 0.7, 95% CI: 0.2–2.1). The differences in ethnicity, diet and lifestyle in this cohort would have contributed to the contrasting results from other Asian studies, as GC is a multifactorial process and an interplay of various factors.

5. Conclusions

In conclusion, the gene, TNF-α (at locations, −308 G/A, alleles, −238 G/A and TNF-α T−857C) might not be the potential molecular markers of susceptibility for GC development. As future work, the researchers from the field of gastrointestinal cancer and cancer biologists would widely benefit from reading this study and further experimental studies with larger sample sizes investigating TNF-α, for more conclusive outcomes.

This study comprehensively evaluated the association of TNF-α polymorphisms at three loci and the risk of development of GC in the Eastern Indian population and evidenced a negative association of TNF-α infection from *H. pylori* infection.

This study could be the first Indian study to explore the genetic polymorphism of three promoter regions of the TNF-α gene as per the Scopus, PubMed and Web of Science databases published in various journals.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of IMS & SUM Hospital, Bhubaneswar.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

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References

1. Zhang, X.Y.; Zhang, P.Y. Gastric cancer: Somatic genetics as a guide to therapy. *J. Med. Genet.* **2017**, *54*, 305–312. [CrossRef] [PubMed]
2. Machlowska, J.; Baj, J.; Sitarz, M.; Maciejewski, R.; Sitarz, R. Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics, and treatment strategies. *Int. J. Mol. Sci.* **2020**, *21*, 4012. [CrossRef] [PubMed]
3. Ferlay, J.; Shin, H.R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D.M. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* **2010**, *127*, 2893–2917. [CrossRef] [PubMed]
4. Ang, T.L.; Fock, K.M. Clinical epidemiology of gastric cancer. *Singap. Med. J.* **2014**, *55*, 621. [CrossRef] [PubMed]
5. Ilic, M.; Ilic, I. Epidemiology of stomach cancer. *World J. Gastroenterol.* **2022**, *28*, 1187. [CrossRef]
6. Uthansingh, K.; Parida, P.K.; Pati, G.K.; Sahu, M.K.; Padhy, R.N. Evaluating the Association of Genetic Polymorphism of Cytochrome p450 (CYP2C9*3) in Gastric Cancer Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). *Cureus* **2022**, *14*, e27220. [CrossRef]
7. Muzaheed. *Helicobacter pylori* Oncogenicity: Mechanism, Prevention, and Risk Factors. *Sci. World. J.* **2020**, *2020*, 3018326. [CrossRef]
8. Li, L.; Liu, J.; Liu, C.; Lu, X. The correlation between TNF-α-308 gene polymorphism and susceptibility to cervical cancer. *Oncol. Lett.* **2018**, *15*, 7163–7167. [CrossRef] [PubMed]

9. Singh, P.K.; Bogra, J.; Chandra, G.; Ahmad, M.K.; Gupta, R.; Kumar, V.; Jain, A.; Ali Mahdi, A. Association of TNF-α (−238 and −308) promoter polymorphisms with susceptibility of oral squamous cell carcinoma in North Indian population. *Cancer Biomark.* **2015**, *15*, 125–131. [CrossRef] [PubMed]
10. Xie, H.; Yao, H.; Huo, Y.; Li, N.; Cheng, Y. Association between TNF-α gene 308G>A polymorphism and lung cancer risk: A meta-analysis. *Tumor Biol.* **2014**, *35*, 9693–9699. [CrossRef] [PubMed]
11. Zhao, H.; Liu, L.; Liu, B.; Wang, Y.; Li, F.; Yu, H. An updated association between TNF-α-238G/A polymorphism and gastric cancer susceptibility in East Asians. *Biosci. Rep.* **2018**, *38*, BSR20181231. [CrossRef] [PubMed]
12. Du, L.C.; 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*, 272–282. [CrossRef] [PubMed]
13. Kim, N.; Cho, S.I.; Yim, J.Y.; Kim, J.M.; Lee, D.H.; Park, J.H.; Kim, J.S.; Jung, H.C.; Song, I.S. The Effects of Genetic Polymorphisms of IL-1 and TNF-A on *Helicobacter pylori*-Induced Gastroduodenal Diseases in Korea. *Helicobacter* **2006**, *11*, 105–112. [CrossRef]
14. Jang, W.H.; Yang, Y.I.; Yea, S.S.; Lee, Y.J.; Chun, J.H.; Kim, H.I.; Kim, M.S.; Paik, K.H. The −238 tumor necrosis factor-α promoter polymorphism is associated with decreased susceptibility to cancers. *Cancer Lett.* **2001**, *166*, 41–46. [CrossRef]
15. Lee, S.G.; Kim, B.; Yook, J.H.; Oh, S.T.; Lee, I.; Song, K. TNF/LTA polymorphisms and risk for gastric cancer/duodenal ulcer in the Korean population. *Cytokine* **2004**, *28*, 75–82. [CrossRef]
16. Bhayal, A.C.; Krishnaveni, D.; RangaRao, K.P.; Bogadi, V.; Suman, C.; Jyothy, A.; Nallari, P.; Venkateshwari, A. Role of tumor necrosis factor-α-308 G/A promoter polymorphism in gastric cancer. *Saudi J. Gastroenterol.* **2013**, *19*, 182–186. [PubMed]
17. Lahiri, D.K.; Nurnberger, J.I., Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* **1991**, *19*, 5444. [CrossRef] [PubMed]
18. Ota, M.; Fukushima, H.; Kulski, J.K.; Inoko, H. Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. *Nat. Protoc.* **2007**, *2*, 2857–2864. [CrossRef] [PubMed]
19. Oshima, H.; Ishikawa, T.; Yoshida, G.J.; Naoi, K.; Maeda, Y.; Naka, K.; Ju, X.; Yamada, Y.; Minamoto, T.; Mukaida, N.; et al. TNF-α/TNFR1 signaling promotes gastric tumorigenesis through induction of Noxo1 and Gna14 in tumor cells. *Oncogene* **2014**, *33*, 3820–3829. [CrossRef]
20. Arnott, C.H.; Scott, K.A.; Moore, R.J.; Robinson, S.C.; Thompson, R.G.; Balkwill, F.R. Expression of both TNF-α receptor subtypes is essential for optimal skin tumour development. *Oncogene* **2004**, *23*, 1902–1910. [CrossRef] [PubMed]
21. Popivanova, B.K.; Kitamura, K.; Wu, Y.; Kondo, T.; Kagaya, T.; Kaneko, S.; Oshima, M.; Fujii, C.; Mukaida, N. Blocking TNF-α in mice reduces colorectal carcinogenesis associated with chronic colitis. *J. Clin. Investig.* **2008**, *118*, 560–570. [CrossRef] [PubMed]
22. Jang, D.I.; Lee, A.H.; Shin, H.Y.; Song, H.R.; Park, J.H.; Kang, T.B.; Lee, S.R.; Yang, S.H. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *Int. J. Mol. Sci.* **2021**, *22*, 2719. [CrossRef]
23. Maloy, S.; Hughes, K. (Eds.) *Brenner's Encyclopedia of Genetics*; Academic Press: Cambridge, MA, USA, 2013.
24. Mier-Cabrera, J.; Cruz-Orozco, O.; de la Jara-Díaz, J.; Galicia-Castillo, O.; Buenrostro-Jáuregui, M.; Parra-Carriedo, A.; Hernández-Guerrero, C. Polymorphisms of TNF-alpha (−308), IL-1beta (+3954) and IL1-Ra (VNTR) are associated to severe stage of endometriosis in Mexican women: A case control study. *BMC Women's Health* **2022**, *22*, 356. [CrossRef] [PubMed]
25. Zhao, C.; Lu, X.; Bu, X.; Zhang, N.; Wang, W. Involvement of tumor necrosis factor-α in the upregulation of CXCR4 expression in gastric cancer induced by *Helicobacter pylori*. *BMC Cancer* **2010**, *10*, 419. [CrossRef]
26. Cen, G.; Wu, W. Association between tumor necrosis factor-alpha 857C/T polymorphism and gastric cancer: A meta-analysis. *Tumour Biol.* **2013**, *34*, 3383–3388. [CrossRef]
27. Wang, P.; Wang, J.; Yu, M.; Li, Z. Tumor necrosis factor-α T-857C (rs1799724) polymorphism and risk of cancers: A meta-analysis. *Dis. Markers* **2016**, *2016*, 4580323. [CrossRef] [PubMed]
28. Yang, J.P.; Hyun, M.H.; Yoon, J.M.; Park, M.J.; Kim, D.; Park, S. Association between TNF-α-308 G/A gene polymorphism and gastric cancer risk: A systematic review and meta-analysis. *Cytokine* **2014**, *70*, 104–114. [CrossRef]
29. Zheng, W.; Zhang, S.; Zhang, S.; Min, L.; Wang, Y.; Xie, J.; Hou, Y.; Tian, X.; Cheng, J.; Liu, K.; et al. The relationship between tumor necrosis factor-α polymorphisms and gastric cancer risk: An updated meta-analysis. *Biomed. Rep.* **2017**, *7*, 133–142. [CrossRef]
30. Perri, F.; Piepoli, A.; Bonvicini, C.; Gentile, A.; Quitadamo, M.; Di Candia, M.; Cotugno, R.; Cattaneo, F.; Zagari, M.R.; Ricciardiello, L.; et al. Cytokine gene polymorphisms in gastric cancer patients from two Italian areas at high and low cancer prevalence. *Cytokine* **2005**, *30*, 293–302. [CrossRef]
31. Garza-González, E.; Hold, G.; Pérez-Pérez, G.I.; Bosques-Padilla, F.J.; Tijerina-Menchaca, R.; Maldonado-Garza, H.J.; el-Omar, E. Role of polymorphism of certain cytokines in gastric cancer in Mexico. Preliminary results. *Rev. Gastroenterol.* **2003**, *68*, 107–112.
32. Sugimoto, M.; Furuta, T.; Shirai, N.; Nakamura, A.; Xiao, F.; Kajimura, M.; Sugimura, H.; Hishida, A. Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *J. Gastroenterol. Hepatol.* **2007**, *22*, 51–59. [CrossRef] [PubMed]
33. Sultana, Z.; Bankura, B.; Pattanayak, A.K.; Sengupta, D.; Sengupta, M.; Saha, M.L.; Panda, C.K.; Das, M. Association of Interleukin-1 beta and tumor necrosis factor-alpha genetic polymorphisms with gastric cancer in India. *Environ. Mol. Mutagen.* **2018**, *59*, 653–667. [CrossRef] [PubMed]
34. Jiang, X.; Naikoo, N.A.; Gao, S. A meta-analysis of tumor necrosis factor-α-308 G>A polymorphism in gastric cancer. *Asian Biomed.* **2020**, *14*, 91–96. [CrossRef]

35. Rokkas, T.; Sechopoulos, P.; Pistiolas, D.; Kothonas, F.; Margantinis, G.; Koukoulis, G. Population differences concerning TNF-α gene polymorphisms in gastric carcinogenesis based on meta-analysis. Annals of gastroenterology: Quarterly publication of the Hellenic Society of Gastroenterology. *Ann. Gastroenterol.* **2014**, *27*, 139–148. [PubMed]
36. Guo, X.F.; Wang, J.; Yu, S.J.; Song, J.; Ji, M.Y.; Cao, Z.; Zhang, J.X.; Wang, J.; Dong, W.G. TNF-α-308 polymorphism and risk of digestive system cancers: A meta-analysis. *World. J. Gastroenterol.* **2013**, *19*, 9461–9471. [CrossRef] [PubMed]
37. Barua, R.R.; Barua, S.; Barua, H.R.; Barua, A.K.; Ansari, M.J.; Chong, J.M.; Uozaki, H.; Fukayama, M. Tumor necrosis factor-α polymorphism in *Helicobacter pylori* associated gastric carcinoma. *Bangladesh Med. Res. Counc. Bull.* **2019**, *45*, 170–174. [CrossRef]