Association of TGFB1 Polymorphisms rs1800469 and rs1800470 with Gastric Cancer Susceptibility in a South Indian Population: A Hospital-Based Case-Control Study

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Abstract

Background: TGFB1 variants may influence gastric cancer susceptibility through altered TGF-β1 signalling. TGF-β1 has a context-dependent role in carcinogenesis, acting as a tumour suppressor during early stages while promoting tumour progression in established cancers. Genetic variants that alter its expression or function may therefore contribute to interindividual differences in gastric cancer susceptibility.

Methods: This hospital-based case-control study included 110 histopathologically confirmed gastric cancer cases and 110 unrelated controls from South India. TGFB1 rs1800469 (C−509T) and rs1800470 (T869C; Leu10Pro) were genotyped using PCR-based restriction methods. Odds ratios (ORs) with 95% confidence intervals (CIs) were evaluated under codominant, dominant, recessive, overdominant, and allelic genetic models.

Results: For rs1800469, the overall genotype distribution differed significantly between cases and controls (P=0.025). The T allele was more frequent among cases than controls (44.5% vs 31.8%; OR=1.72, 95% CI=1.17–2.54; P=0.006). Significant associations were also observed for CT versus CC (OR=1.80, 95% CI=1.00–3.22; P=0.0497), TT versus CC (OR=2.80, 95% CI=1.23–6.40; P=0.014), and the dominant model (CT+TT vs CC: OR=2.00, 95% CI=1.16–3.48; P=0.013). rs1800470 showed no significant association under any of the genetic models examined. Within cases, rs1800469 genotype distributions did not differ significantly across the evaluated demographic, lifestyle, or clinicopathological characteristics.

Conclusion: TGFB1 rs1800469 was associated with gastric cancer susceptibility in this South Indian population, whereas rs1800470 showed no significant association. These findings warrant confirmation in larger, independent populations.

Keywords: TGFB1; Gastric cancer; rs1800469; rs1800470; Polymorphism; Genetic susceptibility; South India

I. Introduction

Gastric cancer is a biologically heterogeneous malignancy that develops through interactions among environmental exposures, infectious agents, host inflammatory responses, and inherited susceptibility. Helicobacter pylori infection, tobacco smoking, alcohol consumption, dietary factors, and family history contribute to risk, although the relative importance of these factors varies across populations [1–4]. India shows substantial regional heterogeneity in gastric cancer burden and exposure patterns, making population-specific molecular epidemiological studies relevant.

Transforming growth factor beta 1 (TGF-β1), encoded by TGFB1, is a multifunctional cytokine involved in epithelial homeostasis, immune regulation, apoptosis, extracellular-matrix remodeling, angiogenesis, and epithelial-mesenchymal transition. TGF-β signaling has a context-dependent role in cancer: it can restrain epithelial proliferation during early tumorigenesis but may facilitate immune evasion, stromal remodelling, invasion, and metastasis during later stages [5,6].

Two common TGFB1 variants have been investigated in relation to gastric cancer susceptibility. rs1800469 (C−509T), located in the promoter region, has been studied for its potential influence on TGFB1 transcription and TGF-β1 levels, whereas rs1800470 (T869C; Leu10Pro) is a coding polymorphism in the signal peptide region that may influence protein processing or secretion. However, association studies of these variants with gastric cancer have produced inconsistent findings across populations [7–11]. These differences may reflect variation in genetic background, environmental exposures, study design, control selection, and sample size.

The present study evaluated TGFB1 rs1800469 and rs1800470 in gastric cancer cases and controls from a South Indian population. In addition to case-control comparisons, rs1800469 genotype distributions were examined among cases according to demographic, lifestyle, and clinicopathological characteristics.

II. Materials and Methods

A. Study design and participants

A hospital-based case-control study was conducted in Visakhapatnam, Andhra Pradesh, India. Gastric cancer cases were recruited from King George Hospital, Mahatma Gandhi Cancer Hospital and Research Institute, and Pinnacle Cancer Hospital. The study included 110 histopathologically confirmed gastric adenocarcinoma cases and 110 apparently healthy unrelated controls from the same geographic region. Adults with newly diagnosed or untreated gastric adenocarcinoma were eligible as cases. Controls had no known history of malignancy and were unrelated to enrolled cases. All participants provided written informed consent. The study received institutional ethics approval (IEC53; 01-04-2022).

B. Data collection

Demographic and lifestyle variables included age, sex, rural or urban residence, smoking, alcohol use, tobacco chewing, and family history of cancer. Clinicopathological information among cases included tumor site, Lauren histological type, TNM stage, lymph-node metastasis, and distant metastasis. Data were obtained from the structured study proforma and available clinical or pathological records.

C. Blood collection and DNA extraction

Approximately 5 mL of peripheral venous blood was collected into EDTA tubes. Genomic DNA was extracted using a modified non-enzymatic salting-out method [12]. DNA concentration and purity were assessed spectrophotometrically, and DNA integrity was evaluated by agarose gel electrophoresis before genotyping.

D. Genotyping of TGFB1 rs1800469 (C−509T)

rs1800469 was genotyped by PCR-RFLP. A 150-bp fragment containing the polymorphic site was amplified using forward primer 5′-GAGACGCCTTGAAGTAACTG-3′ and reverse primer 5′-AACCAAAGATGTTCTGAACTGA-3′. PCR was performed in a 25-µL reaction containing 12.5 µL of 2× PCR master mix, 0.5 µL of each primer, 2.0 µL of template DNA, and 9.5 µL of nuclease-free water. Cycling comprised initial denaturation at 94°C for 10 min; 38 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s; and final extension at 72°C for 7 min. Amplicons were digested overnight at 37°C with Bsu36I and resolved on 3% agarose gel. Genotypes were assigned as CC (89 and 61 bp), CT (150, 89, and 61 bp), and TT (150 bp).

E. Genotyping of TGFB1 rs1800470 (T869C; Leu10Pro)

rs1800470 was genotyped using PIRA-PCR, in which a deliberate primer mismatch creates a restriction site that permits allele discrimination. The expected amplicon size was 136 bp. The forward primer was 5′-CTCCGGGCTGCGGCTGCAGC-3′ and the reverse primer was 5′-GGCCTCGATGCGCTTCCGCTTCA-3′. PCR was performed in a 25-µL reaction containing 2 µL genomic DNA, 12.5 µL of 2× PCR master mix, 0.5 µL of each primer, and 9.5 µL nuclease-free water. Cycling comprised 95°C for 5 min; 35 cycles of 95°C for 30 s, 62°C for 45 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. Products were digested overnight at 37°C with PvuII and separated on 3% agarose gel. Genotypes were assigned as TT (119 and 17 bp), TC (136, 119, and 17 bp), and CC (136 bp); the 17-bp fragment may not be clearly visualized because of its small size.

F. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0. Continuous variables were summarized as mean ±standard deviation, and categorical variables as frequencies and percentages.

Mean age was compared between cases and controls using Student's t-test, while categorical variables were compared using Pearson's chi-square test. For each TGFB1 variant, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated under codominant, dominant, recessive, overdominant, and allelic genetic models. Case-only analyses were performed to compare rs1800469 genotype distributions across demographic, lifestyle, and clinicopathological characteristics. All tests were two-sided, and P<0.05 was considered statistically significant.

III. Results

A. Participant characteristics

Table 1. Demographic and lifestyle characteristics of gastric cancer cases and controls

Characteristic	Category	Cases n (%)	Controls n (%)	P value
Age group	≤40 years	8 (7.3)	22 (20.0)	**0.004**
	41–50 years	20 (18.2)	28 (25.5)	
	51–60 years	36 (32.7)	34 (30.9)	
	>60 years	46 (41.8)	26 (23.6)	
Mean age ± SD, years		56.8 ± 10.9	56.2 ± 11.3	0.680
Sex	Male	78 (70.9)	75 (68.2)	0.660
	Female	32 (29.1)	35 (31.8)	
Residence	Rural	78 (70.9)	48 (43.6)	**<0.001**
	Urban	32 (29.1)	62 (56.4)	
Smoking	Non-smoker	58 (52.7)	82 (74.5)	**<0.001**
	Smoker	52 (47.3)	28 (25.5)	
Alcohol use	No	60 (54.5)	82 (74.5)	**0.002**
	Yes	50 (45.5)	28 (25.5)	
Tobacco chewing	No	72 (65.5)	92 (83.6)	**0.002**
	Yes	38 (34.5)	18 (16.4)	
Family history of cancer	No	90 (81.8)	102 (92.7)	**0.015**
	Yes	20 (18.2)	8 (7.3)	

Bold values indicate statistical significance at P<0.05.

Table 1 describes the demographic and lifestyle characteristics of gastric cancer cases and controls. The study included 110 gastric cancer cases and 110 controls. Mean age was similar between groups (56.8 ±10.9 vs 56.2 ±11.3 years; P=0.68), although the four-category age distribution differed significantly (P=0.004). Sex distribution was comparable between cases and controls. Rural residence, smoking,

alcohol use, tobacco chewing, and family history of cancer were significantly more frequent among cases than controls.

B. Clinicopathological profile

Table 2. Clinicopathological characteristics of 110 gastric cancer cases

Characteristic	Category	Cases n (%)
Tumor site	Cardia	18 (16.4)
	Body	24 (21.8)
	Antrum	58 (52.7)
	Whole stomach	10 (9.1)
Lauren type	Intestinal	68 (61.8)
	Diffuse	34 (30.9)
	Mixed	8 (7.3)
TNM stage	I	12 (10.9)
	II	24 (21.8)
	III	46 (41.8)
	IV	28 (25.5)
Lymph-node metastasis	Present	74 (67.3)
	Absent	36 (32.7)
Distant metastasis	Present	28 (25.5)
	Absent	82 (74.5)

Table 2 describes the clinicopathological characteristics of 110 gastric cancer cases. The antrum was the most frequent tumour site (52.7%), and intestinal-type carcinoma was the predominant Lauren subtype (61.8%). Stage III was the largest single TNM category (41.8%), while 74 patients (67.3%) had stage III–IV disease. Lymph-node metastasis was present in 67.3% of cases, and distant metastasis was present in 25.5%.

C. TGFB1 genotype and allele distributions

For rs1800469, the overall genotype distribution differed significantly between cases and controls (χ^2=7.349; P=0.025). CT and TT genotypes were more frequent among cases, whereas CC was more frequent among controls. The T allele frequency was also higher among cases than controls (44.5% vs 31.8%; P=0.006). For rs1800470, neither genotype distribution (P=0.839) nor allele distribution (P=0.541) differed significantly between cases and controls (Table 3).

Table 3. TGFB1 genotype and allele distributions among gastric cancer cases and controls

Variant	Genotype / allele	Cases n (%)	Controls n (%)	χ^2	P value
rs1800469	CC	34 (30.9)	52 (47.3)	7.349	**0.025**
	CT	54 (49.1)	46 (41.8)		
	TT	22 (20.0)	12 (10.9)		
	C allele	122 (55.5)	150 (68.2)	7.549	**0.006**
	T allele	98 (44.5)	70 (31.8)		
rs1800470	TT	50 (45.5)	54 (49.1)	0.352	0.839
	TC	46 (41.8)	44 (40.0)		
	CC	14 (12.7)	12 (10.9)		
	T allele	146 (66.4)	152 (69.1)	0.374	0.541
	C allele	74 (33.6)	68 (30.9)		

Bold values indicate statistical significance at P<0.05.

D. Genetic-model associations

Table 4. Associations of TGFB1 polymorphisms with gastric cancer susceptibility under different genetic models

Variant	Model	Comparison	OR	95% CI	P value
rs1800469	Codominant	CT vs CC	1.80	1.00–3.22	**0.0497**
	Codominant	TT vs CC	2.80	1.23–6.40	**0.014**
	Dominant	CT+TT vs CC	2.00	1.16–3.48	**0.013**
	Recessive	TT vs CC+CT	2.04	0.95–4.37	0.066
	Over dominant	CT vs CC+TT	1.34	0.79–2.28	0.279
	Allelic	T vs C	1.72	1.17–2.54	**0.006**
rs1800470	Codominant	TC vs TT	1.13	0.64–1.99	0.673
	Codominant	CC vs TT	1.26	0.53–2.98	0.599
	Dominant	TC+CC vs TT	1.16	0.68–1.97	0.589
	Recessive	CC vs TT+TC	1.19	0.52–2.71	0.676
	Over dominant	TC vs TT+CC	1.08	0.63–1.85	0.784
	Allelic	C vs T	1.13	0.76–1.69	0.541

Bold values indicate statistical significance at P<0.05.

Table 4 depicts the associations of TGFB1 polymorphisms with gastric cancer susceptibility under different genetic models. Analysis under different genetic models showed significant associations for rs1800469 in the CT-versus-CC and TT-versus-CC codominant comparisons, the dominant model, and the allelic model. The recessive and over dominant models were not significant. For rs1800470, no significant association was observed under any of the genetic models examined.

E. Case-only rs1800469 comparisons

Among gastric cancer cases, rs1800469 genotype distributions did not differ significantly according to age group, sex, smoking, alcohol use, or tobacco chewing. Likewise, no significant genotype differences were observed according to tumor site, Lauren histological type, TNM stage, lymph-node metastasis, or distant metastasis (Tables 5 and 6).

Table 5. TGFB1 rs1800469 genotype distribution by demographic and lifestyle characteristics among gastric cancer cases

Variable	Category	CC n (%)	CT n (%)	TT n (%)	P value
Age group	≤40	3 (37.5)	2 (25.0)	3 (37.5)	0.475
	41–50	6 (30.0)	9 (45.0)	5 (25.0)	
	51–60	8 (22.2)	22 (61.1)	6 (16.7)	
	>60	17 (37.0)	21 (45.7)	8 (17.4)	
Sex	Male	23 (29.5)	41 (52.6)	14 (17.9)	0.497
	Female	11 (34.4)	13 (40.6)	8 (25.0)	
Smoking	Non-smoker	17 (29.3)	27 (46.6)	14 (24.1)	0.519
	Smoker	17 (32.7)	27 (51.9)	8 (15.4)	
Alcohol use	No	21 (35.0)	29 (48.3)	10 (16.7)	0.481
	Yes	13 (26.0)	25 (50.0)	12 (24.0)	
Tobacco chewing	No	23 (31.9)	37 (51.4)	12 (16.7)	0.483
	Yes	11 (28.9)	17 (44.7)	10 (26.3)	

Table 6. TGFB1 rs1800469 genotype distribution by clinicopathological characteristics among gastric cancer cases

Variable	Category	CC n (%)	CT n (%)	TT n (%)	P value
Tumour site	Cardia	6 (33.3)	10 (55.6)	2 (11.1)	0.537
	Body	10 (41.7)	10 (41.7)	4 (16.7)	
	Antrum	16 (27.6)	30 (51.7)	12 (20.7)	
	Whole stomach	2 (20.0)	4 (40.0)	4 (40.0)	
Lauren type	Intestinal	17 (25.0)	35 (51.5)	16 (23.5)	0.491
	Diffuse	14 (41.2)	15 (44.1)	5 (14.7)	
	Mixed	3 (37.5)	4 (50.0)	1 (12.5)	
TNM stage	I	4 (33.3)	5 (41.7)	3 (25.0)	0.485
	II	10 (41.7)	8 (33.3)	6 (25.0)	
	III	13 (28.3)	27 (58.7)	6 (13.0)	
	IV	7 (25.0)	14 (50.0)	7 (25.0)	
Lymph-node metastasis	Present	22 (29.7)	39 (52.7)	13 (17.6)	0.504
	Absent	12 (33.3)	15 (41.7)	9 (25.0)	
Distant metastasis	Present	9 (32.1)	14 (50.0)	5 (17.9)	0.946
	Absent	25 (30.5)	40 (48.8)	17 (20.7)	

IV. Discussion

This study evaluated two TGFB1 polymorphisms in a South Indian hospital-based case-control population. The principal finding was an association between rs1800469 (C−509T) and gastric cancer susceptibility, whereas rs1800470 (T869C; Leu10Pro) showed no significant association. For rs1800469, the T allele and both CT and TT genotypes were more frequent among cases than controls. Significant associations were observed in the CT-versus-CC and TT-versus-CC comparisons, the dominant model, and the allelic model.

The rs1800469 finding is biologically plausible because this variant is located in the TGFB1 promoter and has been investigated for its potential influence on transcription and TGF-β1 levels. TGF-β signalling has a context-dependent role in cancer. During early tumorigenesis, it can constrain epithelial proliferation, whereas in established cancers it may contribute to immune suppression, stromal

remodeling, epithelial-mesenchymal transition, invasion, and metastasis [5,6]. Variation in TGFB1 regulation may therefore contribute to interindividual differences in gastric cancer susceptibility.

Published studies of rs1800469 and gastric cancer have reported inconsistent findings. Bhayal et al. reported an association of the −509 C/T polymorphism with gastric cancer in a South Indian population [7]. A meta-analysis by Li et al. supported increased gastric cancer susceptibility associated with the −509T variant, particularly under a recessive model [8]. An updated meta-analysis by Chang et al. similarly reported increased risk for the TT genotype under a recessive model [10]. In contrast, Niu et al. reported no significant overall association for C−509T, T869C, or G915C [9]. Differences among studies may reflect variation in genetic background, environmental exposures, sample size, and study design.

For rs1800470, genotype and allele frequencies were similar between cases and controls, and none of the tested genetic models showed a significant association. This finding is consistent with meta-analyses reporting no clear association between T869C and gastric cancer [9,10]. A broader field synopsis of genetic susceptibility to gastric cancer also highlighted heterogeneity in the evidence across candidate polymorphisms and populations [11].

Rural residence, smoking, alcohol use, tobacco chewing, and family history of cancer were significantly more frequent among cases than controls. These findings provide additional context for the multifactorial nature of gastric cancer susceptibility and are consistent with the contribution of environmental and lifestyle exposures described in previous studies [1,3,4].

Clinicopathologically, antral tumors were the most common, and intestinal-type carcinoma was the predominant histological type. A substantial proportion of patients presented with stage III–IV disease and lymph-node metastasis. However, rs1800469 genotype distributions did not differ significantly according to tumor site, Lauren type, TNM stage, lymph-node metastasis, or distant metastasis. Similarly, no significant genotype differences were observed across the evaluated demographic and lifestyle characteristics among cases.

The strengths of this study include histopathological confirmation of gastric cancer cases and the use of defined PCR-based restriction methods for genotyping. Reporting genotype and allele frequencies together with multiple genetic models enabled a comprehensive evaluation of the selected TGFB1 variants. Detailed demographic, lifestyle, and clinicopathological information also allowed exploratory evaluation of rs1800469 genotype distributions among cases.

This study has some limitations. The relatively modest sample size may have limited statistical power for less frequent genotypes and subgroup comparisons. The hospital-based design may also limit the

generalizability of the findings. In addition, information on certain potentially relevant factors, including H. pylori infection and detailed dietary exposures, was not available.

Future studies with larger, multicenter cohorts are needed to confirm these findings across diverse populations. Further investigation of gene–environment interactions and functional studies may help clarify the biological significance of TGFB1 variants in gastric cancer susceptibility.

V. Conclusion

In this South Indian hospital-based case-control study, TGFB1 rs1800469 was associated with gastric cancer susceptibility under codominant, dominant, and allelic comparisons, whereas rs1800470 showed no significant association under the genetic models examined. Within cases, rs1800469 genotype distributions were not significantly associated with the evaluated demographic, lifestyle, or clinicopathological characteristics. These findings suggest a possible role of rs1800469 in gastric cancer susceptibility and warrant confirmation in larger, independent populations.

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