Association between the MTHFR C677T polymorphism and gastric cancer susceptibility: A meta-analysis of 5,757 cases and 8,501 controls

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Abstract. Current data regarding the association between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and the risk of developing gastric cancer are insufficient to draw definite conclusions. Therefore, the present meta-analysis was conducted to achieve a more precise estimation of the association. MEDLINE, EMBASE and Wanfang database searches resulted in the identification of 28 eligible studies describing 5,757 cases and 8,501 controls. The strength of the association between the MTHFR C677T polymorphism and gastric cancer risk were evaluated using crude odds ratios (ORs), with 95% confidence intervals (CIs). The pooled ORs were determined using homozygous (TT vs. CC), heterozygous (CT vs. CC), dominant (TT+CT vs. CC) and recessive (TT vs. CC+CT) models. When all studies were pooled into the meta-analysis, significant associations were identified between the MTHFR C677T polymorphism and the risk of gastric cancer (homozygous model: OR, 1.39; 95% CI, 1.20-1.62; heterozygous model: OR, 1.18; 95% CI, 1.05-1.32; dominant model: OR, 1.23; 95% CI, 1.10-1.38; recessive model: OR, 1.26; 95% CI, 1.12-1.42). Stratification of the data by ethnicity identified a statistically significantly elevated risk of gastric cancer in Asian MTHFR C677T polymorphism populations (homozygous model: OR, 1.64; 95% CI, 1.43-1.90; heterozygous model: OR, 1.30; 95% CI, 1.16-1.45; dominant model: OR, 1.39; 95% CI, 1.25-1.54; recessive model: OR, 1.41; 95% CI, 1.25-1.51), but not in Caucasian populations (homozygous model: OR, 1.15; 95% CI, 0.89-1.48; heterozygous model: OR, 1.03; 95% CI, 0.84-1.25; dominant model: OR, 1.05; 95% CI, 0.86-1.28; recessive model: OR, 1.09; 95% CI, 0.91-1.31). Following adjustment for heterogeneity, the current meta-analysis demonstrated that the MTHFR C677T polymorphism was not associated with the risk of gastric cancer in Caucasian individuals. Furthermore, no evidence of publication bias was observed. Thus, the current meta-analysis indicates that the MTHFR C677T allele may be a low-penetrant risk factor for the development of gastric cancer in Asian populations.

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Introduction

Gastric cancer is the second most common cause of cancer-associated mortality in the world. In particular, it is one of the predominant cancer types in Korean and East Asian populations (1-4). Gastric cancer is a multifactorial malignant disorder caused by a wide range of risk factors, including genetic predisposition, the environment and *Helicobacter pylori* infection (1). Persistent *H. pylori* infection in the human stomach elicits a chronic inflammatory response, the extent of which may vary between individuals depending on the genetic makeup of the host. This phenomenon may aid in explaining the diverse range of outcomes observed in individuals infected with *H. pylori*. Therefore, polymorphisms in genes that are important in the host inflammatory response to this infection may alter an individual's susceptibility to gastric cancer (2). Notably, associations have been identified between gastric cancer and the expression of various genes involved in folate metabolism, such as methionine synthase (MTR), methylenetetrahydrofolate reductase (MTHFR) and MTR reductase (MTRR) (3).

MTHFR is an essential component of folate metabolism that has been indicated to be involved in DNA methylation and synthesis (4). The common MTHFR C677T polymorphism results in the substitution of alanine by valine, producing of a thermolabile variant that retains only ~30% of the activity of the wild-type MTHFR enzyme (5). The association between this gene polymorphism and the risk of gastric cancer has drawn increasing attention in the scientific community and has been investigated extensively, with 27 original studies (3,6-32) examining the role of the

MTHFR C667T polymorphism in the development of gastric cancer. However, these studies have yielded conflicting results, partially due to the small effect of the gene polymorphism on the risk of gastric cancer and the relatively small sample sizes used. Therefore, the aim of the current meta-analysis was to determine a more precise estimation of the association between the MTHFR C677T polymorphism and gastric cancer risk.

Materials and methods

Identification and eligibility of relevant studies. The current meta-analysis was performed according to the guidelines for systematic reviews of genetic association studies (33). Two investigators independently searched the MEDLINE, EMBASE and Wanfang electronic databases for studies published from inception to May 2013. Combining text words and Medical Subject Headings (MESH) terms, the following keywords were used to perform the literature search: 'MTHFR' or 'methylenetetrahydrofolate reductase' to search for MTHFR; 'gastric cancer' or 'stomach cancer' to search for gastric cancer; and 'gene', or 'polymorphism' or 'genetic variation' to search for genetic variations. The aforementioned search terms were used in conjunction with the 'explode' feature where applicable. Full studies published in the English and Chinese languages were considered for inclusion in the present study. In addition, the reference lists of all primary studies and reviews were manually searched. All case-control studies that investigated the association between the MTHFR 677C>T polymorphism and gastric cancer were included. Furthermore, when the same series was used in more than one case-control study, the study with the largest cohort was selected.

Data extraction. The following data was extracted from each of the selected studies: First author, year of publication, ethnicity of study population, and the number of cases and controls for each C677T genotype.

Statistical analysis. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the association between the MTHFR 677C>T polymorphism and the risk of gastric cancer. The pooled ORs were obtained for homozygous (TT vs. CC), heterozygous (CT vs. CC), dominant (TT+CT vs. CC) and recessive (TT vs. CC +CT) models. Heterogeneity assumption was examined using the χ^2-based Q test (34), with P≤0.01 considered to indicate heterogeneity among studies. Subsequently, the pooled OR estimate was calculated for each study using the fixed-effects model (Mantel-Haenszel method) (35). Otherwise, the random-effects model (DerSimonian and Laird method) was used (36). To evaluate the source of between-study heterogeneity, Galbraith plots was constructed to identify outliers that may be acting as major sources of between-study heterogeneity. In addition, subgroup analyses by ethnicity were performed. The potential publication bias of the present study was estimated by constructing a funnel plot in which the standard error of log(OR) was plotted against log(OR), for each study. Funnel plot asymmetry, which indicates a possible publication bias, was evaluated using Egger's linear regression test. Furthermore, the significance of the intercept was determined by performing a t-test, as proposed by Egger (37). P<0.05 was considered to indicate a statistically significant publication bias (38). All statistical analyses were performed using Stata software (version 10.0; Stata Corporation, College Station, TX, USA).

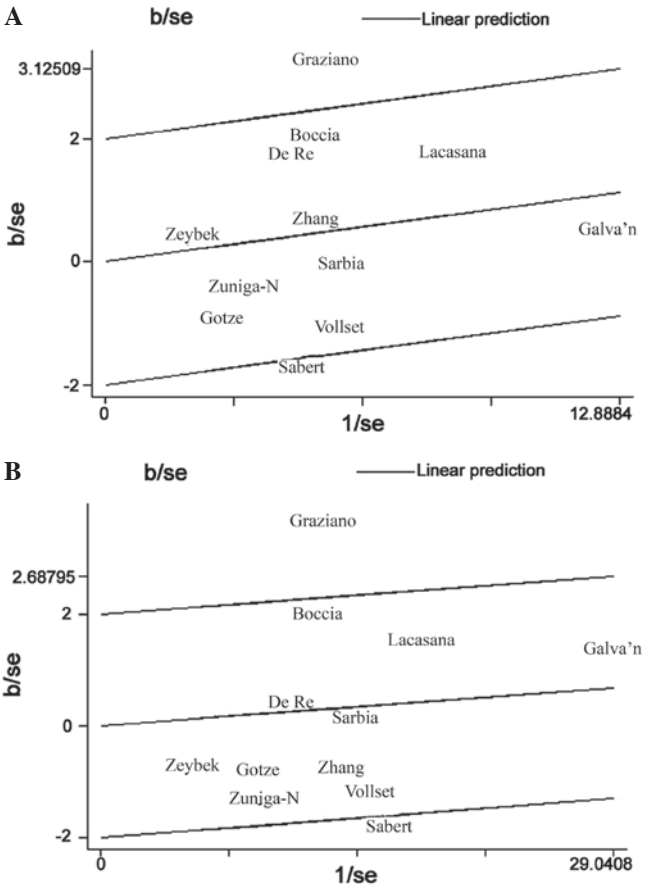

Figure 1. Galbraith plots of the association between the methylenetetrahydrofolate reductase T677C polymorphism and gastric cancer risk in Caucasian populations, using a (A) homozygous and (B) dominant model. Each author name represents a respective and separate study included in the current meta-analysis. b, bias; se, standard error.

Results

Study characteristics. A total of 27 publications met the inclusion criteria of the current meta-analysis (3,6-32), thus, a total of 5,757 cases and 8,501 controls were used in the pooled analyses. Tables I and II list the included studies and their major characteristics. In the 27 studies, the sample sizes ranged between 72 and 1,230 individuals. Furthermore, the studies included 12 European and 17 Asian populations, and the majority of controls were matched for gender and age.

Meta-analysis of the MTHFR C677T polymorphism. Table III indicates the major results of the current meta-analysis. When all the studies were pooled into the meta-analysis, the MTHFR T allele was determined to be associated with a significantly increased risk of developing gastric cancer (homozygous model: OR, 1.39; 95% CI, 1.20-1.62; heterozygous model: OR, 1.18; 95% CI, 1.05-1.32; dominant model: OR, 1.23; 95% CI, 1.10-1.38; recessive model: OR, 1.26; 95% CI, 1.12-1.42) (P<0.001). In the subgroup analysis by ethnicity, no significantly

Table I. Major characteristics of all studies included in the current meta-analysis.

First author (ref.)	Year of publication	Country	Ethnicity	Cases, n	Controls, n
Miao *et al* (8)	2002	China	Asian	217	468
Gao *et al* (7)	2002	China	Asian	107	200
Gao *et al* (32)	2001	China	Asian	107	200
Stolzenberg-Solomon *et al* (21)	2003	China	Asian	90	398
Bi *et al* (9)	2005	China	Asian	309	188
Shen *et al* (10)	2005	China	Asian	320	313
Sarbia *et al* (14)	2005	Germany	Caucasian	332	255
Wang *et al* (20)	2005	China	Asian	129	315
Si *et al* (18)	2005	China	Asian	122	101
Kim *et al* (19)	2005	Korea	Asian	133	445
Li *et al* (30)	2006	China	Asian	170	140
Graziano *et al* (13)	2006	Italy	Caucasian	162	164
Lacasaña-Navarro *et al* (24)	2006	Mexico	Caucasian	201	427
Weng *et al* (17)	2006	China	Asian	38	34
Zeybek *et al* (26)	2007	Turky	Caucasian	35	144
Wang *et al* (16)	2007	China	Asian	467	540
Götze *et al* (12)	2007	Germany	Caucasian	103	106
Zhang *et al* (3)	2007	USA	Caucasian	295	399
Mu *et al* (6)	2007	China	Asian	194	391
Boccia *et al* (11)	2007	Italy	Caucasian	102	254
Vollset *et al* (25)	2007	Europe	Caucasian	295	399
Li *et al* (15)	2007	China	Asian	170	140
Zúñiga-Noriega *et al* (23)	2008	Mexico	Caucasian	51	83
Galván-Portillo *et al* (22)	2009	Mexico	Caucasian	248	478
Yang *et al* (31)	2010	China	Asian	139	165
De Re *et al* (27)	2010	Italy	Caucasian	57	454
Saberi *et al* (29)	2012	Iran	Caucasian	450	780
Gao *et al* (28)	2013	China	Asian	264	535

increased risk of gastric cancer was identified in Caucasians with the MTHFR C677T polymorphism [homozygous model: OR, 1.15; 95% CI, 0.89-1.48 (Fig. 1A); heterozygous model: OR, 1.03; 95% CI, 0.84-1.25; dominant model: OR, 1.05; 95% CI, 0.86-1.28 (Fig. 1B); recessive model: OR, 1.09; 95% CI, 0.91-1.31]; however, significantly increased risks were identified in Asian populations (homozygous model: OR, 1.64; 95% CI, 1.43-1.90; heterozygous model: OR, 1.30; 95% CI, 1.16-1.45; dominant model: OR, 1.39; 95% CI, 1.25-1.54; recessive model: OR, 1.41; 95% CI, 1.25-1.51).

Publication bias. Begg's funnel plot and Egger's test were performed to assess the publication bias of studies included in the current meta-analysis. No evidence of marked asymmetry was observed in the funnel plot (Fig. 2). Furthermore, Egger's test did not indicate any statistical evidence of asymmetry and therefore, publication bias (homozygous model, P=0.866; heterozygous model, P=0.940; dominant model, P=0.851; recessive model, P=0.358).

Discussion

It is well documented that individual susceptibility to the development of cancer can vary, even when exposed to the same environmental carcinogens (2,33). This difference in susceptibility may be associated with genetic variations, such as polymorphisms, in genes involved in carcinogenesis. Therefore, genetic susceptibility to the development of cancer has been the focus of considerable scientific research. Recently, extensive investigation of genetic variants of the MTHFR gene has taken place to determine its role in the etiology of gastric cancer. Numerous studies have examined the role of the MTHFR C677T polymorphism in gastric cancer risk, however, the data is contradictory. Therefore, to improve understanding of the association between the MTHFR C677T polymorphism and the risk of gastric cancer, the present meta-analysis of pooled data from a large sample was conducted. To the best of our knowledge, this is the first meta-analysis regarding the association between the MTHFR C677T polymorphism and the risk of gastric cancer to be conducted. In addition, subgroup analysis and heterogeneity evaluations were performed. The results indicated that the MTHFR 677 T allele is associated with a significantly increased risk of developing gastric cancer. Furthermore, significant associations were identified in Asian individuals, but not in Caucasian individuals, indicating a possible role of ethnicity in the risk of gastric cancer, due to differences in genetic backgrounds, geography and environment (37). However, it is possible that the effect

Table II. Genotypes of the methylenetetrahydrofolate reductase C677T polymorphism included in the meta-analysis.

First author (ref.)	Year of publication	Cases, n			Controls, n		
		CC	CT	TT	CC	CT	TT
Gao *et al* (32)	2001	22	61	24	63	99	38
Miao *et al* (8)	2002	47	107	63	151	217	100
Gao *et al* (7)	2002	22	61	24	63	99	38
Stolzenberg-Solomon *et al* (21)	2003	17	36	37	65	209	124
Bi *et al* (9)	2005	139	150	20	97	76	15
Shen *et al* (10)	2005	105	171	44	113	172	28
Sarbia *et al* (14)	2005	138	153	41	107	115	33
Wang *et al* (20)	2005	25	45	59	74	143	98
Si *et al* (18)	2005	58	48	16	49	43	9
Kim *et al* (19)	2005	42	64	27	143	239	63
Li *et al* (30)	2006	61	78	31	67	56	17
Graziano *et al* (13)	2006	34	86	42	67	68	29
Lacasaña-Navarro *et al* (24)	2006	56	85	60	144	179	104
Weng *et al* (17)	2006	14	19	5	15	11	8
Zeybek *et al* (26)	2007	18	12	5	64	65	15
Wang *et al* (16)	2007	74	203	190	119	234	187
Götze *et al* (12)	2007	46	45	12	41	49	16
Zhang *et al* (3)	2007	146	116	33	185	178	36
Mu *et al* (6)	2007	50	106	38	135	199	57
Boccia *et al* (11)	2007	29	51	22	98	115	41
Vollset *et al* (25)	2007	109	104	32	248	277	94
Li *et al* (15)	2007	61	78	31	67	56	17
Zúñiga-Noriega *et al* (23)	2008	16	23	12	17	49	17
Galván-Portillo *et al* (22)	2009	37	132	79	89	217	172
Yang *et al* (31)	2010	44	80	15	62	75	28
De Re *et al* (27)	2010	18	25	14	152	238	64
Saberi *et al* (29)	2012	422	308	50	198	172	35
Gao *et al* (28)	2013	115	105	44	277	207	51

of the MTHFR 677 C allele is masked by the expression of thus far unidentified causal genes involved in the development of gastric cancer in Caucasian individuals. In addition, the ethnic differences observed in the present study may be due to chance, as studies with small sample sizes typically lack the statistical power to detect marginal effects and may generate a fluctuated risk estimate (39). Considering the limited number of studies included in the present meta-analysis and the small Caucasian populations, the current results should be interpreted with caution.

Heterogeneity is a potential problem that may affect the interpretation of the results of all meta-analyses. In the present meta-analysis, significant between-study heterogeneity for OR was identified in the overall comparisons (homozygous model, P=0.011; heterozygous model, P=0.003; dominant model, P=0.016; recessive model, P=0.039). However, subgroup analysis by ethnicity demonstrated that heterogeneity was only evident between studies involving Caucasian populations (homozygous model, P=0.006; recessive model, P=0.002) but not for those involving Asian populations (Table III) . Heterogeneity may also occur in poorly-designed studies that do not exclude biases, as these biases may affect the estimation of the real effects and cause incorrect conclusions to be drawn (40,41). Therefore, Galbraith plots were used to identify the outlier studies with poor quality designs. Following subgroup analysis of Caucasian studies, the Galbraith plot identified two studies that appeared to be major sources of heterogeneity (Fig. 1), with no between-study heterogeneity observed among the remaining 10 studies (homozygous model, P=0.345; recessive model, P=0.190). As a result, the fixed-effects model was used to pool the ORs from the two outlier studies, effectively removing heterogeneity from the current meta-analysis and thus confirming that the two excluded studies contributed the heterogeneity. Following adjustment for heterogeneity, the current data demonstrated that the MTHFR MTHFR C677T polymorphism was significantly associated with an increased risk of gastric cancer in Asian individuals, but not in Caucasian individuals.

A number of limitations should be taken into consideration when interpreting the findings of the current meta-analysis. First, the controls were not uniformly defined. Although the majority of the control subjects were recruited from healthy populations, certain individuals exhibited benign medical disorders. As a number of studies in the present meta-analysis included control groups that may have different risks of developing gastric cancer,

Table III. Pooled OR data obtained in the current meta-analysis.

Contrast model	Studies, n	OR (95% CI)	P-value	Model	I^2, %	P-value
Total studies						
Homozygous	27	1.39 (1.20-1.62)	<0.001	Random	41.5	0.011
Heterozygous	27	1.18 (1.05-1.32)	0.006	Random	47.3	0.003
Recessive	27	1.26 (1.12-1.42)	<0.001	Random	34.1	0.039
Dominant	27	1.23 (1.10-1.38)	<0.001	Random	52.8	0.016
Caucasian						
Homozygous	12	1.15 (0.89-1.48)	0.791	Random	58.2	0.006
Homozygous (adjusted for heterogeneity)	10	1.13 (0.93-1.36)	0.215	Fixed	10.6	0.345
Heterozygous	12	1.03 (0.84-1.25)	<0.001	Fixed	0.0	0.674
Recessive	12	1.09 (0.91-1.31)	0.367	Fixed	32.1	0.134
Dominant	12	1.05 (0.86-1.28)	0.609	Random	63.3	0.002
Dominant (adjusted for heterogeneity)	10	1.00 (0.88-1.14)	0.968	Fixed	27.6	0.19
Asian						
Homozygous	17	1.64 (1.43-1.90)	<0.001	Fixed	0.0	0.674
Heterozygous	17	1.30 (1.16-1.45)	<0.001	Fixed	2.6	0.423
Recessive	17	1.41 (1.25-1.61)	<0.001	Fixed	8.1	0.361
Dominant	17	1.39 (1.25-1.54)	<0.001	Fixed	0.0	0.729

OR, odds ratio; CI, confidence interval; I^2, index of heterogeneity.

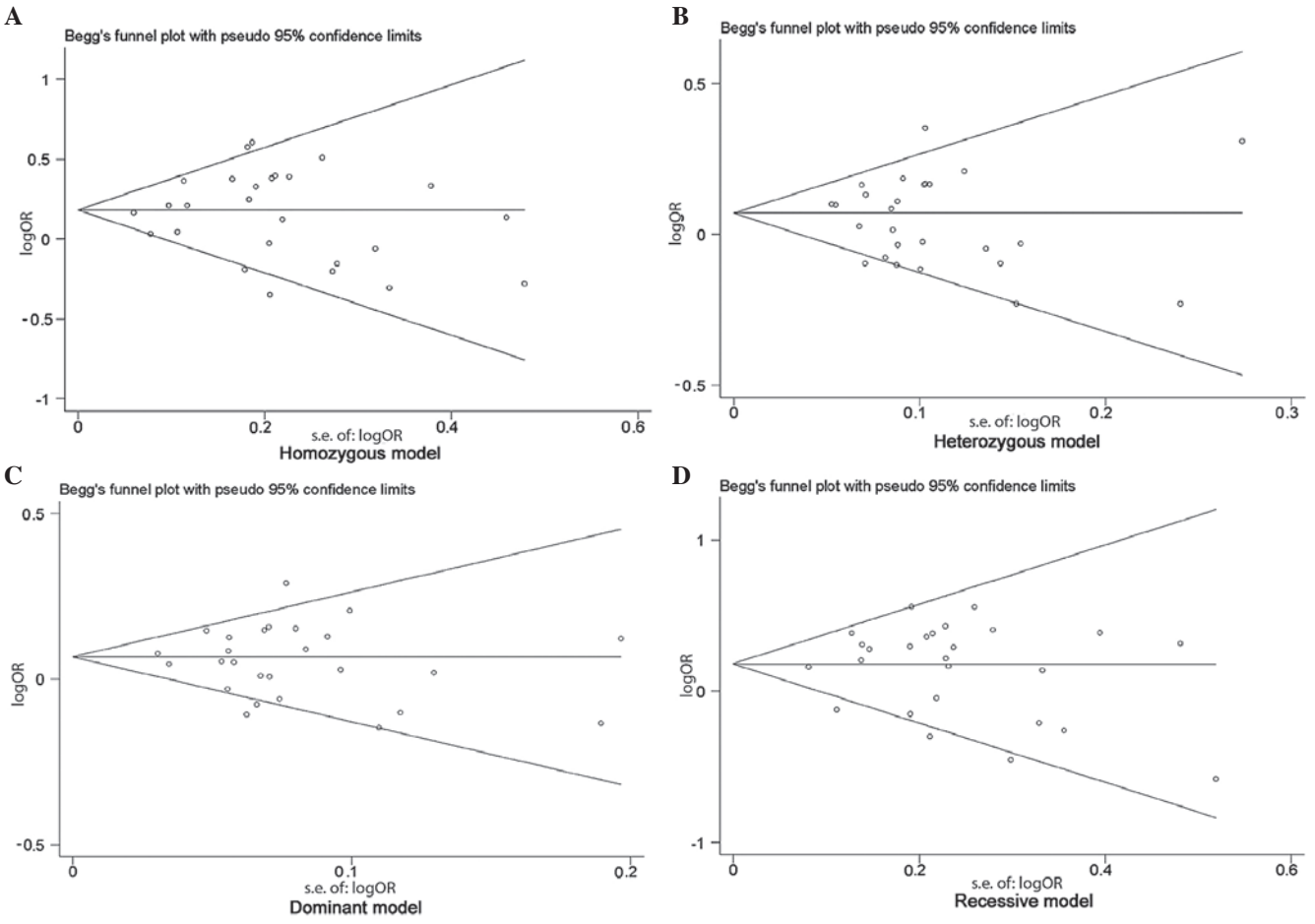

Figure 2. Begg's funnel plots for assessing the publication bias risk, using (A) homozygous (P=0.866), (B) heterozygous (P=0.940) (C) dominant (P=0.851) and (D) recessive (P=0.358) models. s.e., standard error; OR, odds ratio.

non-differential misclassification bias may have occurred. Second, the current results were based on unadjusted estimates. If individual data is made available, future studies should consider using it to perform more precise analyses, as individual data would allow for the adjustment for additional co-variates, such as age, smoking status, environmental factors and lifestyle. Despite the aforementioned limitations, the current meta-analysis exhibited high statistical power, as a large number of cases and controls were pooled from different studies. In addition, no publication bias was detected, indicating that the overall pooled effects were unbiased.

In conclusion, the current meta-analysis indicated that the MTHFR T allele is a low-penetrant genetic risk factor for the development of gastric cancer. However, well-matched case-control studies with homogeneous cancer patients of multi-ethnic groups using standardized unbiased genotyping methods are warranted in the future. Furthermore, it is recommended that investigations should be conducted into the effects of gene-gene and gene-environment interactions on the development of gastric cancer.

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