

## Research Article

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# Runt-related transcription factor 3 promoter hypermethylation and gastric cancer risk: A meta-analysis

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**Abstract:** Objective: The aim of this study was to investigate the correlation between runt-related transcription factor 3 (RUNX3) gene promoter hypermethylation and gastric cancer risk by meta-analysis. Methods: By searching Medline, PubMed, Embase, Cochrane, Ovid and CNKI electronic databases, the open published studies about the association between RUNX3 gene promoter hypermethylation and gastric cancer risk were screened. The hypermethylation rate in cancer tissue and autologous control tissue (normal gastric tissue of gastric cancer patients) were extracted from each included study. The odds ratio (OR) and corresponding 95% confidence interval (95% CI) of RUNX3 gene promoter hypermethylation in cancer tissue versus autologous control tissue of gastric cancer patients were pooled with random or fixed effect models. The publication bias was evaluated by Begg's funnel plot and Egger's line regression test. Results: Finally, twenty three relevant studies were included in this meta-analysis. The hypermethylation rate in cancer tissue and autologous control tissue of gastric cancer patients were  $0.56 \pm 0.16$  and  $0.18 \pm 0.22$  respectively, which demonstrated a hypermethylation rate in cancer tissue significantly higher than that of autologous controls ( $P < 0.05$ ). A significant positive correlation of hypermethylation rate between cancer tissue and autologous control existed for the included 23 studies ( $r_{\text{pearson}} = 0.62$ ,  $P < 0.05$ ). For significant heterogeneity across the studies, the OR was pooled by random effects model. The combined OR was

8.06 with the 95% CI of 5.73~11.32, which indicated the hypermethylation frequency in cancer tissue was higher than that of autologous controls. Conclusion: The RUNX3 gene promoter hypermethylation rate was much higher in cancer tissue than that of normal gastric tissue in patients with gastric cancer, which indicates a close association between gastric cancer and RUNX3 gene promoter hypermethylation. Furthermore, RUNX3 gene promoter hypermethylation may be a potential biomarker for gastric cancer diagnosis.

**Keywords:** gastric cancer; RUNX3 gene; hypermethylation; meta-analysis

## 1 Introduction

Gastric cancer is the most diagnosed common malignant tumor in the digestive system. Its morbidity and mortality have declined in recent years, but it is still the most diagnosed malignant carcinoma in the digestive system, which seriously affects people's health. Recently, many published studies have confirmed that hypermethylation of CpG islands plays an important role in carcinogenesis and cancer development. Runt-related transcription factor 3 (RUNX3) gene is a member of the runt domain family of transcription factors, also known as polyomavirus enhancer-binding protein 2 (PEBP2)/core binding factors (CBF) [1]. Several studies have shown that hypermethylation in the promoter region of RUNX3 can inactivate it and lead to the occurrence, development and metastasis of gastric cancer [2]. However, the hypermethylation frequency of RUNX3 was quite different in the previously published relevant studies, and whether the hypermethylation frequency in cancer tissue was higher than in the autologous controls was not clear.

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## 2 Materials and Methods

### 2.1 Relevant publication searching

The electronic databases of Medline, Pubmed, Embase, Cochrane, Ovid and CNKI were searched by two reviewers independently for the open published studies about RUNX3 gene promoter hypermethylation and gastric cancer. The text words for electronic database searching were as follows: “runt-related transcription factor 3/RUNX3”, “AML2”, “CBFA3”, “PEPB”, “gastric cancer”, “stomach cancer”, “gastric neoplasm”, “stomach neoplasm”. Furthermore, the references of the identified studies were also screened for potential suitable publications.

### 2.2 Study inclusion criteria

The publication inclusion criteria were as follows: (1) study type: prospective or retrospective clinical observation or cross-sectional study; (2) patients: the patients included in each study was confirmed by cytology or pathology; (3) Promoter hypermethylation methods: methylation specific polymerase chain reaction (MSP); (4) Results: hypermethylation frequency of cancer tissue and autologous controls were provided in each study or could be calculated. Exclusion criteria: (1) study type: Case report or review publications; (2) patients: the gastric cancer diagnosis was not confirmed by cytology or pathology; (3) Promoter hypermethylation methods: Other than methylation specific polymerase chain reaction; (4) Results: hypermethylation frequency of cancer tissue and autologous controls can't be extracted or calculated from each individual study.

### 2.3 Data extraction

The data and general characteristics of each included study were extracted by two reviewers independently. The extracted information includes: (1) General information, such as title, first and corresponding authors, date of paper publication and journal name; (2) Study character: patients' race, sample size, hypermethylation detection methods; (3) Outcomes: hypermethylation frequency of cancer tissue and autologous control of gastric cancer patients.

### 2.4 Statistical analysis

The data was analyzed by STATA 11.0 (for meta-analysis) software. The association between RUNX3 gene promoter hypermethylation and gastric cancer was expressed by odds ratio(OR) and corresponding 95% confidence interval (95% CI). The heterogeneity across the included 23 studies was assessed by  $I^2$  test. The correlation between cancer tissue and autologous control tissue was examined by Pearson's correlation test. Two tails  $P < 0.05$  was considered statistically different.

## 3 Results

### 3.1 Publication searching

One hundred and sixty two studies were initially identified from searching the related databases. 27 publications were first excluded for duplicated publication. After reading the title and abstract, 80 studies were excluded for reasons. After reading the full text of the paper, 32 publications were further excluded for not fulfilling the inclusion criteria. Finally, 23 studies [3-25] were included in this study. The publication searching process is demonstrated in Figure 1. The general features of the included 23 publications are shown in Table 1.

### 3.2 Hypermethylation rate in cancer tissue and autologous controls

The hypermethylation rates in cancer tissue and autologous control tissue for gastric cancer patients were  $0.56 \pm 0.16$  and  $0.18 \pm 0.22$  respectively, which demonstrates that the hypermethylation rate in cancer tissue is significant higher than that of autologous controls ( $P < 0.05$ , Figure 2).

### 3.3 Hypermethylation correlation analysis

The correlation of hypermethylation rate between cancer tissue and autologous control tissue was examined by Pearson's correlation test. Significant positive correlation of hypermethylation rate between cancer tissue and autologous control existed for the included 23 studies ( $r_{\text{pearson}} = 0.62$ ,  $P < 0.05$ , Figure 3).

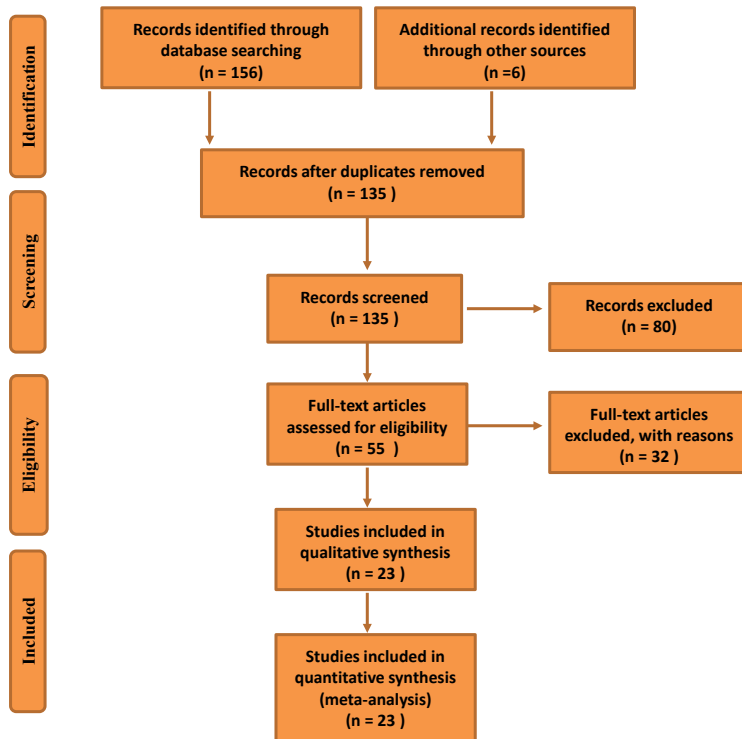
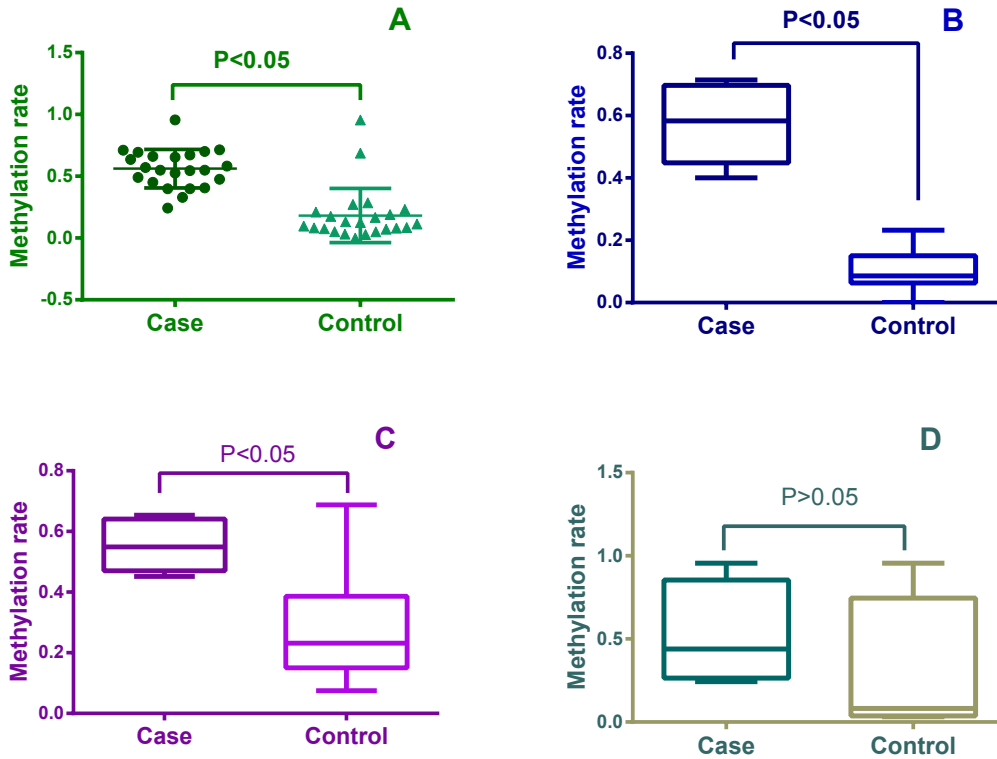


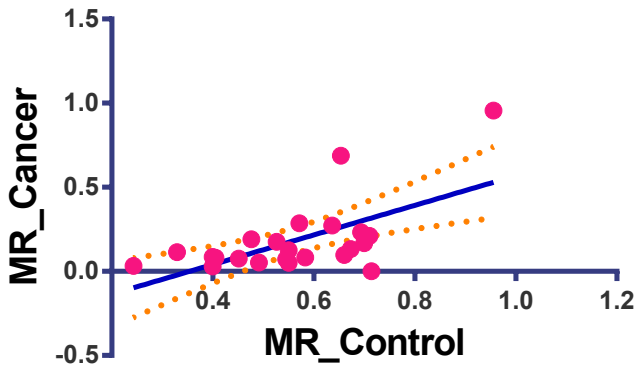
Figure 1. The publication searching flow chart of publication identification

Table 1. The general features of the included 23 studies

Authors	Tumor	Control	Age (mean/median)	Methods	Region	Year
	(M+/M-)	(M+/M-)				
Waki [3]	42/51	7/86	64.3	MSP	Japan	2003
Nakase [4]	14/8	6/16	65.4	MSP, qRT-PCT	Japan	2005
Homma [5]	43/2	43/2	63	MSP	U.S	2006
So [6]	17/9	11/5	64	MSP, microarray	Japan	2007
Li Q [7]	15/22	3/34	60.2	MSP	China	2007
Yang SH [8]	27/11	8/30	57	MSP	China	2007
Gargano [9]	22/18	2/38	Na	MSP	Italy	2007
Li LY [10]	22/18	5/35	62	MSP	China	2008
Kitajima [11]	30/27	10/47	65.7	MSP,PCR	Japan	2008
Song [12]	26/53	9/70	64	MSP	Korea	2008
Kim [13]	18/56	2/61	57.7	MSP	Korea	2008
Chen [14]	28/42	2/68	53	MSP, RT-PCT	China	2010
Hiraki [15]	28/21	14/35	68.6	qRT-PCT	Japan	2010
Lin H [16]	43/19	13/43	61.5	MSP	China	2010
Hu [17]	68/35	12/111	64.1	MSP, RT-PCR	China	2010
Mikata [18]	10/11	4/17	70	MSP, qRT-PCT	Japan	2010
Hu SL [19]	7/5	1/11	Na	MSP	China	2011
Tang H [20]	101/49	20/130	60.9	MSP	China	2012
He XB [21]	14/21	3/32	59	MSP	China	2012
Huang P [22]	21/9	5/25	55.2 (25-80)	MSP	China	2012
Liu JS[23]	30/12	0/42	53 (19-76)	MSP	China	2009
Liu P[24]	28/29	3/54	62.3 (34-81)	MSP	China	2013
Li Y[25]	30/25	4/51	60.0±8.6	MSP	China	2013



**Figure 2.** Scatter and box plot of hypermethylation rate between cancer tissue and autologous controls (A: scatter plot for all the included 23 studies; B: Box plot for China patients; C: Box plot for Japan patients; D: Box plot for U.S and Italy patients.)



**Figure 3.** Scatter plot for correlation of hypermethylation rate between cancer tissue and autologous control tissue

**Table 2.** The sub-group analysis of hypermethylation for RUNX3 gene in cancer tissue and autologous control tissue.

Region	Cancer tissue (M+/M-)	Control tissue (M+/M-)	OR	95%CI	P
China	434/297	79/666	12.46	9.33~16.64	<0.05
Japan	141/127	52/206	4.08	2.23~7.48	<0.05
Korea	44/109	11/131	4.96	2.14~11.47	<0.05
U.S	43/2	43/2	1.00	0.13~7.43	>0.05
Italy	22/18	2/38	23.22	4.92~109.67	<0.05

### 3.4 Meta-analysis

We first evaluated the statistical heterogeneity through  $I^2$  test. We found significant statistical heterogeneity across the included 23 publications. Therefore, the OR was pooled by random effect model. The combined OR was 8.06 with the 95% CI of 5.73~11.32, which indicates that the hypermethylation frequency in cancer tissue is higher than that of autologous controls (Figure 4).

### 3.5 Subgroup analysis

The odds ratios (OR) of hypermethylation for RUNX3 in cancer tissue and autologous control tissue for different geographic regions were also calculated for subgroup analysis. The hypermethylation frequency in cancer tissue was higher than in autologous controls for China, Japan, Korea and Italy ( $P < 0.05$ ), but not in the U.S.

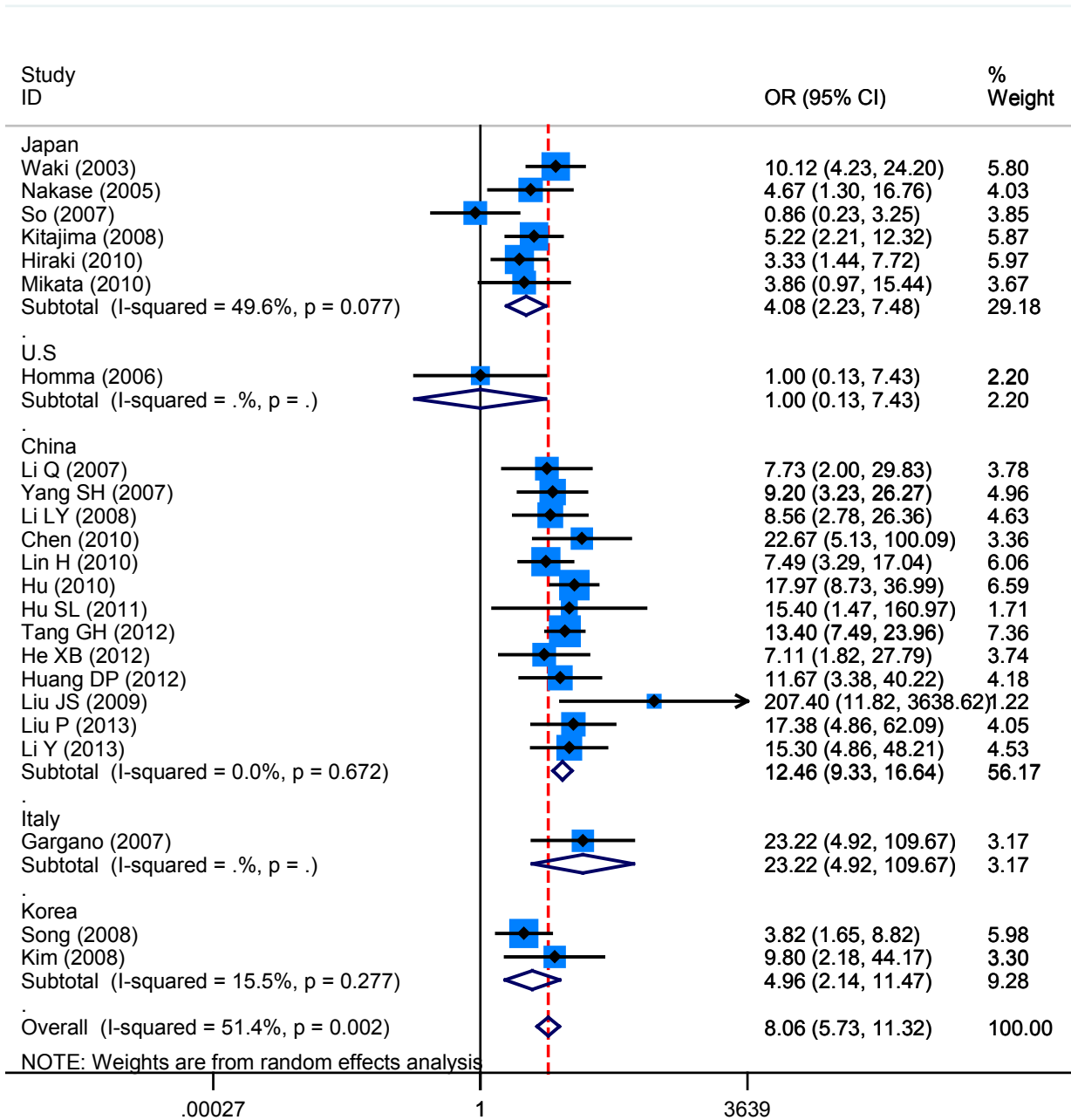


Figure 4. Forest plot of hypermethylation of RUNX3 in cancer tissue and autologous control tissue

### 3.6 Publication bias analysis

The publications bias across the included studies for the effect size of OR was calculated through Begg's funnel plot (Figure 5) and Egger's line regression test. No significant publication bias was found in this meta-analysis for the effect of OR ( $t=1.77$ ,  $P=0.09$ ).

## 4 Discussion

Previously published studies showed that human runt-related transcription factor 3 (RUNX3) gene participates in many genes' expression and regulation during human growth and development. This gene is a key one in mediating the TGF- $\beta$  signaling pathway. RUNX3 also plays an important role in the regulation of gastric mucosal growth. It can mediate the growth inhibition and apoptosis induced by TGF- $\beta$ , and regulate the differentiation and maturation of gastric mucosal epithelial cells. Inactivation of this gene or loss of heterozygosity may lead to intestinal metaplasia and dysplasia of the gastric mucosa. Hypermethylation of the RUNX3 gene promoter region is a major cause of inactivation of this gene and may lead to carcinogenesis in the normal gastric mucosa.

Waki and colleagues [3] evaluated the hypermethylation status of 10 gastric cancer cell lines and found 7 cell lines (7/10) were hypermethylated in the promoter region of RUNX3. Another study [21] evaluated the hypermethylation status of RUNX3 promoter region in human gastric cancer tissue and found that 45% of cancer tissue was hypermethylated, which was significantly higher than corresponding autologous controls tissues [21]. Nakase [4] found that the hypermethylation rates of RUNX3 in cancer and corresponding normal gastric tissue were 64% and 27% respectively. The results indicated that RUNX3 promoter hypermethylation was closely correlated with the development of human gastric cancer.

In our present study, we included the previously published studies and compared the hypermethylation frequency in cancer tissue and autologous controls in order to evaluate the correlation between human gastric cancer and RUNX3 promoter hypermethylation. However, there was significant statistical heterogeneity across the included studies. The heterogeneity may come from the different hypermethylation detection methods. 23 open published studies were included in this meta-analysis and we found that the hypermethylation rate in cancer tissue was much higher than that of the autologous controls. This indicates that RUNX3 promoter hypermethylation may play an important role in human gastric cancer

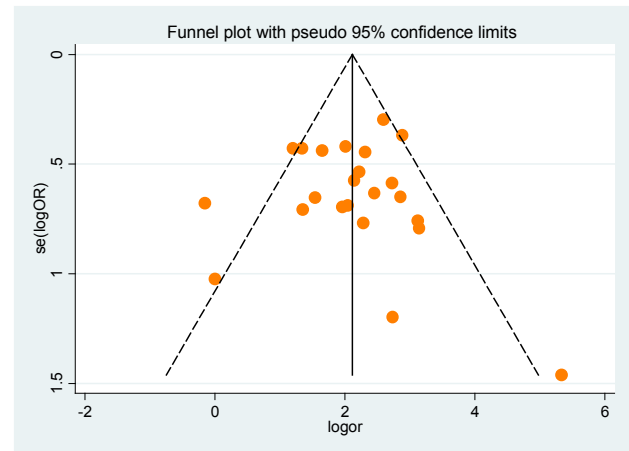


Figure 5. Begg's funnel plot for evaluation of publication bias

development. These results also reveal that the reversal of tumor suppressor gene promoter hypermethylation may open up new areas for cancer treatment [26, 27].

**Conflict of interest:** Authors state no conflict of interest

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