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Research Paper

MLH1 Promoter Methylation and Prediction/Prognosis of Gastric Cancer: A Systematic Review and Meta and Bioinformatic Analysis

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Abstract

Background: The promoter methylation of *MLH1* gene and gastric cancer (GC)has been investigated previously. To get a more credible conclusion, we performed a systematic review and meta and bioinformatic analysis to clarify the role of *MLH1* methylation in the prediction and prognosis of GC.

Methods: Eligible studies were targeted after searching the PubMed, Web of Science, Embase, BIOSIS, CNKI and Wanfang Data to collect the information of *MLH1* methylation and GC. The link strength between the two was estimated by odds ratio with its 95% confidence interval. The Newcastle–Ottawa scale was used for quantity assessment**.** Subgroup and sensitivity analysis were conducted to explore sources of heterogeneity. The Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) were employed for bioinformatics analysis on the correlation between *MLH1* methylation and GC risk, clinicopathological behavior as well as prognosis.

Results: 2365 GC and 1563 controls were included in the meta-analysis. The pooled OR of *MLH1* methylation in GC was 4.895 (95% CI: 3.149-7.611, P<0.001), which considerably associated with increased GC risk. No significant difference was found in relation to Lauren classification, tumor invasion, lymph node/distant metastasis and tumor stage in GC. Analysis based on GEO and TCGA showed that high *MLH1* methylation enhanced GC risk but might not related with GC clinicopathological features and prognosis.

Conclusion: *MLH1* methylation is an alive biomarker for the prediction of GC and it might not affect GC behavior. Further study could be conducted to verify the impact of MLH1 methylation on GC prognosis.

Key words: *MLH1*, methylation, gastric cancer, risk, prognosis

Introduction

DNA methylation is a major epigenetic alteration that plays a key role in the occurrence of cancer [1]. It is a genetically modified style with reversibility and heredity and has important biological significance, manifested in the control of tissue-specific gene expression, maintenance of chromosomal integrity [2]. The methylation of tumor-associated genes has been shown to be one of the important mechanisms involved in the process of gene transcriptional silencing and regulating gene expression then results in tumor suppressor gene inactivation, oncogene activation, eventually leading to tumorigenesis [3, 4]. The feasible technology to detect methylated DNA allows us to use DNA

methylation as a molecular biomarker for cancer prediction and prognosis [5-7].

DNA mismatch repair (MMR) system is one of key link in suppressing tumor formation, which can repair mismatched DNA in DNA replication to maintain genome stability. The MMR system contains a few key genes like *MLH1, MSH2, MSH6* and *PMS2 etc.*, from which encoding protein can form heterodimer to identified mismatched bases, together with other repair proteins, to complete DNA repair [8]. It is well accepted that the inactivation of MMR function is derived from germline mutation, somatic mutations or epigenetic silencing. The abnormalities of MMR can lead to microsatellite instability (MSI), which is short (1-6 base pairs) tandem repeats, spreading throughout the genome, being the identified heteromorphosis related to the occurrence and development of cancer. And colorectal cancers with MSI-H have an improved prognosis [9].MMR preferentially protects genes from mutation and has important consequences for understanding the evolution of genomes during both natural selection and human tumor growth. MMR deficiency disproportionately increases the numbers of single-nucleotide variants in genes [10].

MLH1 gene is localized at chromosome 3p22.2. It is responsible for the replacement of the mispaired nucleotides in the genome during the replication [11]. As a key member of MMR system, *MLH1* is epigenetically inactivated via methylation of the gene promoter that lead to the deficiency of MMR. For example, in colorectal cancer (CRC) MSI resulted from methylation of *MLH1* gene promoter, can cause its transcriptional silencing and affect other growth regulation and apoptosis-related genes, leading to the carcinogenesis of CRC. The majority of sporadic MSI tumors are caused by an epigenetic inactivation of MLH1 or MSH2. MMR deficient tumors have 10-100 times more somatic mutations than MMR proficient (pMMR) tumors leading to increased neoantigen burden and immunogenicity [12]. Similarly, *MLH1* hypermethylation is also preceded by malignant proliferation of other cancers such as endometrial cancer, lung cancer, breast cancer, esophageal cancer and gastric cancer [13-20]. So the detection of *MLH1* methylation can be used for prediction of tumorigenesis.

Gastric cancer (GC) is the third major cause of cancer-related deaths in the world [21]. Environmental, genetic, diet and other predisposing factors contribute to the development of gastric cancer. In recent years, more and more evidence shows that methylation of tumor suppressor gene is not be ignored risk factor in gastric carcinogenesis. In 2014, Cancer Genome Atlas Research classified GC into four pathological subtypes, in which MSI type including MMR methylation was proposed for the first time [22]. Along with the popularization of the classification, MMR methylation in gastric cancer had been widely studied. But the relevance between *MLH1* methylation and GC, especially the role of *MLH1* methylation on the risk prediction and prognosis of GC, remains controversial.

Here, we conducted a systematic review and meta and bioinformatic analysis to evaluate the correlation between *MLH1* promoter methylation and GC through comparing cancer with healthy controls. Moreover, we also assessed the correlation between *MLH1* promoter methylation and biological behavior as well as prognosis of GC by comparing cancer with different clinical pathological parameters and survival status. This study expects to get more credible information to assess the role of *MLH1* methylation in gastric cancer prediction and prognosis.

Methods

Search strategy

Electronic databases, including PubMed, Web of Science, Embase, BIOSIS, Chinese National Knowledge Infrastructure (CNKI), Wanfang Data were used to systematically look for related studies published in English and Chinese until May 1, 2017. The following terms were searched: methylation or DNA methylation or hypermethylation, gastric cancer or gastric carcinoma, and MLH1 or hMLH1. Furthermore, references that were cited in each included study were also searched manually to identify potential relevant studies.

Inclusion and exclusion criteria

Eligible studies had to meet the following inclusion criteria: 1) Research topic focused on the *MLH1* methylation and gastric cancer; 2) Case–control or cohort studies; 3) The studies with sufficient data for calculating odds ratios (ORs) and 95% confidence intervals (CIs); 4) Subjects investigated had a defined diagnosis by pathology. 1) Researches not related to methylation; 2) Researches not related to MLH1 methylation or methylation sites were not in the promoter region; 3) Researches not focus on GC, such as gastric ulcer and gastric functional dyspepsia and precancerous lesions; 4) Researches that selected subgroups (such as selected based on age, sex, and tumor stage); 5) Case reports and reviews; 6) Animal and cell studies. 7) Paper with insufficient or duplicated data. For duplicated data, only the most comprehensive studies were included.

Data extraction

Data from the included studies were extracted

independently by two authors, Shixuan Shen and Xiaohui Chen. The information was collected from extracted data including: the first author's name, publication year, country where study conducted, detection method, sample type, the frequency of *MLH1* methylation in case and control groups, clinicopathological parameters (i.e., Lauren classification, tumor invasion, lymph node status, distant metastasis and tumor stage) and survival status. The two authors reached a consensus on each item. If the data could not be obtained from the original studies, we would contact the corresponding author on reasonable request. If the authors are not convenient or willing to cooperate, we would exclude this study.

Quantity assessment

The Newcastle–Ottawa scale (NOS) with eight items was used to evaluate the quality of the included studies using three parameters: selection (four items, each awarded one star), comparability (one item, which can be awarded up to two stars) and exposure/outcome (three items, each awarded one star) [23]. NOS scores of 1–3, 4–6 and 7–9 were considered low, medium and high quality, respectively. Only studies with scores ≥ 7 were included in the analysis.

Bioinformatical analysis

We screened the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) which is a public repository that archives and freely distributes microarray [24] and use GEO2R (NCBI) to compare the methylation level in GC and normal tissues and then analyzed the association between *MLH1* promoter methylation and the GC risk.

The information of 338 GC patients was downloaded from the Cancer Genome Atlas (TCGA) database by TCGA-assembler in R software. We put the raw data into analyzing the role of *MLH1* promoter methylation in the GC risk prediction, behavior determination and prognosis evaluation.

Statistical analyses

Stata 11.0 (Stata Corporation, TX, USA) were used in this meta-analysis. The link strength between *MLH1* methylation and GC risk or clinicopathologic features was estimated by odds ratio (OR) with its 95% CI. The heterogeneity among the studies was assessed by Q-test and further quantified by the I^2 metric [25]. If there was substantial heterogeneity ($P<0.05$ or $I^2 > 50\%$), a random effect model was used to pool the ORs; otherwise, a fixed effect model was employed [26]. P value < 0.05 was considered statistically significant. Egger's linear regression test were applied to examine whether the results existed publication bias [27]. All tests were two-sided, and $P<0.05$ indicated statistical significance. When heterogeneity was shown, sensitivity analysis was performed to identify heterogeneity sources. Subgroup analysis was carried out to explore the effect of country, ethnicity, and methylation testing methods.

SPSS 22.0 software (SPSS, Chicago, IBM, USA) were used in the current bioinformatics analysis. Person χ^2 test was applied to evaluate the association of *MLH1* methylation with clinicopathologic features. Kaplan–Meier curves were drew to outline the survival status and the differences between the groups were analyzed using the log-rank test. P values<0.05 were considered statistically significant.

Results

Meta-analysis

Study characteristics

According to the literature selection criteria and search strategy, 26 studies [11, 17, 18, 28-50] were included in the present meta-analysis, including 2365 gastric cancer cases and 1563 nonmalignant controls. The study screening process is shown in Figure 1. Among these studies, 16 studies reporting 1794 cases and 1563 nonmalignant controls were selected to evaluate the relevance between *MLH1* methylation and GC risk. Furthermore, 10 studies, including 476 intestinal GC and 290 diffuse GC, estimated the Lauren classification-based association; 9 studies assessed the tumor invasion-based association; 16 studies explored the lymph node metastasis-based association; 8 studies appraised the distant metastasis-based association and 9 studies including 219 stage I–II patients and 422 stage III–IV patients evaluated the tumor stage-based association. These 26 studies were published between 2008 and 2016. All of them were written in English or Chinese. Of the studies, 10 came from China, 4 came from Brazil ,3 came from Japan, 2 came from Korea, 2 came from India, 1 came from Egypt, 1 came from Iran, 1 came from Lithuania, 1 came from Russia and 1 came from Spain, severally. The basic characteristics of all the included studies were summarized in Table 1. The NOS results showed that all the involved studies were at a higher quality level with scores ≥ 7. Full results of NOS quality assessment were summarized (Table S1).

MLH1 promoter methylation and GC risk

In the identification of GC and controls, slight heterogeneity was existed ($I^2 = 36.46\%$ and $P = 0.006$), therefore a random effect model was performed. Our results exhibited that the frequency of *MLH1* promoter methylation was enhanced in patients with

GC compared with control groups (OR= 4.895, 95% CI: 3.149-7.611, P<0.001, Figure 2, Table 2), showing that the *MLH1* methylation status was significantly associated with the GC risk. We furthermore performed subgroup analyses stratified by country, ethnicity, testing methods, and materials respectively. Country-specific OR showed an increased risk for individuals with the *MLH1* methylation compared with those without *MLH1* methylation in China (OR=15.222, 95% CI: 5.395-42.952, P<0.001) and Japan (OR=2.452, 95% CI: 1.158-5.193, P<0.001). Then we calculated the pool OR for *MLH1* promoter in the Asian subgroup, that was 5.949 (95% CI: 3.393-10.431, P<0.001) within a random effect model, and that for the Negroid subgroup was 5.017 (95% CI: 2.510-10.027, P<0.001) under a random effect model. But for the Caucasian subgroup, the pool OR was 1.744 (95% CI: 0.871-3.491, P= 0.116), showing no significance with *MLH1* promoter methylation. Subgroup analysis based on the testing methods indicated that considerably increased risks were found in both MSP (OR=5.426, 95% CI: 3.215-9.156) and Methylight (OR=3.168, 95% CI:1.521-6.599) groups (Table 2). Testing materials analysis revealed that the pool OR was 4.472 (95% CI: 2.874-6.959, P<0.001) for the tissue and 12.538 (95% CI:1.861-84.463, P=0.009) for the blood. That is, the GC risk was significantly raised in both tissue and blood subgroup.

MLH1 promoter methylation and GC clinicopathologic features

Fixed-effects model was applied for Lauren classification, tumor invasion, distant metastasis status and tumor stage (all P_h > 0.1) and random-effects model was used for lymph node status (P_h < 0.1). There was no significant difference in *MLH1* methylation detected in Lauren classification (OR=0.878, 95% CI: 0.619-1.244, P=0.463, Figure 3a), tumor invasion (OR=0.844, 95% CI: 0.568-1.253, P=0.400, Figure 3b), lymph node status (OR=0.929, 95% CI: 0.620-1.390, P=0.720, Figure 3c), distant metastasis status (OR=0.819, 95% CI: 0.481-1.396, P=0.464, Figure 3d) and tumor stage (OR=0.687, 95% CI: 0.455-1.039, P=0.075, Figure 3e) in gastric cancer(Table 3).

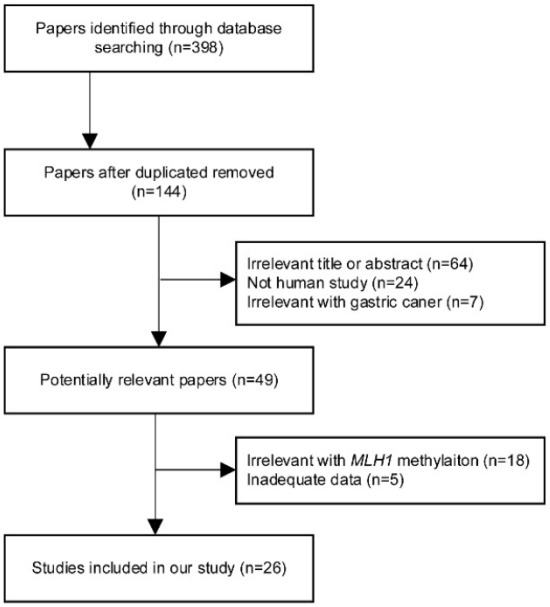

Figure 1. Flow chart of literature search and study selection.

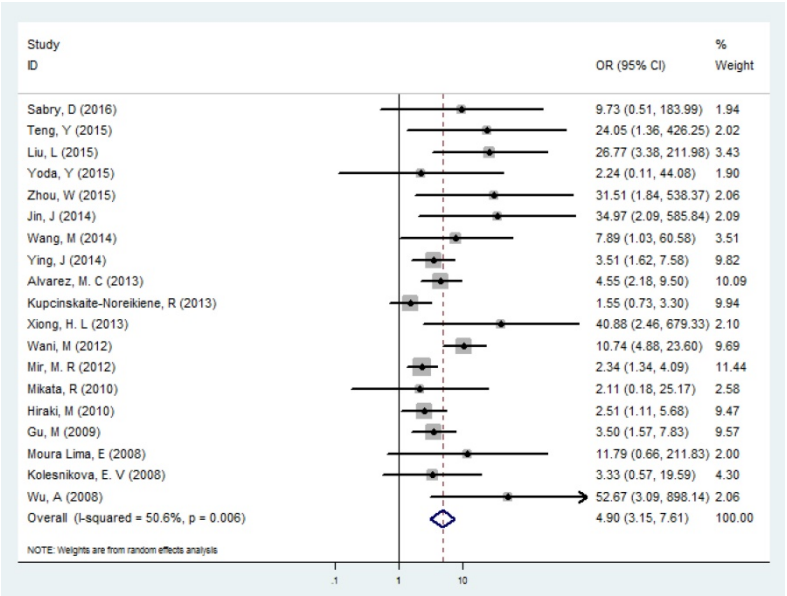

Figure 2. Forest plot of the correlation between *MLH1* methylation and GC.

Sensitivity analysis

For those groups which existed slight heterogeneity (GC risk I^2=36.46%, P_h=0.006, Lymph node status I^2=37.30%, P_h=0.072, Figure 4), Sensitivity analysis was subsequently performed to detect the influence of individual study on the pooled estimate by omitting one study from the pooled analysis each time. The exclusion of each single study did not significantly change the pooled OR, suggesting that the results of the meta-analysis were robust.

Publication bias

As indicated in Table 3, slight publication bias was perceived by Egger's test and Begg's test in the contrast of cancer and control groups, and also in distant metastasis as well as tumor stage subgroups. There was no obvious publication bias stated in other analytic subgroups. (all P > 0.05).

Table 1. Characteristics of the studies included in the meta-analysis

Author	Year	Country	Ethnicity	Method	Sample type	Case (M/U)	Control (M/U)	Lauren classification (M/U)		Tumor invastion (M/U)		Lymph node status(M/U)		Distant metastasis(M/U)		TNM stage (M/U)	
								Intestinal	Diffuse	T1-T2	T3-T4	Negative	Positive	Negative	Positive	Ⅰ-Ⅱ	Ⅲ-Ⅳ
Sabry, D	2016	Egypt	Negroids	Methy Light	Tissue	10/0	20/9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kupcinskaite, R	2016	Spain	Caucasians	MSP	Tissue	25/56	NA	13/29	12/27	7/14	18/42	11/17	14/39	NA	NA	NA	NA
Yoda, Y	2015	Japan	Asians	Bead array	Tissue	7/43	0/6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Teng, Y	2015	China	Asians	MSP	Tissue	13/27	0/24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Liu, L	2015	China	Asians	MSP	Tissue	24/26	1/29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Li, Y	2015	China	Asians	MSP	Tissue	22/80	NA	13/51	9/24	3/10	19/70	10/26	12/54	21/73	1/7	6/13	16/67
Zhou, W	2015	China	Asians	MSP	Blood	36/47	0/20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Wang, M	2014	China	Asians	Methy Light	Tissue	20/114	1/45	5/47	12/55	NA	NA	NA	NA	18/94	2/20	NA	NA
Jin, J	2014	China	Asians	MSP	Tissue	16/267	0/283	NA	NA	6/116	10/151	6/152	10/115	13/245	3/22	NA	NA
Moghbeli, M	2014	Iran	Asians	MSP	Tissue	13/38	NA	7/29	5/8	4/15	9/23	2/6	11/32	NA	NA	3/17	10/21
Guo, H	2014	China	Asians	MSP	Tissue	16/54	NA	NA	NA	6/20	10/34	6/15	10/39	15/51	1/3	8/25	8/29
Ying, J	2014	China	Asians	MSP	Tissue	29/91	10/110	NA	NA	NA	NA	3/13	26/78	22/75	7/16	3/15	26/76
Xiong, H. L	2013	China	Asians	MSP	Blood	19/394	0/413	NA	NA	8/170	11/224	9/222	10/172	15/362	4/32	NA	NA
Kupcinskaite, R	2013	Lithuania	Caucasians	MSP	Tissue	22/47	16/53	10/25	11/21	NA	NA	10/15	11/31	NA	NA	4/9	17/36
Alvarez, M. C	2013	Brazil	Negroids	MSP	Tissue	36/56	12/85	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kim, K. J	2013	South Korea	Asians	Methy Light	Tissue	80/22	NA	72/17	1/3	19/9	61/13	33/9	47/13	NA	NA	47/15	33/7
Wani, M	2012	India	Asians	MSP	Tissue	51/19	14/56	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mir, M. R	2012	India	Asians	MSP	Tissue	104/26	82/48	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alves, M. K	2011	Brazil	Negroids	MSP	Tissue	25/51	NA	15/33	10/18	NA	NA	NA	NA	NA	NA	NA	NA
Mikata, R	2010	Japan	Asians	MSP	Tissue	2/19	1/20	NA	NA	NA	NA	0/6	2/13	NA	NA	0/8	2/11
Hiraki, M	2010	Japan	Asians	Methy Light	Tissue	32/17	21/28	17/10	7/15	NA	NA	7/13	25/4	NA	NA	12/12	20/5
Ferrasi, A. C	2010	Brazil	Negroids	MSP	Tissue	27/62	NA	19/38	8/23	NA	NA	6/11	21/50	NA	NA	NA	NA
Gu, M	2009	Korea	Asians	MSP	Tissue	39/15	23/31	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Moura Lima, E	2008	Brazil	Negroids	MSP	Tissue	10/36	0/20	6/20	4/16	3/9	7/27	5/11	5/25	10/31	0/5	NA	NA
Kolesnikova, E. V	2008	Russia	Caucasians	MSP	Blood	5/15	2/20	NA	NA	2/8	3/7	2/7	3/8	4/12	1/3	NA	NA
Wu, A	2008	China	Asians	MSP	Tissue	18/42	0/60	NA	NA	NA	NA	14/37	4/5	NA	NA	5/17	13/25

Abbreviations: M, methylations; U, unmethylations; MSP, methylation-specifc PCR; NA, not available

Bioinformatical analysis

MLH1 promoter methylation and GC risk

We extracted 2 GEO series (GSEs) within 180 GEO series both related to gastric cancer and methylation. All two GSEs were solely derived from human tissues and used methylation probe to detect the methylation rate. We screened two methylation location, cg18320188 and cg02279071 on the CpG island of *MLH1* DNA sense strand. They located on chromosome 3 (37008972 – 37010459). Their sequences were showed in Table S2. After analyze the data using GEO2R, we found that *MLH1* promoter methylation showed a high level in GC compared to normal tissues (P=0.0149 from GSE30601 probe cg18320188, P=0.0442 from GSE25869 probe cg02279071).

Then we search data covering DNA methylation and gene expression on the website of MethHC (A database of DNA Methylation and gene expression in Human Cancer http://methhc.mbc.nctu.edu.tw/php/index.php) and the TCGA (The Cancer Genome Atlas) [51]. The data showed that there were significant differences in methylation levels between cancer and normal tissues (P<0.005). All the results expounded that *MLH1* methylation status was considerably related with the GC risk.

Table 2. Subgroup analysis of *MLH1* promoter methylation in gastric cancers compared with controls.

	Studies	Heterogeneity test		Test for overall effect	
		I2 (%)	Ph	OR (95% CI)	P-value
Gastric cancer risk	19	36.46%	0.006	4.895 (3.149-7.611)	<0.001
Subgroup					
Country					
China	8	46.20%	0.072	15.222 (5.395-42.952)	<0.001
Japan	3	0.00%	0.989	2.452(1.158-5.193)	<0.001
Ethnicity					
Asians	14	55.70%	0.006	5.949 (3.393-10.431)	<0.001
Caucasians	2	0.00%	0.436	1.744 (0.871-3.491)	0.116
Negroids	3	0.00%	0.729	5.017 (2.510-10.027)	<0.001
Methods					
MSP	15	59.20%	0.002	5.426 (3.215-9.156)	<0.001
Methylight	3	0.00%	0.408	3.168 (1.521-6.599)	0.002
Bead array	1	NA	NA	2.241 (0.114-44.084)	0.595
Materials					
Tissue	16	50.30%	0.011	4.472 (2.874-6.959)	<0.001
Blood	3	46.00%	0.157	12.538 (1.861-84.463)	0.009

Note: Values in bold indicate statistical significance.
Abbreviations: CI, confidence interval; P_h, P-value of Q test for heterogeneity among studies; OR, odds ratio; NA, not available

Table 3. Association of *MLH1* promoter methylation with clinicopathologic features in gastric cancer.

Clinicopathological features	Studies	Heterogeneity test		Statistical model	Test for overall effect		Begg's test		Egger's test	
		I^2 (%)	P_h		OR (95% CI)	*P*-value	z-value	P-value	t-value	P-value
Gastric cancer risk	19	36.46%	0.006	R	4.895 (3.149-7.611)	**<0.001**	0.190	0.234	3.110	0.006
Lauren classification	10	1.30%	0.426	F	0.878 (0.619-1.244)	0.463	0.000	1.000	1.470	0.180
Tumor invasion	9	0.00%	0.949	F	0.844 (0.568-1.253)	0.400	0.310	0.754	0.250	0.806
Lymph node status	15	37.30%	0.072	R	0.929 (0.620-1.390)	0.720	0.990	0.322	-1.050	0.314
Distant metastasis	8	0.00%	0.479	F	0.819 (0.481-1.396)	0.464	1.360	0.174	2.050	0.087
Tumor stage	9	0.70%	0.428	F	0.687 (0.455-1.039)	0.075	0.620	0.536	-0.160	0.877

Abbreviations: R, random effect model; F, fixed effect model

Table 4. Association of *MLH1* promoter methylation with clinicopathologic features in gastric cancer based on bioinfromatic analysis

Clinical features		Methylation status					
		cg18320188			cg02279071		
		M	U	P value	M	U	P value
Lauren classification	Intestinal	85	68	0.57	82	71	0.27
	Diffuse	36	34		32	38	
Tumor invastion	T1-T2	42	43	0.90	50	35	0.05
	T3-T4	127	126		118	135	
Lymph node status	Negative	55	50	0.56	58	47	0.19
	Positive	111	116		108	119	
Distant metastasis	Negative	152	149	0.79	155	146	0.43
	Positive	9	10		8	11	
TNM stage	stage I-II	79	75	0.90	83	71	0.18
	stage III-IV	88	86		81	93	

MLH1 promoter methylation and GC clinicopathologic features

We analyzed the association between *MLH1* methylation and clinicopathologic features such as Lauren classification, tumor invasion, distant metastasis status and tumor stage using the same methylation probe as GSE30601 cg18320188 and GSE25869 cg02279071. No correlation was found between the two. (Table 4).

MLH1 promoter methylation and GC prognosis

Firstly, the influence of *MLH1* methylation on recurrence free survival (RFS) time was assessed. A total of 338 patients with recurrence free survival time related data were enrolled in this section. Analytic results of Kaplan-Meier curve and Log-Rank test suggested that *MLH1* methylation was not significantly associated with RFS (Table 5, Figure 5). And then, the association between *MLH1* methylation and overall survival time (OS) was also evaluated. Similar to RFS, the results did not show any correlation between *MLH1* methylation and OS of GC (Table 5, Figure 5).

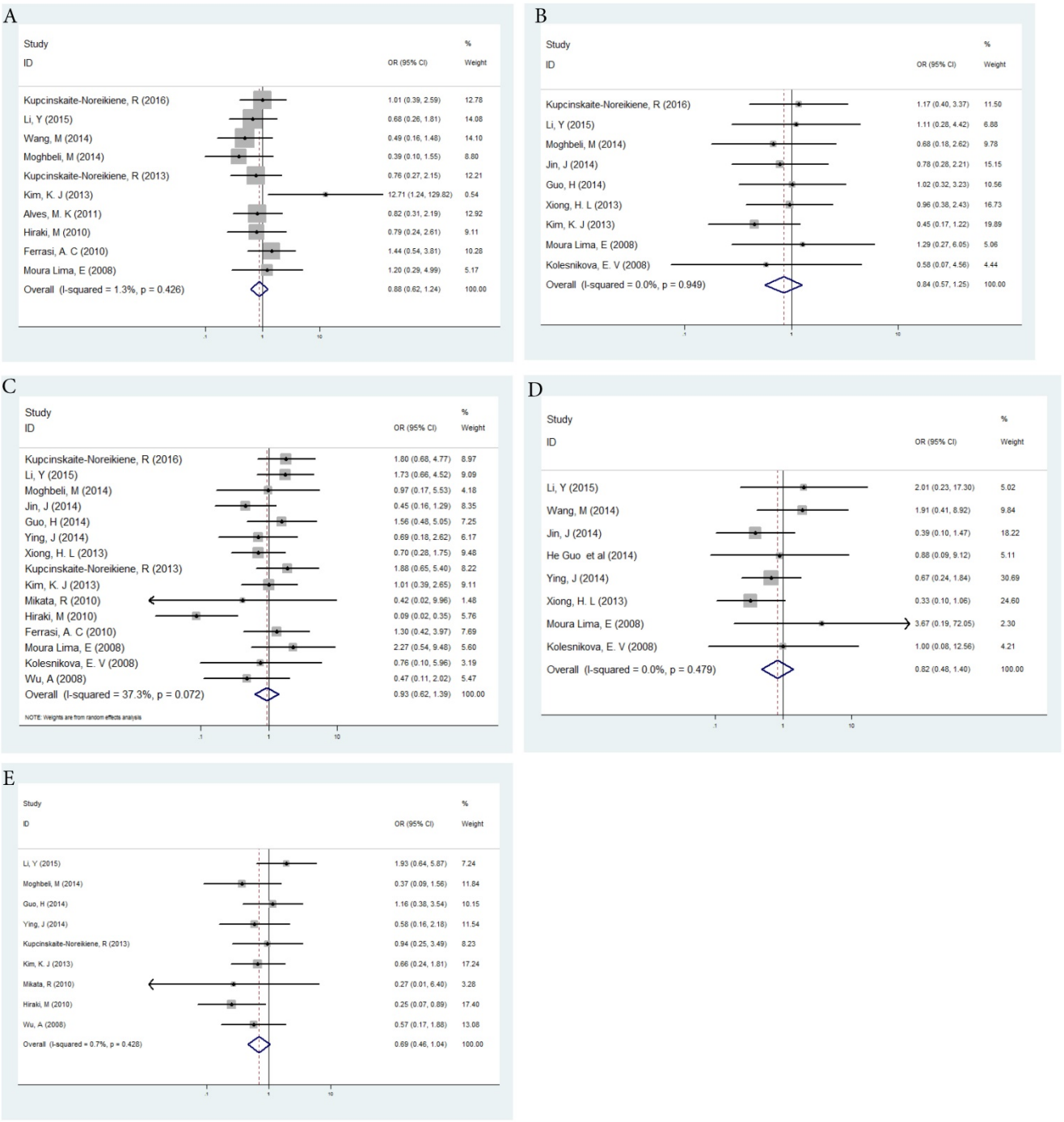

Figure 3. Forest plot of the correlation between *MLH1* methylation and GC clinicopathologic features. **a.** Forest plot of the correlation between *MLH1* methylation and Lauren classification. **b.** Forest plot of the correlation between *MLH1* methylation and tumor invasion. **c.** Forest plot of the correlation between *MLH1* methylation and lymph node status. **d.** Forest plot of the correlation between *MLH1* methylation and distant metastasis status. **e.** Forest plot of the correlation between *MLH1* methylation and tumor stage.

Table 5. Association of *MLH1* promoter methylation with prognosis in gastric cancer based on bioinfromatic analysis.

Methylation probe		RFS			OS		
		Median survival time	X^2 value	P value	Median survival time	X^2 value	P value
cg18320188	Unmethylation	1676	3.09	0.08	1153	0.06	0.81
	Methylation	1376			869		
cg02279071	Unmethylation	1184	2.21	0.14	869	0.63	0.43
	Methylation	NA			1043		

Discussion

The methylation frequency of the *MLH1* promoter was inconsistent in GC with a range from 4% to 100% [37]. Thus, the association between *MLH1* promoter methylation and GC exists controversy. To get a more credible conclusion, we performed a systematic review and meta and bioinformatic analysis using the previously published studies and database to assess the relevance between *MLH1* methylation and GC. Our study showed a strong correlation between *MLH1* methylation and GC risk, indicating that *MLH1* methylation could predict the occurrence of gastric cancer as a convincing biomarker. No significant correlation was found between *MLH1* methylation and GC clinicopathological behavior as well as prognosis.

In the present meta-analysis, 2365 GC cases and 1563 control samples, from 26 studies were selected totally. Compared with the controls, the accumulated OR of *MLH1* methylation in GC patients was 4.895 (95% CI: 3.149-7.611, P<0.001). It was in accordance with earlier studies in which the frequency of *MLH1* promoter methylation in GC was enhanced compared with control groups [30, 34, 37]. Our bioinformatics analysis based on GEO and TCGA also showed that *MLH1* promoter methylation sustained a high level in GC compared to normal tissues (P=0.0149, P=0.0442 respectively). The results drew from both meta and bioinformatics analysis suggested that the methylation of *MLH1* was significantly associated with increased GC risk. The consequence may be caused by two main reasons. Firstly, Mismatch repair (MMR) deficiency leads to a tumour phenotype known as microsatellite instability (MSI), in which cells accumulate genetic errors [19]. *MLH1* is a functional member of DNA MMR system, which is responsible for the replacement of the mispaired nucleotides in the genome during the DNA replication [11]. When performing mismatch repair function, the heteroduplex composed of *MLH1* and *PMS2* could combine with DNA fragment thereby trigger the repair process. In addition to that it reactivates cell cycle arrest and caspase-mediated apoptosis in response to DNA damage, promotes cell mobility and interacts with other significant cell signaling proteins [52-55]. The aberration of the *MLH1* function could lead to the dysfunction of DNA MMR system therefore result in the GC carcinogenesis [56]. Secondly, *MLH1* is also a tumor suppressor gene, which expression is repressed by promoter methylation. And that's exactly one of the key features of cancer [57]. As a tumor suppressor gene *MLH1* silencing mediated by aberrant promoter DNA hypermethylation could lead to the tumor information [58]. Based on the present results and analysis, we could conclude that *MLH1* methylation significantly elevated the risk of GC and might be a probable biomarker for the prediction of GC.

The current meta and bioinformatic analysis revealed that no significant difference of *MLH1* methylation in relation to clinicopathological features, such as Lauren classification, tumor invasion, lymph node status, distant metastasis and tumor stage in GC (all P > 0.1), suggesting that the methylation status of *MLH1* promoter may not affect the biological behavior of GC. The phenomenon that

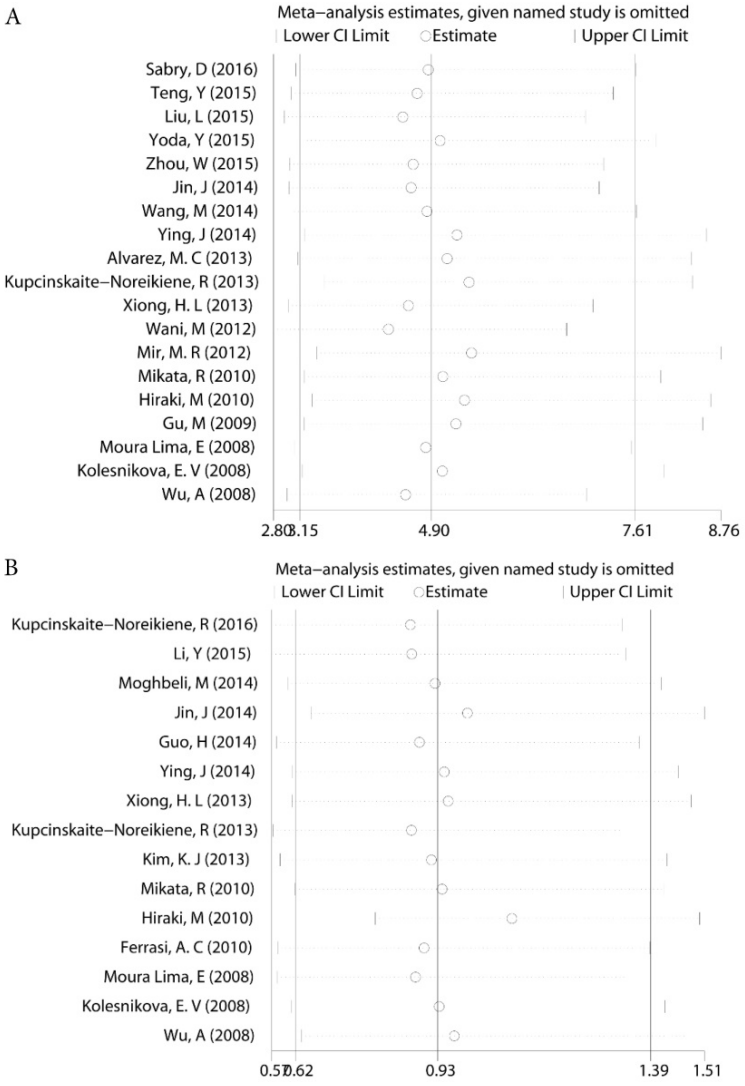

Figure 4. Sensitivity analysis **a.** The sensitivity analysis of MLH1 methylation and GC **b.** The sensitivity analysis of MLH1 methylation and lymph node status.

MLH1 methylation increased the risk of GC but not related with clinicopathological features hinted that DNA methylation occurs early in the multistep process of gastric carcinogenesis. Bischoff *et al.* reported that *MLH1* methylation with a consequent protein decrease occurred early during endometrial carcinogenesis [13]. And the coherent conclusions were also elucidated in lung cancer and breast cancer. [14-16]. Thus, we can infer that *MLH1* methylation may contribute to initial carcinogenesis but not progression of GC.

It has been reported that *MLH1* methylation are associated with poor prognosis in cancers, such as in non-small cell lung cancer and ovarian cancer after chemotherapy [59, 60]. But the current bioinformatics analysis revealed that no relationship between *MLH1* methylation with the prognosis of GC including RFS and OS based on the data from TCGA database. Only one study in the meta-analysis revealed that among oxaliplatin-treated patients, OS was longer in the *MLH1* unmethylated group than in the *MLH1* methylated group [32]. The phenomenon suggested that *MLH1* methylation may not affect the prognosis of GC. The different effects of *MLH1* methylation on prognosis in different tumors may be due to the organ specificity. There may exist some gastric specific indicators commonly affected the consequence of *MLH1* methylation and prognosis [61]. Further expanding of the sample size could be conduct to verify the impact of *MLH1* methylation on GC prognosis.

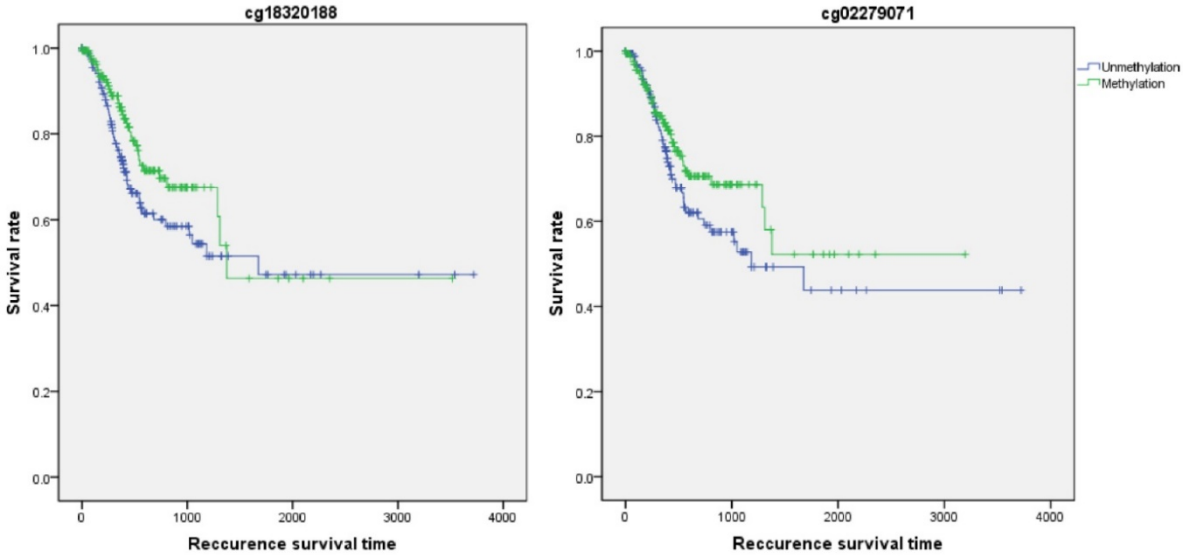

Figure 5. Recurrence survival time analysis of GC correlated with MLH1 methylation.

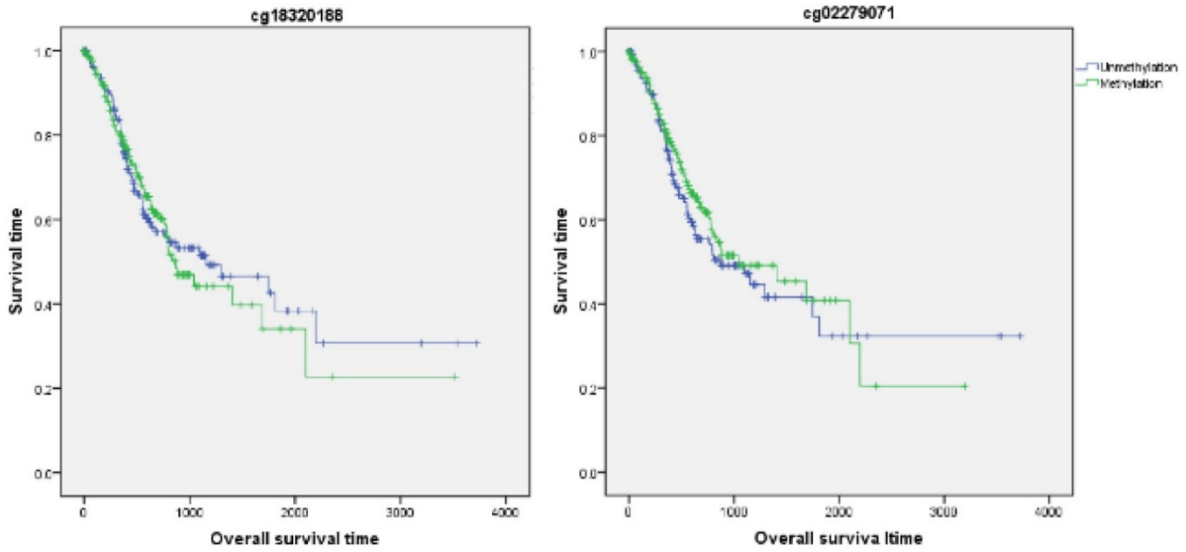

Figure 6. Overall survival time analysis of GC correlated with MLH1 methylation.

Our study had some limitations. First, our meta-analysis could not adjust for confounding factors such as age, sex, smoking behavior, or *H.pylori* infection due to some relevant data could not be extracted. Second, the studies included was only searched by English and Chinese, the other language of studies were not included therefore some important researches may be omitted. Third, up to now, few studies reported the association of *MLH1* methylation with prognosis of GC. On this point, we did only the bioinformatics analysis and failed to meta-analysis. There is a need to strengthen the prognosis-based association study in the future. Fourth, Heterogeneity existed in our meta-analysis. Although we try to eliminate the heterogeneity by subgroup analysis according to the potential heterogeneous factors, such as geographic region, ethnicity, testing methods and materials, there is still some heterogeneity in this meta-analysis because some original studies did not provide the necessary information.

In summary, this systematic review and meta and bioinformatic analysis showed a strong correlation between *MLH1* methylation and GC risk and no significant correlation was found between *MLH1* methylation and GC clinicopathological behavior as well as prognosis. The present results suggest that *MLH1* methylation can be used as a favorable molecular marker for the prediction of GC and it might not affect GC behavior. Further study could be conducted to verify the impact of MLH1 methylation on GC prognosis.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v09p1932s1.pdf

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Author Contributions

Conceived and designed the experiments: Yuan Yuan and Liping Sun. Analyzed the data: Shixuan Shen, Xiaohui Chen and Hao Li. Wrote the paper: Shixuan Shen. Revised the manuscript: Yuan Yuan.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing Interests

The authors have declared that no competing interest exists.

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