Relationship Between Human mutL Homolog 1 (hMLH1) Hypermethylation and Colorectal Cancer: A Meta-Analysis

Authors' Contribution:
Study Design **A**
Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
Literature Search **F**
Funds Collection **G**

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Background: Hypermethylation of CpG islands in gene promoter regions is an important mechanism of gene inactivation in cancers. Promoter hypermethylation of human mutL homolog 1 (hMLH1) has been implicated in a subset of colorectal cancers that show microsatellite instability (MSI), while the connection of the epigenetic inactivation of *hMLH1* in colorectal cancers remains unknown. The aim of this study was to evaluate the relationship between the promoter hypermethylation of hMLH1 and colorectal cancers by performing a meta-analysis.

Material/Methods: Eligible studies were identified through searching PubMed, Cochrane Library, Web of Science, and Google Scholar databases. R Software including meta packages was used to calculate the pooled and odds ratios (ORs) with corresponding confidence intervals (CIs). Funnel plots were also performed to evaluate publication bias.

Results: This meta-analysis obtained 45 articles, including 4096 colorectal cancer patients, and identified a significant association between hMLH1 hypermethylation and colorectal cancer risk using the fixed-effects model (OR=8.3820; 95% CI, 6.9202~10.1527; z=21.7431; *P*<0.0001) and random effects model pooled (OR=10.0963; 95% CI, 6.1919~16.4626; z=9.2688; *P*<0.0001). The significant relationship was found in subgroup analyses.

Conclusions: The results of this meta-analysis show a significant association between *hMLH1* hypermethylation and colorectal cancer risk.

MeSH Keywords: **Colorectal Neoplasms • Human Genome Project • Promoter Regions, Genetic**

Abbreviations: **hMLH1** – human mutL homolog 1; **MSI** – microsatellite instability; **MSS** – microsatellite stability; **OR** – odds ratio; **95% CI** – 95% confidence interval

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895643

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Background

Colorectal cancer is usually an adenocarcinoma that arises from the colon or rectum. It is the second and the third most common cancer in women and men in the world, respectively [1]. Colorectal cancer is also the fourth leading cause of cancer-related death worldwide, with an estimated 694 000 (8.5%) deaths in 2012 [2,3]. Surgery is the main treatment for colorectal cancer, but post-operative recurrence and metastasis lead to a poor prognosis for patients with this disease [4]. Anatomic and pathological stages are still the most accurate predictors of colorectal cancer prognosis, and once tumor cells have spread, the long-term prognosis is poor because no curative treatments are available. Therefore, novel molecular biomarkers for colorectal cancer are needed for accurate prediction.

Tumorigenesis in humans is a multistep process, reflecting an accumulation of genetic changes that lead normal cells to transform into cancer cells [5]. The causes of genetic alterations are multifactorial, with exogenous and endogenous factors known to induce a variety of genetic alterations, including DNA methylation, insertions, base substitutions, and deletions [6,7]. The abnormal methylation of tumor suppressor gene promoter regions can increase the degree of chromatin spiral, and can lead to gene transcription inhibition, which is closely related to tumorigenesis. Colorectal cancer is one of the best characterized tumors with regard to the genetic mechanisms involved in its development [8–12]. However, the clinical significance of these genetic alterations is still unclear. Human mutL homolog 1, known as *hMLH1*, can mediate protein-protein interactions during mismatch recognition, strand discrimination, and strand removal. It is a human gene located on chromosome 3 and is commonly associated with hereditary nonpolyposis colorectal cancer. Aberrant hypermethylation of CpG islands in *hMLH1* promoter regions is known to play an important role in the tumorigenesis of human colorectal cancer; its epigenetic alterations may affect DNA stability, such as chromosomal instability and microsatellite instability (MSI) [13]. About 15% of colorectal cancers show a high level of MSI, reflecting dysfunction of the post-replicative DNA mismatch repair system, mainly through the CpG methylation-mediated silencing of the *hMLH1* gene [7,14–16].

This systematic literature review aimed to quantify the impacts of *hMLH1* hypermethylation on the risk of colorectal cancer and MSI based on the above results and through selecting a large number of published articles on colorectal cancer.

Material and Methods

Literature search

This pooled study involved searching a range of computerized databases, including PubMed, Cochrane Library, Web of Science, and Google Scholar for articles published in English before March 2015. The study used a subject and text word strategy with "colorectal cancer or colon cancer or colorectal carcinoma" and "hMLH1 or human mutL homolog 1" and "methylation or hypermethylation or epigenetic".

Inclusion and exclusion criteria

The included articles had to meet the following criteria: (1) original study; (2) the diagnosis of colorectal cancer was based on clinical diagnosis through histopathology; (3) studies with a case–control design and available frequency of the *hMLH1* promoter methylation; (4) only the data from articles with full text in English were included in the analysis. The article title, author names, year published, research institutions, and procedures for enrolling participants were checked to avoid duplication of data. If several publications were reported with the same population data, only the most complete study with more information was included. For case group and control group, we defined the number of *hMLH1* hypermethylation of colorectal cancer tissues/blood (or high-level MSI in colorectal cancer) as case groups and the number of *hMLH1* hypermethylation of normal (corresponding adjacent non-cancer tissues or healthy tissues/blood from a healthy person) (or microsatellite stability in colorectal cancer) as control groups in individuals. Exclusion criteria were: (1) studies not focused on the association of colorectal cancer or *hMLH1*; (2) *hMLH1* methylation conducted only in the cell lines; (3) no raw data available or cannot retrieve any raw data; (4) conference papers, case reports, letters, or reviews papers.

Data extraction

Data were extracted from each study by 2 independent reviewers (HF Zhang and YW Lu) using the selection standards described above. Decisions were made and disagreements about study selection were resolved by discussing with ZR Xie and KH Wang. The following information was extracted from the studies: the first author's last name, publication year, original country of patients in the subjects, sex, age, and the number of *hMLH1* hypermethylation of cases and controls in individuals.

Statistical analysis

This meta-analysis used pooled odds ratio (OR) with its 95% confidence interval (CI) to measure the strength of the association between the *hMLH1* promoter methylation and colorectal
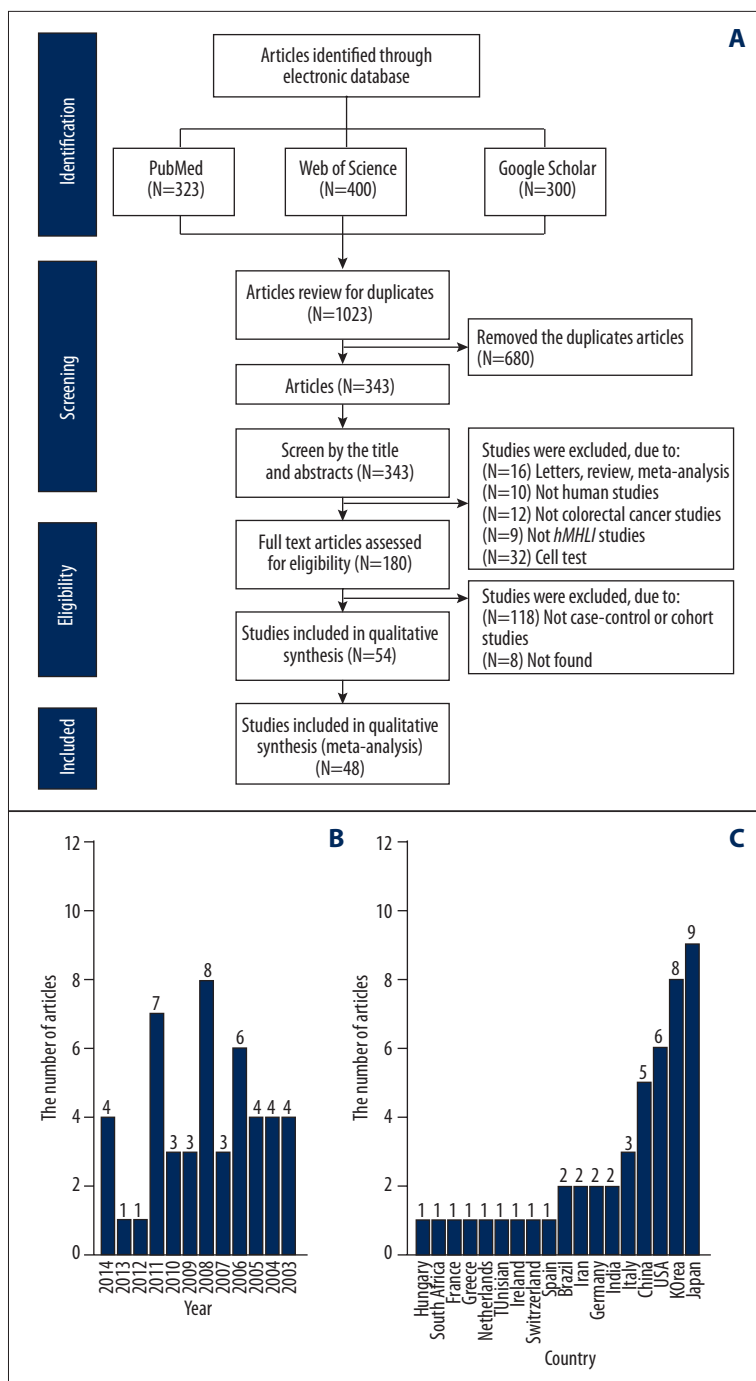
Figure 1. (**A–C**)Flow chart shows study selection procedure and the distribution of the number of topic-related articles in the electronic database during the last decade.

cancer. The significance of the pooled OR was determined by $p < 0.05$, which was considered as statistically significant. I^2 statistic with values over 50% and chi-squared test with $p \leq 0.1$ were considered to show strong heterogeneity between studies [17]. According to the heterogeneity statistic I^2, the data were pooled using the random-effects model when $I^2 > 50\%$ and $p \leq 0.1$, or the fixed-effects model when $I^2 < 50\%$ [18]. Subgroup analyses were performed according to different ethnic groups and the specimen source in consideration of the source of

heterogeneity. Tau-squared (χ^2) was used to determine how much heterogeneity was explained by subgroup differences. To assess the contributions of single studies to the final results, sensitivity analyses were performed. Begg's test and Egger's test were carried out to examine whether the results of a meta-analysis had been affected by publication bias and funnel plot asymmetry [19]. All statistical analyses were performed using *R* Software (*R* version 3.1.2) including meta packages.

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Table 1. Main characteristics of the studies included in the meta-analysis.

Author	Year	Country	Method	Sample	Median age (year)	TNM. stage	Male/female	Case		Control	
								M	U	M	U
Morimoto et al.	2014	Japan	MSP	Tissue	60.5	NA	62/43	41	65	0	5
Malhotra et al.	2014	India	MSP	Tissue	56	I–IV	10/20	15	15	3	27
Kanth et al.	2014	India	MSP	Tissue	40	I–IV	61/30	44	47	12	79
Coppedè et al.	2014	Italy	MS-HRM	Tissue	71.07	I–IV	61/46	13	94	1	79
Vergouwe et al.	2013	South Africa	MSP	Tissue	58.5	I–IV	34/44	45	33	5	12
Huang et al.	2012	China	MSP	Tissue	50	I–IV	17/13	6	24	0	30
Maeda et al.	2011	Japan	Chip	Tissue	60.9	I–IV	27/39	30	33	0	24
Lee et al.	2011	Korea	MSP	Tissue	63.4	I–IV	77/35	28	108	0	112
Kim et al.	2011	Korea	MSP	Blood	61.78	I–IV	35/32	15	36	0	51
Auclair et al.	2011	France	MSP	Blood	50	NA	NA	55	55	18	5
Aoyagi et al.	2011	Japan	QMSP	Tissue	64.5	III–IV	86/48	30	104	0	134
Ahn et al.	2011	Korea	MSP	Tissue	61	III	93/76	6	155	0	161
Psofaki et al.	2010	Greece	MSP	Tissue	62.5	I–IV	44/35	36	43	2	18
Miladi-Abdennadher et al.	2011	Tunisian	MSP	Tissue	62.9	I–IV	46/26	38	34	5	15
Mirchev et al.	2010	Germany	MSP	Tissue	73.8	I–IV	67/83	150	0	39	111
Hiraki et al.	2010	Japan	QMSP	Tissue	65.5	II–IV	10/17	4	23	1	26
Menigatti et al.	2009	Switzerland	QMSP	Tissue	65	I–IV	53/47	20	213	6	94
Lee et al.	2009	Korea	MSP	Blood	61	I–II	139/104	51	192	4	144
Kawaguchi et al.	2009	Japan	MSP	Tissue	57.7	I–III	NA	17	27	0	44
Ramirez et al.	2008	Spain	MSP	Tissue	67	I–IV	53/29	22	60	9	73
Nagasaka et al.	2008	Japan	COBRA	Tissue	65	I–IV	157/86	15	14	39	168
Mokarram et al.	2008	Iran	MSP	Blood	60.42	I–IV	90/61	20	131	8	73
Kim et al.	2008	Korea	MSP	Tissue	60	I–III	15/10	5	20	0	25
Kakar et al.	2008	USA	MSP	Tissue	NA	NA	NA	2	28	0	32
Ide et al.	2008	Japan	MSP	Tissue	60	I–IV	60/34	87	7	18	76
Fujiwara et al.	2008	Japan	COBRA	Tissue	55	NA	34/23	40	17	0	20
Brim et al.	2008	Iran	MSP	Tissue	65.7	I–IV	39/56	66	4	5	30
Noda et al.	2007	Japan	MSP	Tissue	65	I–IV	14/16	10	20	2	14
Leung et al.	2007	China	MSP	Tissue	69	NA	NA	5	15	1	29
Greenspan et al.	2007	USA	MSP	Tissue	60	NA	NA	8	31	0	39
Zhang et al.	2006	China	MSP	Tissue	62	I–IV	9/11	8	12	0	20
Ye et al.	2006	USA	MSP	Tissue	57.5	NA	71/26	12	85	11	83
Wallner et al.	2006	Germany	QMSP	Tissue	67	I–IV	26/12	19	19	0	20
O'Brien et al.	2006	USA	MSP	Tissue	NA	NA	NA	221	239	9	56
Fox et al.	2006	Ireland	MSP	Tissue	69	I–IV	52/58	13	97	3	107

Table 1 continued. Main characteristics of the studies included in the meta-analysis.

Author	Year	Country	Method	Sample	Median age (year)	TNM. stage	Male/female	Case		Control	
								M	U	M	U
Derks et al.	2006	Netherlands	MSP	Tissue	67	I–IV	NA	13	5	9	9
Leung et al.	2005	China	MSP	Blood	57	I–IV	18/31	19	30	4	37
Ashktorab et al.	2005	USA	MSP	Tissue	68	I–IV	15/19	29	5	0	34
Anacleto et al.	2005	Brazil	MMSP	Tissue	NA	NA	NA	16	82	0	30
Anacleto et al.	2005	Brazil	MSP	Tissue	NA	NA	NA	19	90	0	30
Xu et al.	2004	China	MSP	Tissue	60	I–IV	37/28	12	53	1	5
Kim et al.	2004	Korea	MSP	Tissue	56	I–IV	71/63	30	104	0	64
Arnold et al.	2004	USA	MSP	Tissue	NA	NA	70/79	46	127	0	173
Lee et al.	2004	Korea	MSP	Tissue	58	I–IV	70/79	30	119	0	24
Strazzullo et al.	2003	Italy	MSP	Tissue	NA	NA	NA	8	34	0	42
Roh et al.	2003	Korea	MSP	Tissue	25	I–IV	15/6	3	18	0	21
Ricciardiello et al.	2003	Italy	MSP	Tissue	62	NA	40/30	9	61	0	70
Kamory et al.	2003	Hungary	MSP	Tissue	65	NA	19/18	7	30	0	37

MSP – methylation specific polymerase chain reaction; QMSP – quantitative methylation specific polymerase chain reaction; MS-HRM – Methylation sensitive-high resolution melting; COBRA – Combined Bisulfite Restriction Assays; M – the number of patients with methylation; U – the number of patients with unmethylation; NA – not available.

Table 2. The article features of the relationship between *hMHL1* gene promoter hypermethylation and MSI in colorectal cancer.

Author	Year	Country	Method	Sample	Median age (year)	TNM. stage	Male/female	MSI-H		MSS	
								M	U	M	U
Kanth et al.	2014	India	MSP	Tissue	40	I–IV	61/30	27	17	11	36
Maeda et al.	2011	Japan	Chip	Tissue	60.9	I–IV	27/39	12	12	0	42
Kawaguchi et al.	2009	Japan	MSP	Tissue	57.7	I–III	NA	10	7	7	20
Nagasaka et al.	2008	Japan	COBRA	Tissue	65	I–IV	157/86	15	21	0	207
Kim et al.	2008	Korea	MSP	Tissue	60	I–III	15/10	5	4	3	13
Fujiwara et al.	2008	Japan	COBRA	Tissue	55	NA	34/23	13	10	3	30
Brim et al.	2008	Iranian	MSP	Tissue	65.7	I–IV	39/56	48	2	18	2
Greenspan et al.	2007	USA	MSP	Tissue	60	NA	NA	2	9	7	14
Fox et al.	2006	Ireland	MSP	Tissue	69	I–IV	52/58	8	2	5	95
Ashktorab et al.	2005	USA	MSP	Tissue	68	I–IV	15/19	16	3	13	2
Kim et al.	2004	Korea	MSP	Tissue	56	I–IV	71/63	10	13	20	91
Arnold et al.	2004	USA	MSP	Tissue	NA	NA	70/79	19	8	27	119
Roh et al.	2003	Korea	MSP	Tissue	25	I–IV	15/6	3	9	0	9
Ricciardiello et al.	2003	Italy	MSP	Tissue	62	NA	40/30	6	3	0	61

MSP – methylation specific polymerase chain reaction; COBRA – Combined Bisulfite Restriction Assays; M – the number of patients with methylation; U – the number of patients with unmethylation; MSI-H – high-level MSI; MSS – microsatellite stability; NA – not available.

Study	Experimental Events	Total	Control Events	Total	Odds ratio	OR	95%-CI	W(fixed)	W(random)
Morimoto et al. (2014, Japan)	41	106	0	5		6.97	[0.38; 129.36]	0.5%	1.4%
Malhotra et al. (2014 ,India)	15	30	3	30		9.00	[2.24; 36.17]	1.4%	2.7%
Kanth et al. (2014, India)	44	91	12	91		6.16	[2.98; 12.83]	5.8%	3.2%
Coppedè et al. (2014, Italy)	13	107	1	80		10.93	[1.40; 85.38]	0.9%	2.1%
Vergouwe et al. (2013, South Africa)	45	78	5	17		3.27	[1.05; 10.19]	3.3%	2.9%
Huang et al. (2012, China)	6	30	0	30		16.18	[0.87; 301.62]	0.4%	1.4%
Maeda et al. (2011, Japan)	30	63	0	24		44.61	[2.60; 765.55]	0.4%	1.5%
Lee et al. (2011, Korea)	28	136	0	112		59.10	[3.56; 980.13]	0.4%	1.5%
Kim et al. (2011, Korea)	15	51	0	51		43.74	[2.54; 754.58]	0.3%	1.5%
Auclair et al. (2011, France)	55	110	18	23		0.28	[0.10; 0.80]	14.0%	3.0%
Aoyagi et al. (2011, Japan)	30	134	0	134		78.51	[4.78; 1299.00]	0.4%	1.5%
Ahn et al. (2011, Korea)	6	161	0	161		13.50	[0.75; 241.69]	0.5%	1.5%
Psofaki et al. (2010, Greece)	36	79	2	20		7.53	[1.64; 34.68]	1.6%	2.5%
Miladi-Abdennadher et al. (2011, Tnuisian)	38	72	5	20		3.35	[1.10; 10.20]	3.5%	2.9%
Mirchev et al. (2010, Germany)	150	150	39	150		849.66	[51.67; 13972.70]	0.1%	1.5%
Hiraki et al. (2010, Japan)	4	27	1	27		4.52	[0.47; 43.42]	0.8%	1.9%
Menigatti et al. (2009, Switzerland)	20	233	6	100		1.47	[0.57; 3.78]	7.2%	3.1%
Lee et al. (2009, Korea)	51	243	4	148		9.56	[3.38; 27.06]	3.7%	3.0%
Kawaguchi et al. (2009, Japan)	17	44	0	44		56.64	[3.27; 980.14]	0.3%	1.5%
Ramirez et al. (2008, Spain)	22	82	9	82		2.97	[1.27; 6.94]	6.2%	3.1%
Nagasaka et al. (2008, Japan)	15	29	39	207		4.62	[2.06; 10.35]	4.3%	3.2%
Mokarram et al. (2008, Iran)	20	151	8	81		1.39	[0.58; 3.32]	8.5%	3.1%
Kim et al. (2008, Korea)	5	25	0	25		13.68	[0.71; 262.17]	0.4%	1.4%
Kakar et al. (2008, USA)	2	30	0	32		5.70	[0.26; 123.78]	0.4%	1.4%
Ide et al. (2008, Japan)	87	94	18	94		52.48	[20.79; 132.43]	1.3%	3.1%
Fujiwara et al. (2008, Japan)	40	57	0	20		94.89	[5.43; 1658.32]	0.2%	1.5%
Brim et al. (2008, Iran)	66	70	5	35		99.00	[24.81; 395.01]	0.4%	2.7%
Noda et al. (2007, Japan)	10	30	2	16		3.50	[0.68; 18.49]	1.6%	2.4%
Leung et al. (2007, China)	5	20	1	30		9.67	[1.03; 90.41]	0.6%	1.9%
Greenspan et al. (2007, USA)	8	39	0	39		21.32	[1.18; 383.70]	0.4%	1.5%
Zhang et al. (2008, China)	8	20	0	20		27.88	[1.48; 526.12]	0.3%	1.4%
Ye et al. (2006, USA)	12	97	11	94		1.07	[0.45; 2.55]	9.2%	3.1%
Wallner et al. (2006, Germany)	19	38	0	20		41.00	[2.31; 726.58]	0.3%	1.5%
O'Brien et al. (2006, USA)	221	460	9	65		5.75	[2.78; 11.91]	7.7%	3.2%
Fox et al. (2006, Ireland)	13	110	3	110		4.78	[1.32; 17.28]	2.5%	2.8%
Derks et al. (2006, Netherlands)	13	18	9	18		2.60	[0.65; 10.38]	2.3%	2.7%
Leung et al. (2005, China)	19	49	4	41		5.80	[1.80; 19.08]	2.5%	2.8%
Ashktorab et al. (2005, USA)	29	34	0	34		370.09	[19.63; 6976.00]	0.1%	1.4%
Anacleto et al. (2005, Brazil)	16	98	0	30		12.20	[0.71; 209.55]	0.6%	1.5%
Anacleto et al. (2005, Brazil)	19	109	0	30		13.14	[0.77; 224.28]	0.6%	1.5%
Xu et al. (2004, China)	12	65	1	6		1.13	[0.12; 10.60]	1.4%	1.9%
Kim et al. (2004, Korea)	30	134	0	64		37.65	[2.26; 626.39]	0.5%	1.5%
Arnold et al. (2005, Brazil)	46	173	0	173		126.55	[27.73; 2072.88]	0.3%	1.5%
Lee et al. (2004, Korea)	30	149	0	24		12.51	[0.74; 211.51]	0.6%	1.5%
Strazzullo et al. (2003, Italy)	8	42	0	42		20.94	[1.17; 375.81]	0.4%	1.5%
Roh et al. (2003, Korea)	3	21	0	21		8.14	[0.39; 167.98]	0.4%	1.4%
Ricciardiello et al. (2003, Italy)	9	70	0	70		21.78	[1.24; 381.96]	0.4%	1.5%
Kamory et al. (2003, Hungary)	7	37	0	37		18.44	[1.01; 335.96]	0.4%	1.5%
Fixed effect model	**1438**	**4296**	**215**	**2827**		**8.11**	**[6.74; 9.76]**	**100.0%**	**–**
Random effects model						**9.24**	**[5.84; 14.62]**	**–**	**100.0%**

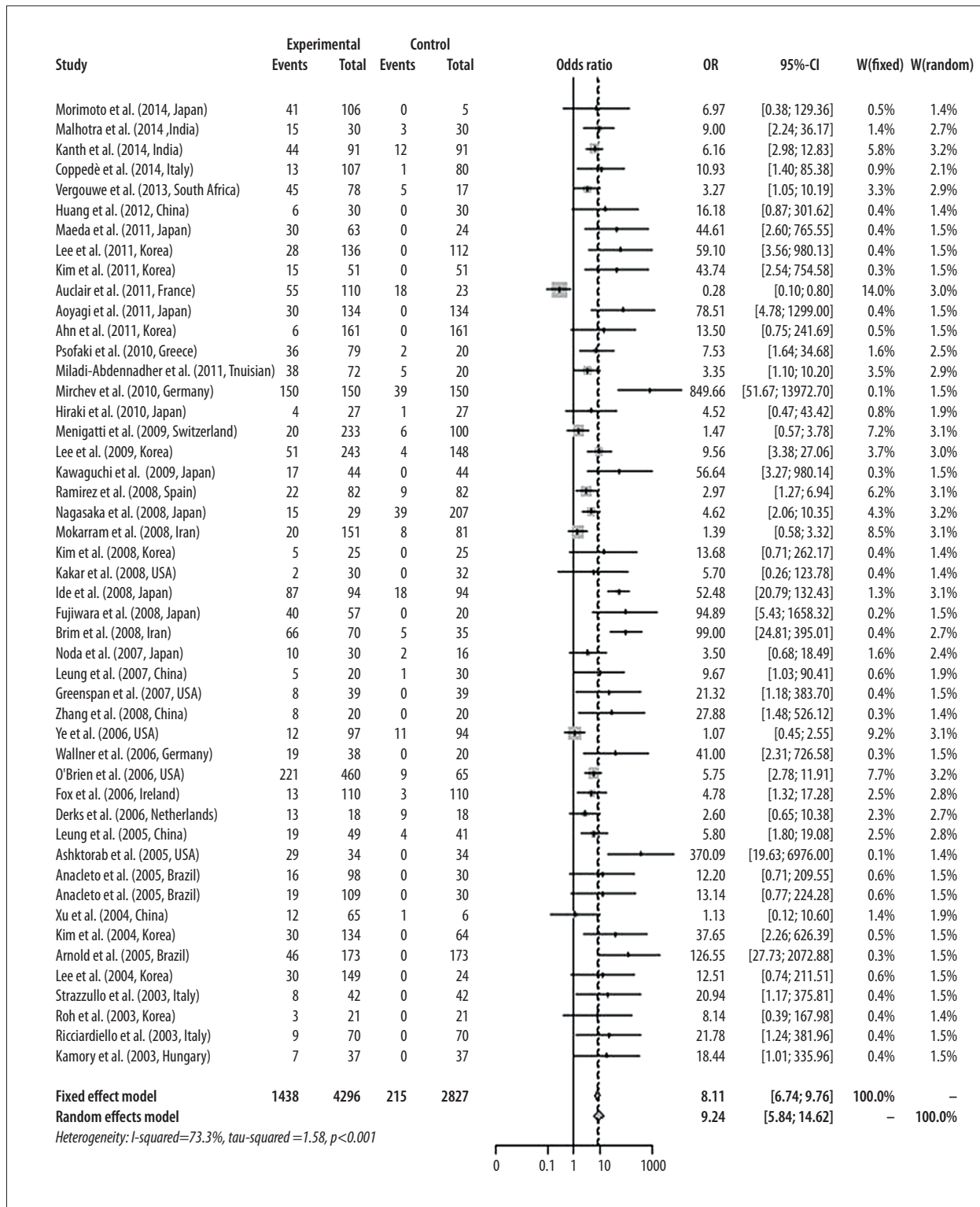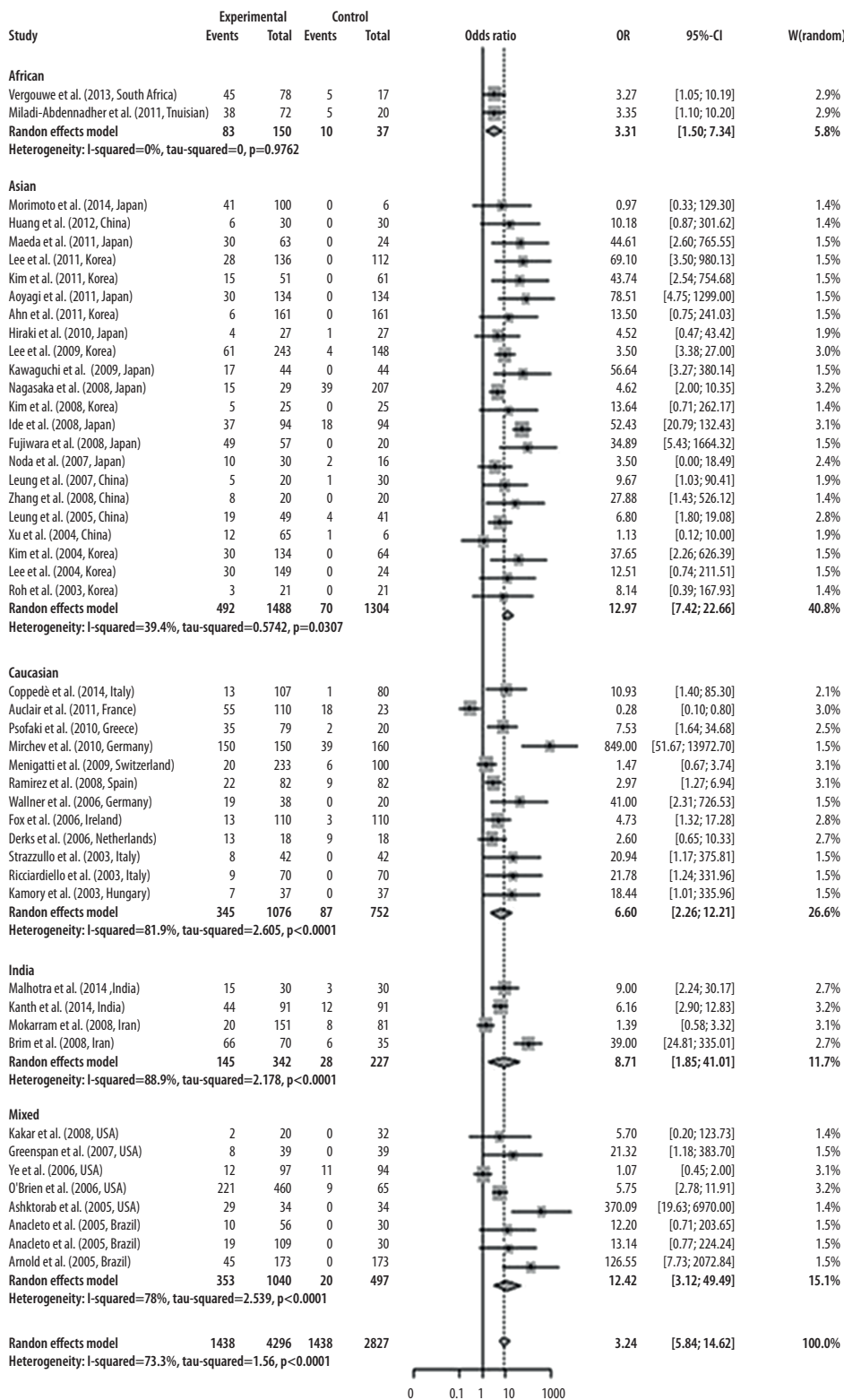*Heterogeneity: I-squared=73.3%, tau-squared =1.58, p<0.001*

0   0.1   1   10   1000

Figure 2. The combined estimates for the association between *hMHL1* gene promoter hypermethylation and colorectal cancer with forest plot.
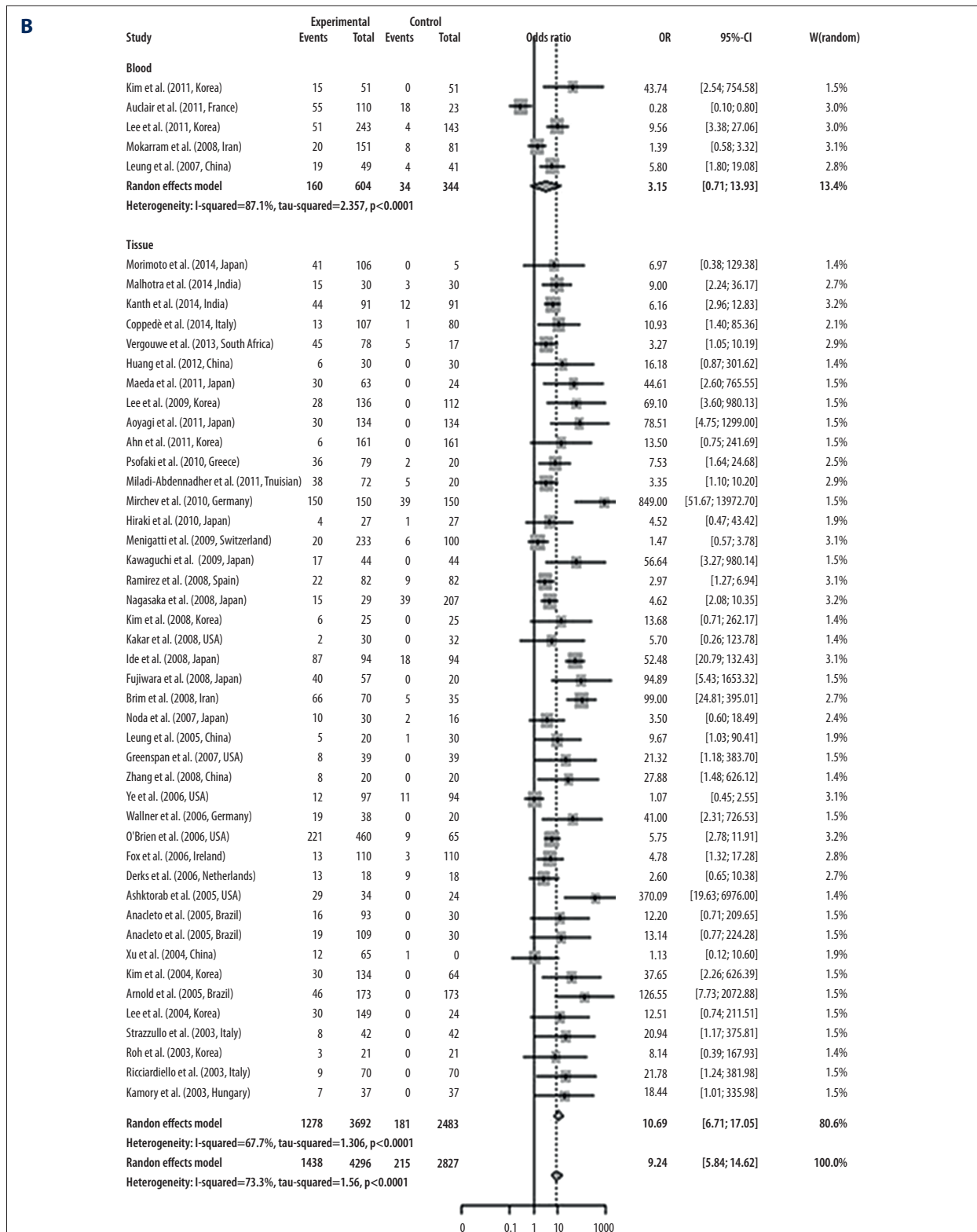
A

Study	Experimental Events	Total	Control Events	Total	Odds ratio	OR	95%-CI	W(random)
African								
Vergouwe et al. (2013, South Africa)	45	78	5	17		3.27	[1.05; 10.19]	2.9%
Miladi-Abdennadher et al. (2011, Tnuisian)	38	72	5	20		3.35	[1.10; 10.20]	2.9%
Randon effects model	83	150	10	37		3.31	[1.50; 7.34]	5.8%
Heterogeneity: I-squared=0%, tau-squared=0, p=0.9762								
Asian								
Morimoto et al. (2014, Japan)	41	100	0	6		0.97	[0.33; 129.30]	1.4%
Huang et al. (2012, China)	6	30	0	30		10.18	[0.87; 301.62]	1.4%
Maeda et al. (2011, Japan)	30	63	0	24		44.61	[2.60; 765.55]	1.5%
Lee et al. (2011, Korea)	28	136	0	112		69.10	[3.50; 980.13]	1.5%
Kim et al. (2011, Korea)	15	51	0	61		43.74	[2.54; 754.68]	1.5%
Aoyagi et al. (2011, Japan)	30	134	0	134		78.51	[4.75; 1299.00]	1.5%
Ahn et al. (2011, Korea)	6	161	0	161		13.50	[0.75; 241.03]	1.5%
Hiraki et al. (2010, Japan)	4	27	1	27		4.52	[0.47; 43.42]	1.9%
Lee et al. (2009, Korea)	61	243	4	148		3.50	[3.38; 27.00]	3.0%
Kawaguchi et al. (2009, Japan)	17	44	0	44		56.64	[3.27; 380.14]	1.5%
Nagasaka et al. (2008, Japan)	15	29	39	207		4.62	[2.00; 10.35]	3.2%
Kim et al. (2008, Korea)	5	25	0	25		13.64	[0.71; 262.17]	1.4%
Ide et al. (2008, Japan)	37	94	18	94		52.43	[20.79; 132.43]	3.1%
Fujiwara et al. (2008, Japan)	49	57	0	20		34.89	[5.43; 1664.32]	1.5%
Noda et al. (2007, Japan)	10	30	2	16		3.50	[0.00; 18.49]	2.4%
Leung et al. (2007, China)	5	20	1	30		9.67	[1.03; 90.41]	1.9%
Zhang et al. (2008, China)	8	20	0	20		27.88	[1.43; 526.12]	1.4%
Leung et al. (2005, China)	19	49	4	41		6.80	[1.80; 19.08]	2.8%
Xu et al. (2004, China)	12	65	1	6		1.13	[0.12; 10.00]	1.9%
Kim et al. (2004, Korea)	30	134	0	64		37.65	[2.26; 626.39]	1.5%
Lee et al. (2004, Korea)	30	149	0	24		12.51	[0.74; 211.51]	1.5%
Roh et al. (2003, Korea)	3	21	0	21		8.14	[0.39; 167.93]	1.4%
Randon effects model	492	1488	70	1304		12.97	[7.42; 22.66]	40.8%
Heterogeneity: I-squared=39.4%, tau-squared=0.5742, p=0.0307								
Caucasian								
Coppedè et al. (2014, Italy)	13	107	1	80		10.93	[1.40; 85.30]	2.1%
Auclair et al. (2011, France)	55	110	18	23		0.28	[0.10; 0.80]	3.0%
Psofaki et al. (2010, Greece)	35	79	2	20		7.53	[1.64; 34.68]	2.5%
Mirchev et al. (2010, Germany)	150	150	39	160		849.00	[51.67; 13972.70]	1.5%
Menigatti et al. (2009, Switzerland)	20	233	6	100		1.47	[0.67; 3.74]	3.1%
Ramirez et al. (2008, Spain)	22	82	9	82		2.97	[1.27; 6.94]	3.1%
Wallner et al. (2006, Germany)	19	38	0	20		41.00	[2.31; 726.53]	1.5%
Fox et al. (2006, Ireland)	13	110	3	110		4.73	[1.32; 17.28]	2.8%
Derks et al. (2006, Netherlands)	13	18	9	18		2.60	[0.65; 10.33]	2.7%
Strazzullo et al. (2003, Italy)	8	42	0	42		20.94	[1.17; 375.81]	1.5%
Ricciardiello et al. (2003, Italy)	9	70	0	70		21.78	[1.24; 331.96]	1.5%
Kamory et al. (2003, Hungary)	7	37	0	37		18.44	[1.01; 335.96]	1.5%
Randon effects model	345	1076	87	752		6.60	[2.26; 12.21]	26.6%
Heterogeneity: I-squared=81.9%, tau-squared=2.605, p<0.0001								
India								
Malhotra et al. (2014 ,India)	15	30	3	30		9.00	[2.24; 30.17]	2.7%
Kanth et al. (2014, India)	44	91	12	91		6.16	[2.90; 12.83]	3.2%
Mokarram et al. (2008, Iran)	20	151	8	81		1.39	[0.58; 3.32]	3.1%
Brim et al. (2008, Iran)	66	70	6	35		39.00	[24.81; 335.01]	2.7%
Randon effects model	145	342	28	227		8.71	[1.85; 41.01]	11.7%
Heterogeneity: I-squared=88.9%, tau-squared=2.178, p<0.0001								
Mixed								
Kakar et al. (2008, USA)	2	20	0	32		5.70	[0.20; 123.73]	1.4%
Greenspan et al. (2007, USA)	8	39	0	39		21.32	[1.18; 383.70]	1.5%
Ye et al. (2006, USA)	12	97	11	94		1.07	[0.45; 2.00]	3.1%
O'Brien et al. (2006, USA)	221	460	9	65		5.75	[2.78; 11.91]	3.2%
Ashktorab et al. (2005, USA)	29	34	0	34		370.09	[19.63; 6970.00]	1.4%
Anacleto et al. (2005, Brazil)	10	56	0	30		12.20	[0.71; 203.65]	1.5%
Anacleto et al. (2005, Brazil)	19	109	0	30		13.14	[0.77; 224.24]	1.5%
Arnold et al. (2005, Brazil)	45	173	0	173		126.55	[7.73; 2072.84]	1.5%
Randon effects model	353	1040	20	497		12.42	[3.12; 49.49]	15.1%
Heterogeneity: I-squared=78%, tau-squared=2.539, p<0.0001								
Randon effects model	1438	4296	1438	2827		3.24	[5.84; 14.62]	100.0%
Heterogeneity: I-squared=73.3%, tau-squared=1.56, p<0.0001								

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B

Study	Experimental Events	Total	Control Events	Total	Odds ratio	OR	95%-CI	W(random)
Blood								
Kim et al. (2011, Korea)	15	51	0	51		43.74	[2.54; 754.58]	1.5%
Auclair et al. (2011, France)	55	110	18	23		0.28	[0.10; 0.80]	3.0%
Lee et al. (2011, Korea)	51	243	4	143		9.56	[3.38; 27.06]	3.0%
Mokarram et al. (2008, Iran)	20	151	8	81		1.39	[0.58; 3.32]	3.1%
Leung et al. (2007, China)	19	49	4	41		5.80	[1.80; 19.08]	2.8%
Randon effects model	**160**	**604**	**34**	**344**		**3.15**	**[0.71; 13.93]**	**13.4%**
Heterogeneity: I-squared=87.1%, tau-squared=2.357, p<0.0001								
Tissue								
Morimoto et al. (2014, Japan)	41	106	0	5		6.97	[0.38; 129.38]	1.4%
Malhotra et al. (2014 ,India)	15	30	3	30		9.00	[2.24; 36.17]	2.7%
Kanth et al. (2014, India)	44	91	12	91		6.16	[2.96; 12.83]	3.2%
Coppedè et al. (2014, Italy)	13	107	1	80		10.93	[1.40; 85.36]	2.1%
Vergouwe et al. (2013, South Africa)	45	78	5	17		3.27	[1.05; 10.19]	2.9%
Huang et al. (2012, China)	6	30	0	30		16.18	[0.87; 301.62]	1.4%
Maeda et al. (2011, Japan)	30	63	0	24		44.61	[2.60; 765.55]	1.5%
Lee et al. (2009, Korea)	28	136	0	112		69.10	[3.60; 980.13]	1.5%
Aoyagi et al. (2011, Japan)	30	134	0	134		78.51	[4.75; 1299.00]	1.5%
Ahn et al. (2011, Korea)	6	161	0	161		13.50	[0.75; 241.69]	1.5%
Psofaki et al. (2010, Greece)	36	79	2	20		7.53	[1.64; 24.68]	2.5%
Miladi-Abdennadher et al. (2011, Tnuisian)	38	72	5	20		3.35	[1.10; 10.20]	2.9%
Mirchev et al. (2010, Germany)	150	150	39	150		849.00	[51.67; 13972.70]	1.5%
Hiraki et al. (2010, Japan)	4	27	1	27		4.52	[0.47; 43.42]	1.9%
Menigatti et al. (2009, Switzerland)	20	233	6	100		1.47	[0.57; 3.78]	3.1%
Kawaguchi et al. (2009, Japan)	17	44	0	44		56.64	[3.27; 980.14]	1.5%
Ramirez et al. (2008, Spain)	22	82	9	82		2.97	[1.27; 6.94]	3.1%
Nagasaka et al. (2008, Japan)	15	29	39	207		4.62	[2.08; 10.35]	3.2%
Kim et al. (2008, Korea)	6	25	0	25		13.68	[0.71; 262.17]	1.4%
Kakar et al. (2008, USA)	2	30	0	32		5.70	[0.26; 123.78]	1.4%
Ide et al. (2008, Japan)	87	94	18	94		52.48	[20.79; 132.43]	3.1%
Fujiwara et al. (2008, Japan)	40	57	0	20		94.89	[5.43; 1653.32]	1.5%
Brim et al. (2008, Iran)	66	70	5	35		99.00	[24.81; 395.01]	2.7%
Noda et al. (2007, Japan)	10	30	2	16		3.50	[0.60; 18.49]	2.4%
Leung et al. (2005, China)	5	20	1	30		9.67	[1.03; 90.41]	1.9%
Greenspan et al. (2007, USA)	8	39	0	39		21.32	[1.18; 383.70]	1.5%
Zhang et al. (2008, China)	8	20	0	20		27.88	[1.48; 626.12]	1.4%
Ye et al. (2006, USA)	12	97	11	94		1.07	[0.45; 2.55]	3.1%
Wallner et al. (2006, Germany)	19	38	0	20		41.00	[2.31; 726.53]	1.5%
O'Brien et al. (2006, USA)	221	460	9	65		5.75	[2.78; 11.91]	3.2%
Fox et al. (2006, Ireland)	13	110	3	110		4.78	[1.32; 17.28]	2.8%
Derks et al. (2006, Netherlands)	13	18	9	18		2.60	[0.65; 10.38]	2.7%
Ashktorab et al. (2005, USA)	29	34	0	24		370.09	[19.63; 6976.00]	1.4%
Anacleto et al. (2005, Brazil)	16	93	0	30		12.20	[0.71; 209.65]	1.5%
Anacleto et al. (2005, Brazil)	19	109	0	30		13.14	[0.77; 224.28]	1.5%
Xu et al. (2004, China)	12	65	1	0		1.13	[0.12; 10.60]	1.9%
Kim et al. (2004, Korea)	30	134	0	64		37.65	[2.26; 626.39]	1.5%
Arnold et al. (2005, Brazil)	46	173	0	173		126.55	[7.73; 2072.88]	1.5%
Lee et al. (2004, Korea)	30	149	0	24		12.51	[0.74; 211.51]	1.5%
Strazzullo et al. (2003, Italy)	8	42	0	42		20.94	[1.17; 375.81]	1.5%
Roh et al. (2003, Korea)	3	21	0	21		8.14	[0.39; 167.93]	1.4%
Ricciardiello et al. (2003, Italy)	9	70	0	70		21.78	[1.24; 381.98]	1.5%
Kamory et al. (2003, Hungary)	7	37	0	37		18.44	[1.01; 335.98]	1.5%
Randon effects model	**1278**	**3692**	**181**	**2483**		**10.69**	**[6.71; 17.05]**	**80.6%**
Heterogeneity: I-squared=67.7%, tau-squared=1.306, p<0.0001								
Randon effects model	**1438**	**4296**	**215**	**2827**		**9.24**	**[5.84; 14.62]**	**100.0%**
Heterogeneity: I-squared=73.3%, tau-squared=1.56, p<0.0001								

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Figure 3. Subgroup meta-analysis for the relationship between *hMHL1* gene promoter hypermethylation and colorectal cancer risk. (**A**) Ethnicity was categorized as ''Asian'', ''Caucasian'', "India's race", "African descent" and ''Mixed-Race''. Samples of studies from the USA and Brazil are ''Mixed-Race'' ethnicity, and Iran and India are Indian ethnicity. (**B**) Subgroup meta-analysis based on different samples by random-effects model.
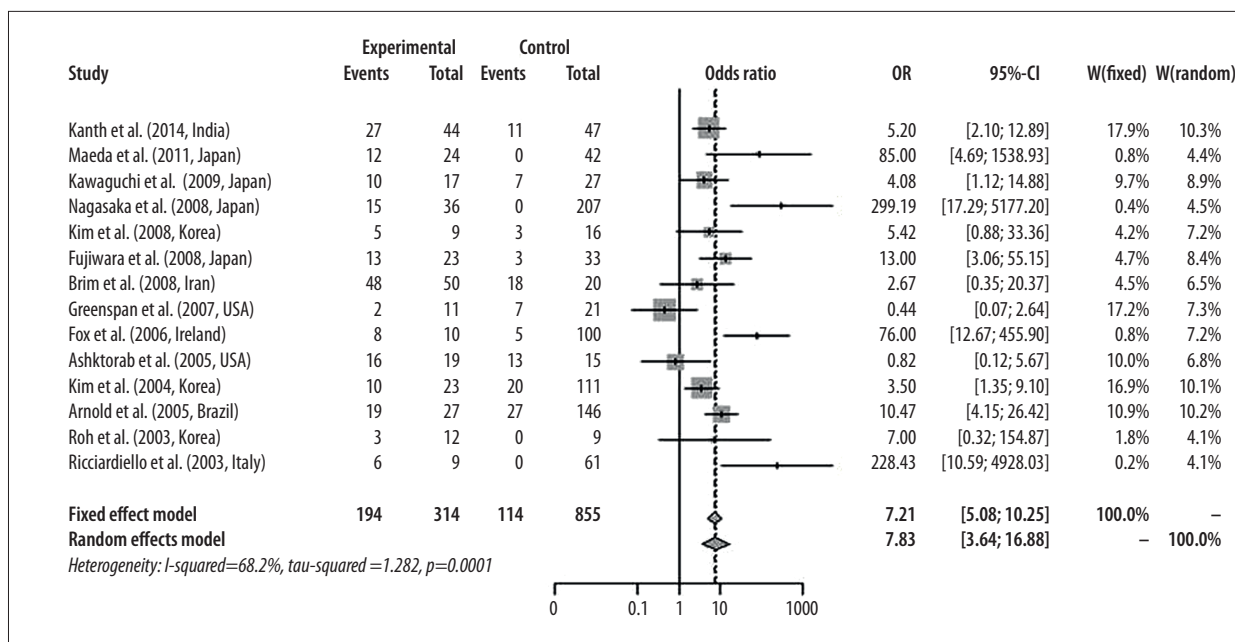
Figure 4. Forest plots for the relationship between *hMHL1* gene promoter hypermethylation and MSI in colorectal cancer.

Results

Eligible sudies and study characteristics

After being selected in accordance with the inclusion criteria, 45 studies met the standard, as shown in Figure 1A [20–66]. The characteristics of the 45 retained studies are listed in Tables 1 and 2. The studies included were published between 2003 and 2014 (Figure 1B) and were conducted in 18 countries (Figure 1C). A total of 33.47% of colorectal cancer patients had the methylated *hMLH1* allele, with a frequency ranging from 3.73% to 100% in individual trials. Among the 45 studies, 13 focused on the MSI, which were divided into MSI-H (high-level MSI) and MSS (microsatellite stability).

Meta-analysis

The main results of this meta-analysis and the heterogeneity test are shown in Figures 2–4. Significant heterogeneity was observed in overall and stratified analyses; the pooled OR for colorectal cancer risk was calculated by fixed-effect model and random-effects model. The combined results based on 45 studies show that the *hMLH1* promoter hypermethylation was significantly associated with the increased risk of colorectal cancer using the fixed-effects model (OR=8.3820; 95%CI, 6.9202~10.1527; z=21.7431; $P<0.0001$) and random-effects model (OR=10.0963; 95%CI, 6.1919~16.4626; z=9.2688; $P<0.0001$). For heterogeneity, pooled tau^2 was 1.7063 and I^2 was 74.6%. The OR showed that colorectal cancer patients have a 10.0963-fold higher risk for *hMLH1* hypermethylation compared with that in adjacent tissues and normal blood by

the random-effects model, suggesting a statistically significant increase in likelihood of methylation in colorectal cancer compared to adjacent tissues and normal blood.

The relationship between subgroup and *hMLH1* promoter hypermethylation among colorectal cancer cases were also conducted. For the subgroup analysis of ethnicity by random-effects model, the OR for the Asian subgroup was 16.83 (95% CI, 10.08~28.09) from 19 studies, the Caucasian subgroup was 6.60 (95% CI, 2.26~19.28) from 12 studies, the Indian subgroup was 8.71 (95% CI, 1.85~41.01) from 4 studies, the African subgroup was 3.31 (95% CI, 1.50~7.34) from 2 studies, and the mixed-race subgroup was 12.42 (95% CI, 3.12~49.49) from 8 studies (all $P<0.0001$) (Figure 3A). In the subgroup analysis of sample source, the OR in the tissues was 11.95 (95% CI, 7.26~19.68; $P<0.0001$) and the OR in the blood was 3.15 (95% CI, 0.71–13.93; $P<0.0001$) (Figure 3B).

For the relationship between *hMHL1* gene promoter and MSI, 13 articles were analyzed showing MSI-H had a 6.09- or 6.48-fold higher significance than MSS by fixed- or random-effects models (Figure 4). Hypermethylation of *hMLH1* gene promoter was the main mechanism underlying the pathogenesis of MSI colorectal cancer [28,62] and this meta-analysis identified the same result.

Publication bias and sensitivity analysis

To ensure the quality of this study, a Begg's funnel plot and Egger's tests were performed to eliminate the publication bias of included studies. Visual assessment of the Begg's test did

Study	Odds ratio	OR	95%-CI
Omitting Morimoto et al.		9.31	[5.85; 14.80]
Omitting Malhotra et al.		9.30	[5.82; 14.89]
Omitting Kanth et al.		9.54	[5.89; 15.44]
Omitting Coppedè et al.		9.24	[5.80; 14.73]
Omitting Vergouwe et al.		9.60	[5.99; 15.37]
Omitting Huang et al.		9.18	[5.78; 14.60]
Omitting Maeda et al.		9.02	[5.68; 14.30]
Omitting Lee et al.		8.95	[5.66; 14.19]
Omitting Kim et al.		9.02	[5.69; 14.31]
Omitting Auclair et al.		9.90	[6.42; 15.29]
Omitting Aoyagi et al.		8.91	[5.63; 14.09]
Omitting Ahn et al.		9.21	[5.79; 14.65]
Omitting Psofaki et al.		9.34	[5.84; 14.94]
Omitting Miladi-Abdennadher et al.		9.59	[5.99; 15.37]
Omitting Mirchev et al.		8.41	[5.41; 13.08]
Omitting Hiraki et al.		9.40	[5.90; 14.98]
Omitting Menigatti et al.		9.78	[6.14; 15.55]
Omitting Lee et al.		9.32	[5.80; 14.95]
Omitting Kawaguchi et al.		8.98	[5.65; 14.23]
Omitting Ramirez et al.		9.68	[6.02; 15.54]
Omitting Nagasaka et al.		9.58	[5.94; 15.46]
Omitting Mokarram et al.		9.78	[6.16; 15.54]
Omitting Kim et al.		9.21	[5.79; 14.64]
Omitting Kakar et al.		9.33	[5.87; 14.83]
Omitting Ide et al.		8.54	[5.45; 13.39]
Omitting Fujiwara et al.		8.89	[5.62; 14.07]
Omitting Brim et al.		8.50	[5.42; 13.35]
Omitting Noda et al.		9.51	[5.95; 15.18]
Omitting Leung et al.		9.26	[5.81; 14.76]
Omitting Greenspan et al.		9.14	[5.75; 14.53]
Omitting Zhang et al.		9.10	[5.73; 14.46]
Omitting Ye et al.		9.80	[6.21; 15.47]
Omitting Wallner et al.		9.04	[5.69; 14.34]
Omitting O'Brien et al.		9.56	[5.91; 15.49]
Omitting Fox et al.		9.48	[5.92; 15.19]
Omitting Derks et al.		9.61	[6.01; 15.34]
Omitting Leung et al.		9.44	[5.89; 15.14]
Omitting Ashktorab et al.		8.67	[5.51; 13.65]
Omitting Anacleto et al.		9.22	[5.80; 14.67]
Omitting Anacleto et al.		9.21	[5.79; 14.65]
Omitting Xu et al.		9.63	[6.06; 15.31]
Omitting Kim et al.		9.04	[5.70; 14.34]
Omitting Arnold et al.		8.81	[5.58; 13.90]
Omitting Lee et al.		9.22	[5.80; 14.65]
Omitting Strazzullo et al.		9.14	[5.75; 14.53]
Omitting Roh et al.		9.28	[5.84; 14.75]
Omitting Ricciardiello et al.		9.14	[5.75; 14.52]
Omitting Kamory et al.		9.16	[5.76; 14.57]
Random effects model		9.24	[5.84; 14.62]

Figure 5. Begg's funnel plot of publication biases and sensitivity analysis on the relationship between *hMHL1* gene promoter hypermethylation and colorectal cancer susceptibility. (**A**) The natural logarithm of odds ratio (OR) and its standard error were used in the funnel plot. The circles correspond to the log OR from individual trials, and the diagonal lines show the expected 95% confidence interval (CI) around the summary estimate. (**B**) Sensitivity analysis of the summary odds ratio coefficients on the relationship between *hMHL1* promoter methylation and the colorectal cancer patients.

not reveal any evidence of obvious asymmetry in all data analysis (*t*=−0.9897, *P*=0.3279). Meanwhile, Egger's test also provided statistical evidence of funnel plot symmetry and detected little evidence of publication bias (t=3.9443, *P*=0.0002907). Therefore, there was little publication bias for the 45 studies (Figure 5A).

Sensitivity analyses were conducted to determine whether any single study of this meta-analysis affected the final results. According to sensitivity analysis with the 45 studies, the OR ranged from 9.1372 (95% CI, 5.7054~14.6331) to 10.8803 (95% CI, 6.8569~17.2643) by omitting a single study under the random-effects model, which suggested that there was no change in the OR or the 95% CI after each deletion (Figure 5B).

Discussion

Colorectal cancer is a major cause of cancer-associated morbidity and mortality worldwide. The epidemiology of colorectal cancer has been studied by researchers, but the results have been insufficient. This meta-analysis retrospectively included 45 studies, aiming to investigate whether promoter DNA methylation of the *hMHL1* gene has an effect on the risk of colorectal cancer. The meta-analysis results revealed that the frequency of *hMHL1* gene promoter hypermethylation in colorectal cancer tissues/blood was significantly higher than that in normal tissues/blood, suggesting that *hMHL1* gene promoter hypermethylation may be implicated in the development and progression of colorectal cancer. Although the exact role of methylation status of the *hMHL1* gene in colorectal cancer carcinogenesis is still indistinct, CpG island hypermethylation in the *hMHL1* promoter region maybe lead to transcriptional silencing, and thus inhibit its function to the DNA mismatch repair pathway, resulting in the development of colorectal cancer [67]. The results of subgroup analysis based on ethnicity demonstrated that *hMHL1* gene promoter hypermethylation was closely associated with the risk of colorectal cancer among Asians, Caucasians, Indians, Africans and mixed-race people, revealing that there was no ethnic difference in the effects of *hMHL1* gene promoter hypermethylation on colorectal cancer susceptibility.

The development of human cancer is associated with genomic instability, which causes the accumulation of genetic changes that eventually result in the conversion of normal cells to malignant phenotypes. The human DNA repair system plays an important role in reducing mutations and maintaining genomic stability. The 1 mismatch of repair genes, *hMLH1*, is an integral component of the DNA mismatch repair pathway. Defective DNA mismatch repair is most commonly associated with the functional loss of *hMLH1* genes and results in the methylator phenotypes characterized by MSI in colorectal cancer. This meta-analysis result of 13 articles found that MSI-H have a higher frequency of *hMHL1* gene promoter hypermethylation than MSS by fixed- and random-effects models, indicating a significant relationship between *hMHL1* gene promoter hypermethylation and MSI in colorectal cancer.

There also existed 3 limitations in this meta-analysis that should be interpreted. A first potential limitation stemmed from the small numbers of studies and wide standard deviations, thereby limiting confidence in drawing conclusions. Secondly, the meta-analysis is a retrospective study that may lead to subject selection bias, thereby influencing the reliability of the meta-analysis results. Thirdly, the results this meta-analysis have insufficient statistical power to assess the correlations between *hMHL1* gene promoter hypermethylation and the development and progression of colorectal cancer. Although this study has the above limitations, this meta-analysis has a high value for the risk of colorectal cancer. Furthermore, additional studies with a larger sample size are still required to provide a more representative and convincing statistical analysis.

Conclusions

This meta-analysis showed a positive association between *hMHL1* gene promoter hypermethylation and colorectal cancer risk. Thus, *hMHL1* gene promoter hypermethylation might be a valuable diagnostic biomarker for the early detection of colorectal cancer.

Disclosure

The authors declare that they have no competing interests.

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