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ORIGINAL RESEARCH

Prognostic value of the combination of microsatellite instability and *BRAF* mutation in colorectal cancer

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Purpose: The aim of this study was to investigate the prognostic value of the combination of microsatellite instability (MSI) and *BRAF* V600E mutation in colorectal cancer (CRC).

Materials and methods: We compare the prognosis difference among CRC patients with four subtypes according to MSI and *BRAF* mutation, ie, microsatellite stable/*BRAF* wild type (MSS/*BRAF*wt), MSS/*BRAF* mutation (MSS/*BRAF*mut), MSI/*BRAF*wt, and MSI/*BRAF*mut, by pooling the previous related reports and public available data sets till December 2017 for the first time.

Results: Twenty-seven independent studies comprising 24,067 CRC patients were included. Meta-analysis suggested that, compared with MSS/*BRAF*wt subtype, MSS/*BRAF*mut was associated with shorter overall survival (OS) (N=25, HR = 2.018, 95% CI = 1.706–2.388, P=2.220E-16), while there was a trend of association of MSI/*BRAF*mut with OS (N=13, HR = 1.324, 95% CI = 0.938–1.868, *P*=1.096E-01) and no association of MSI/*BRAF*wt with OS (N=17, HR = 0.996, 95% CI = 0.801–1.240, *P*=9.761E-01). Compared with MSI/*BRAF*wt subtype, MSI/*BRAF*mut was a poor factor for OS (N=22, HR = 1.470, 95% CI = 1.243–1.740, *P*=7.122E-06). Compared with MSS/*BRAF*mut subtype, both MSI/*BRAF*wt (N=11, HR = 0.560, 95% CI = 0.433–0.725, *P*=1.034E-05) and MSI/*BRAF*mut (N=16, HR = 0.741, 95% CI = 0.567–0.968, *P*=2.781E-02) were favorable for OS. Subgroup analysis revealed similar results in all subgroups except the subgroup of stage IV cancer, in which MSI showed poor effects on OS in *BRAF* wild-type patients (N=6, HR = 1.493, 95% CI = 1.187–1.879, *P*=6.262E-04) but not in *BRAF*-mutated patients (N=5, HR = 1.143, 95% CI = 0.789–1.655, *P*=4.839E-01). Meta-analysis regression and test of interaction revealed no interaction of MSI with *BRAF* mutation when evaluating the associations of MSI/*BRAF* mutation subtypes with OS in CRC.

Conclusion: Among the four subtypes according to MSI and *BRAF* mutation, MSS/*BRAF*mut was a poor prognostic factor, while MSS/*BRAF*wt and MSI/*BRAF*wt were comparable and favorable and MSI/*BRAF*mut was moderate in CRC. The combination of MSI/*BRAF* mutations could facilitate the planning of individualized treatment strategies and prognosis improvement in CRC.

Keywords: meta-analysis, microsatellite instability, colorectal cancer, *BRAF* mutation, prognosis

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Introduction

Colorectal cancer (CRC) is the third most common malignancy in adults worldwide, with more than 6,000 million deaths each year.[1] Currently, the American Joint Committee on Cancer (AJCC) TNM staging system remains the strongest prognostic marker. However, growing evidence revealed that due to the considerable heterogeneity in CRC clinical presentation and prognosis, CRC should be subdivided into different prognostic

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groups by additional prognostic or predictive factors such as molecular biomarkers that reflect the development pathways of CRC.[2–5] In the past cascades, the researchers have investigated a series of biomarkers and pathways including microsatellite instability (MSI) and mutation in B-type Raf kinase (*BRAF*), which are used as significant prognostic factors in intrinsic CRC subtypes.[6–9]

MSI, the molecular fingerprint of the deficient DNA mismatch repair (MMR) system, is caused by a lack of or alteration in MMR genes, which causes the accumulation of insertion and/or deletion in the microsatellite DNA domain.[10,11] MSI, ie, deficient MMR, results from inheritance of a germline mutation in one of the MMR genes *(MLH1*, *MSH2*, *MSH6*, and *PMS2*) or more commonly from epigenetic inactivation of MLH.[12,13] MSI occurs in ~15% of cases in CRC and usually favorable for the clinical outcomes in CRC in contrast to microsatellite stable (MSS).[14–16] MSI can be evaluated by immunohistochemistry (IHC) analysis of *MLH1*, *MSH2*, *MSH6*, and *PMS2* or by MSI testing.

BRAF, a member of the RAF family, is an essential intermediary in the RAS/RAF/mitogen-activated protein kinase (MAPK) signaling cascade and is commonly activated by its mutations.[17–19] *BRAF* V600E missense mutation is a valine (V) to glutamic acid (E) switch at codon 600 caused by the c.1799T>A transversion and accounts for 80% of *BRAF* mutations.[20] *BRAF* V600E mutation occurs in about 14% of CRC cases and more frequently in sporadic CRC with MSI.[21] However, in contrast to MSI, *BRAF* V600E mutation shows an independent negative prognostic association with survival in CRC.[22–25] Recent findings indicate that the prognostic potential of MSI can override the negative prognostic potential of *BRAF*V600E.[7,26] A number of studies have been performed to investigate the clinical utility of the combination of MSI status and *BRAF* mutation; however, the results are inconsistent and inconclusive and the sample size of the individual study is limited.

In the present study, we performed a meta-analysis for the first time to explore the prognostic role of the combination of MSI status and *BRAF* V600E mutation in CRC.

Materials and methods

Publication search

A systematic search was performed to obtain the published articles that report the prognostic effects of the combination of MSI status and *BRAF* mutation in CRC in three English databases (PubMed, EMBASE, and Web of Science) up to December 30, 2017. The following terms were used as keywords in searching: "colorectal OR colon OR rectal", "cancer OR tumor OR carcinoma", "mismatch repair OR MMR OR microsatellite instability OR MSI", "B-type Raf kinase OR *BRAF*", and "prognosis OR survival OR overall survival OR recurrence-free survival OR RFS OR progression-free survival OR PFS". The retrieved articles were screened and selected by two independent investigators according to the inclusion and exclusion criteria. We also screened the review articles and the references of selected articles to identify additional eligible studies. In addition, the public databases, TCGA and NCBI GEO, were utilized to search for eligible studies.

Inclusion and exclusion criteria

Inclusion criteria were as follows: 1) the histologic type of the tumors was limited to CRC; 2) the associations of the combination of MSI and *BRAF* mutation with RFS, PFS, and/or overall survival (OS) in CRC were evaluated; 3) the sample size of the study was more than 200, and when one study reported only MSI CRCs, the sample size should be more than 50; 4) full peer-reviewed articles that have been published as full texts; and 5) only the studies published in English. Exclusion criteria were as follows: 1) study with insufficient or duplicate data and 2) abstracts, letters, or review articles.

Data extraction

The following characteristics of eligible studies and related data were collected by two independent authors carefully: name of the first author; year of publication; country of origin; patient sex and age; cancer type; TNM stage; frequency of MSI/*BRAF*mut, MSI/*BRAF*wt, MSS/*BRAF*mut, and MSS/*BRAF*wt; sample size; and HRs with their 95% CI that reflect the associations of the combination of MSI and *BRAF* with RFS, PFS, or OS. If the study reports both the data calculated by univariate and multivariate analyses, only the adjusted HRs and 95% CIs in multivariate analysis were selected. If one study only reported a Kaplan–Meier plot curve, HR and its 95% CI were evaluated according to the previous reports.[27] Inconsistency was solved by discussion among the authors.

Quality score assessment

The Newcastle Ottawa Scale (NOS) was used to evaluate the methodological quality of each eligible article in our meta-analysis according to the following three aspects: selection, comparability, and exposure, with scores ranging from 0 to 9. The study was defined as high quality if the NOS score was more than 7.

Statistical analysis

Stata 12.0 software (StataCorp, College Station, TX, USA) was used to perform all the statistical analyses. The crude HRs and their 95% CIs were pooled to assess and compare the associations of RFS, PFS, or OS with the combination of MSI and *BRAF* mutation in CRC. The statistical significant level was determined by *Z*-test. A *P*-value less than 0.05 was considered as statistically significance. The heterogeneity among the studies was determined by Q statistic test. Fixed-effects model (Mantel–Haenszel method) was used when $P>0.1$ or $I^2<50\%$, otherwise, the pooled HRs were calculated by random-effects model.[28] The stabilities of the pooled HRs were evaluated by sensitivity analysis by omitting each study in every turn. At the same time, subgroup analyses were performed according to the cancer type (colon cancer [CC] and mixed types, which included both CC and rectal cancer, and the results of CC and rectal cancer could not be separated), tumor stage (TNM I–III and IV), therapeutic strategy (surgery with or without adjuvant chemotherapy and first-line chemotherapy), sample size (<500, 500–1000, and >1,000 cases), and methods for obtaining HR (M, U, and KM, which were obtained from multivariate analysis, univariate analysis, and Kaplan–Meier plot curves, respectively). Potential publication bias was explored by Begg's funnel plots and Egger's test.[29,30] Influence of a covariate on the association of one factor with OS in CRC was analyzed by meta-analysis regression and test of interaction.[31]

Results

Study characteristics

The literature selection process is shown in Figure 1. A total of 3,493 documents were initially identified from three English databases. After excluding those duplicated papers, reviews or meeting abstracts, and irrelevant papers, 44 articles were further evaluated. Subsequently, 21 articles were excluded according to the inclusion and exclusion criteria, of which three articles did not report RFS, PFS, or OS outcomes related to the MSI/*BRAF* mutation status,[32–34] nine had overlapped data with other studies,[35–43] five articles included small sample size,[44–48] six reported no sufficient data,[49–54] and HR in one article could not be calculated correctly from the Kaplan–Meier plot curve.[55] Finally, 23 eligible articles containing 26 cohort studies were included in the present meta-analysis.[7,23,25,56–75] Venderbosch et al's[70] article consisted of four independent cohort studies. Nam et al's[73] report contained 197 stage IV CRC cases and was included because the sample size was very close to 200. In addition, three cohort studies with overlapped data with other studies

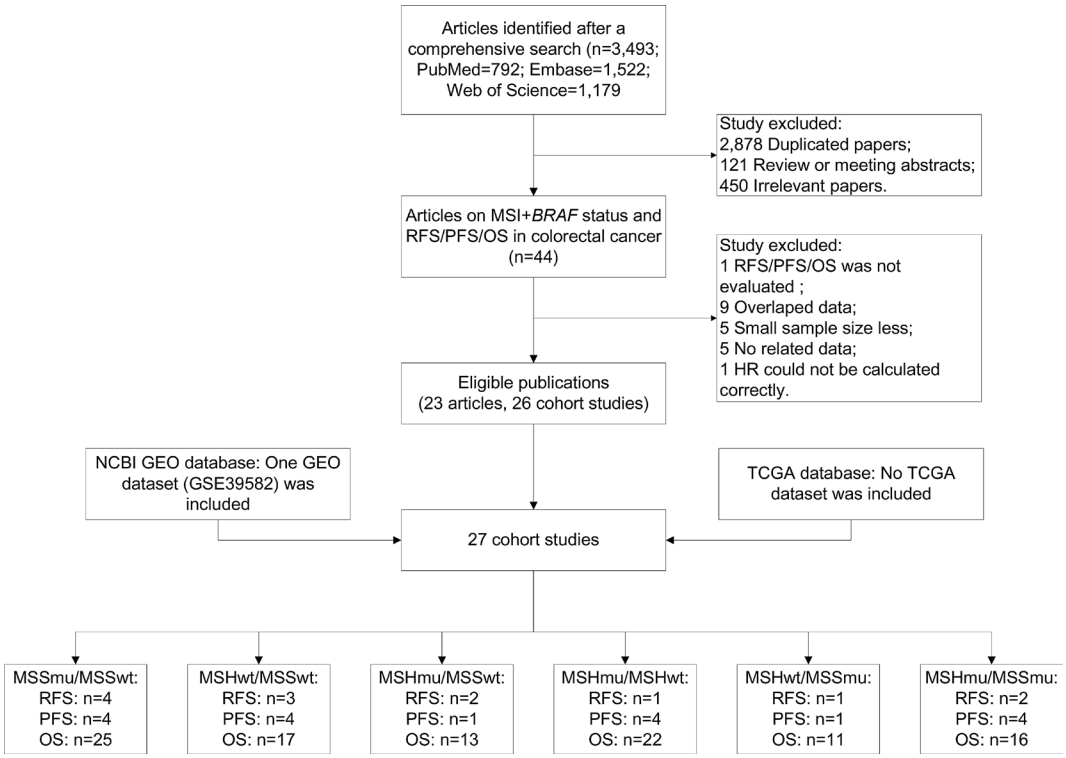

Figure 1 Flow chart of study selection.

Abbreviations: MSI, microsatellite instability; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival.

were also included in subgroup analysis stratified by cancer type and stage.[38,39,42] Furthermore, public available data sets from online databases, TCGA and NCBI GEO, were also screened according to the criteria, and only a data set, GSE39582, was selected.[76] In total, 27 independent cohort studies containing 24,067 cases were included. Quality assessment was performed, and all the studies achieved a high NOS score (≥7, data not shown). The characteristics of the included studies are summarized in Table 1.

Associations of MSI/*BRAF* mutation status with RFS and OS in CC in GSE39582

There were 465 CC cases in GSE39582. The average age of the patients was 67.2 years. Two hundred fifty-seven cases were males and 208 were females. Two hundred sixty-five patients received adjuvant chemotherapy after resection. Kaplan–Meier plot curve and Cox proportional hazards models were performed to clarify the associations of the MSI/*BRAF* mutation status with RFS and OS (Figure 2 and Table 2). In univariate analysis, compared with MSS/*BRAF*wt, MSS/*BRAF*mut was poor for RFS (HR = 2.586, 95% CI = 1.262–5.296, *P*=9.408E-03), while MSI/*BRAF*wt was favorable for RFS (HR = 0.338, 95% CI = 0.138–0.827, *P*=1.746E-02) and MSI/*BRAF*mut showed a trend of favorable effects on RFS (HR = 0.396, 95% CI = 0.146–1.073, *P*=6.858E-02). *BRAF* mutation had no effects on RFS in patient with MSI phenotype. Compared with MSS/*BRAF*mut, both MSI/*BRAF*wt (HR = 0.134, 95% CI = 0.044–0.411, *P*=4.454E-04) and MSI/*BRAF*mut (HR = 0.158, 95% CI = 0.047–0.525, *P*=2.631E-03) were favorable factors. After adjusting for TNM stage and adjuvant chemotherapy, only significant associations of RFS with MSI/*BRAF*wt (HR = 0.156, 95% CI = 0.036–0.616, *P*=8.069E-03) and MSI/*BRAF*mut (HR = 0.196, 95% CI = 0.051–0.737, *P*=1.612E-02) compared with MSS/*BRAF*mut were found. For OS, MSS/*BRAF*mut was an unfavorable factor for the prognosis compared with MSS/*BRAF*wt phenotype in both univariate analysis (HR = 3.050, 95% CI = 1.548–6.007, *P*=1.264E-03) and multivariate analysis (HR = 2.600, 95% CI = 1.313–5.150, *P*=6.132E-03). Compared with MSS/*BRAF*mut, both MSI/*BRAF*wt and MSI/*BRAF*mut were favorable factors in univariate analysis (MSI/*BRAF*wt vs MSS/*BRAF*mut: HR = 0.268, 95% CI = 0.108–0.660, *P*=4.199E-03; MSI/*BRAF*mut vs MSS/*BRAF*mut: HR = 0.264, 95% CI = 0.098–0.710, *P*=8.322E-03) and multivariate analysis (MSI/*BRAF*wt vs MSS/*BRAF*mut: HR = 0.315, 95% CI = 0.115–0.863, *P*=2.461E-02; MSI/*BRAF*mut vs MSS/*BRAF*mut: HR = 0.287, 95% CI = 0.104–0.793, *P*=1.604E-02). At the same time, no interaction between MSI and *BRAF* mutation was observed.

Meta-analysis results

Rfs/PFS

There were four studies evaluating the associations of the MSI/*BRAF* mutation status with RFS in CRC. Comparisons were performed in six pairs (MSS/*BRAF*mut vs MSS/*BRAF*wt, MSI/*BRAF*wt vs MSS/*BRAF*wt, MSI/*BRAF*mut vs MSS/*BRAF*wt, MSI/*BRAF*mut vs MSI/*BRAF*wt, MSI/*BRAF*wt vs MSS/*BRAF*mut, and MSI/*BRAF*mut vs MSS/*BRAF*mut). Overall, HRs of MSS/*BRAF*mut, MSI/*BRAF*wt, and MSI/*BRAF*mut were 1.543 (95% CI = 1.159–2.053, *P*=2.978E-03), 0.505 (95% CI = 0.308–0.828, *P*=6.728E-03), and 0.535 (95% CI = 0.302–0.943, *P*=3.235E-02), respectively, compared to MSS/*BRAF*wt (Figure 3A–C and Table 3). MSI was also a favorable factor for RFS in *BRAF*-mutated patients (HR = 0.233, 95% CI = 0.069–0.783, *P*=1.827E-02; Figure 3D and Table 3) as well as wild-type patients. For the metastatic CRC (mCRC) treated with first-line chemotherapy, there were four studies evaluating the associations of MSI/*BRAF* mutation status with PFS. The mCRC patients with MSS/*BRAF*mut (HR = 1.452, 95% CI = 1.020–0.2.067, *P*=3.845E-02) or MSI/*BRAF*mut (HR = 1.380, 95% CI = 1.070–0.1.770, *P*=1.207E-02) exhibited poor PFS compared with those with MSS/*BRAF* wild type (Figure 4 and Table 3). In contrast, there was no significant difference in PFS in other comparisons. Subgroup analysis was not performed due to the limited included studies.

OS

Next, the prognostic value of the combination of MSI and *BRAF* mutation in OS in CRC was analyzed. Overall, *BRAF* mutation was a poor factor for OS in both MSS (MSS/*BRAF*mut vs MSS/*BRAF*wt: N=25, HR = 2.018, 95% CI = 1.706–2.388, *P*=2.220E-16; Figure 5A and Table 4) and MSI patients (MSI/*BRAF*mut vs MSI/*BRAF*wt: N=22, HR = 1.470, 95% CI = 1.243–1.740, *P*=7.122E-06; Figure 5B). MSI was a favorable factor for OS in *BRAF*-mutated patients (MSI/*BRAF*mut vs MSS/*BRAF*mut: N=16, HR = 0.741, 95% CI = 0.567–0.968, *P*=2.781E-02; Figure 5C) but not in *BRAF* wild-type patients (MSI/*BRAF*wt vs MSS/*BRAF*wt: N=17, HR = 0.996, 95% CI = 0.801–1.240, *P*=9.761E-01; Figure 5D). In addition, MSI decreased the negative prognostic potential of *BRAF* mutations to a non-significant

Table 1 Basal characteristics of the included studies in the present meta-analysis

Study	Treatment	Country	Sex (male/female)	Age (years): N	Cancer type	TNM stage	MSImut	MSIwt	MSSmut	MSSwt	Prognosis	HR resource
Samowitz et al (2005)[23]	NA	USA	435/368	<65: 317; ≥65: 486	CC	I–III: 717; IV: 158	40	40	39	758	OS	M
French et al (2008)[56]	S + AC	USA	254/236	61.4	CC	II–III	35	23	42	390	OS	U
Roth et al (2010)[57]	S + AC	Europe	755/552	>60: 659; ≤60: 648	CC	II–III	45	143	53	1,002	RFS and OS	M
Tran et al(2011)[58]	S/chemotherapy	Australia/USA	186/164	63.4	CRC	IV	12	28	30	280	OS	KM
Zlobec et al (2010)[59]	S ± AC	Switzerland	186/215	69.5	CRC	I–IV	19	38	26	291	OS	KM
Gavin et al (2012)[25]	S + AC	USA	1,234/992	<65: 1,516; ≥65: 710	CC	II–III	71	130	176	1,358	OS	KM
Kalady et al (2012)[60]	S ± AC	USA	251/224	67	CRC	I–III: 322	29	47	9	251	OS	M/KM
Ogino et al (2012)[61]	S + AC	USA	274/232	59.7	CC	III	34	43	41	387	RFS and OS	M
Phipps et al (2012)[62]	NA	USA	900/1,080	<60: 955; ≥60: 1,025	CRC	I–III: 1,722; IV: 226	126	172	109	1,494	OS	M
Sylvester et al (2012)[63]	S ± AC	USA	182/245	67.4	CRC	I–III: 341; IV: 78	17	34	13	307	OS	KM
GSE39582[76]	S ± AC	France	257/208	67.2	CC	I–III: 407; IV: 58	31	42	14	378	RFS and OS	M
Lochhead et al (2013)[7]	NA	USA	570/683	68.5	CRC	I–III: 972; IV: 170	101	92	81	979	OS	M
Wangefjord et al (2013)[64]	S ± AC	Sweden	247/277	70.5	CRC	I–III: 433; IV: 91	43	28	29	384	OS	M
Lin et al (2014)[65]	S ± AC	Taiwan, China	689/374	70.3	CC	I–III: 870; IV: 193	26	101	36	900	OS	KM
Luey et al (2014)[66]	S ± AC	Australia	563/546	72.0	CRC	I–III: 1,039; IV: 44	108	32	133	720	OS	M/KM
Malesci et al (2014)[67]	S ± AC	Italy	513/368	64.8	CRC	I–III: 668; IV: 213	37	25	23	764	OS	M/KM
Ooki et al (2014)[68]	S ± AC	Japan	242/163	64.4	CRC	III	5	10	16	374	RFS and OS	M/KM
Toon et al (2014)[69]	S ± AC	Australia	683/743	74	CRC	I–III: 1,364; IV: 62	184	94	91	1,057	OS	M/KM
Venderbosch et al (2014)[70]—CAIRO	Chemotherapy	Netherlands	1,988/1,071	63.9	CRC	IV	12	6	13	291	OS and PFS	U
Venderbosch et al (2014)[70]—CAIRO2	Chemotherapy	Netherlands			CRC	IV	12	17	33	454	OS and PFS	U
Venderbosch et al (2014)[70]—COIN	Chemotherapy	UK			CRC	IV	20	45	100	1,296	OS and PFS	U
Venderbosch et al (2014)[70]—FOCUS	Chemotherapy	UK			CRC	IV	9	31	51	672	OS and PFS	U
Seppälä et al (2015)[71]	S ± AC	Finland	370/392	70.3 (11.5)	CRC	I–III: 652; IV: 100	60	44	34	600	OS	M
de Cuba et al (2016)[72]	S ± AC	Netherlands	62/81	≤68: 72; >69: 71	CC	II–III	73	70	NA	NA	OS	KM
Nam et al (2016)[73]	S ± AC	Korea	103/88	59.77	CRC	IV	0	3	6	188	OS	M
Fujiyoshi et al (2017)[74]	S ± AC	Japan	231/170	63.4	CRC	IV	4	11	21	360	OS	U
Taieb et al (2017)[75]	S + AC	Europe	2,397/2,014	58.1	CC	III	201	276	279	3,655	OS	KM

Abbreviations: AC, adjuvant chemotherapy; CC, colon cancer; CRC, colorectal cancer; KM, HR was obtained indirectly from Kaplan–Meier plot curves; M, HR was obtained from multivariate analysis; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; NA, not applicable; OS, overall survival; RFS, recurrence-free survival; PFS, progression-free survival; S, surgery; U, HR was obtained from univariate analysis.

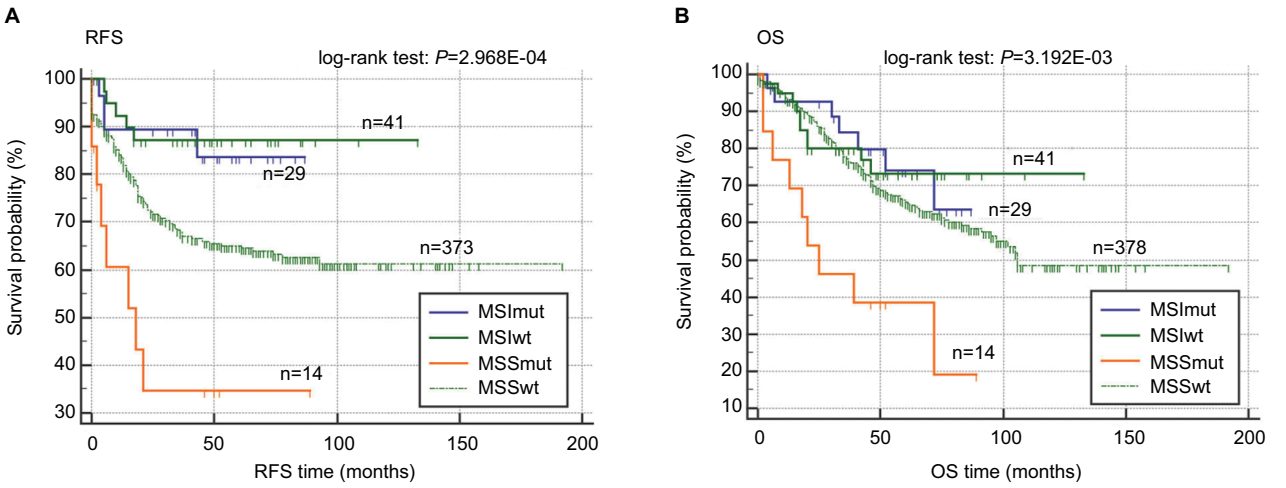

Figure 2 Effect of MSI/*BRAF* mutation status on RFS and OS in colon cancer (GSE39582).
Note: RFS (**A**) and OS (**B**) were compared among the colon cancer patients with four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut.
Abbreviations: MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; RFS, recurrence-free survival; OS, overall survival.

Table 2 Univariate and multivariate Cox proportional hazards regression models for RFS and OS of the patients with colon cancer in GSE39582 cohort

Parameters	Univariate analysis				Multivariate analysis			
	HR	LCI	UCI	*P*-value	HR	LCI	UCI	*P*-value
RFS								
Sex (female/male)	0.923	0.660	1.290	6.396E-01				
Age (≥60/<60 years)	0.763	0.534	1.090	1.373E-01				
TNM stage (III–IV/I–II)	2.730	1.917	3.887	**2.550E-08**				
Tumor location (proximal/distal)	1.304	0.924	1.841	1.303E-01				
Adjuvant chemotherapy (yes/no)	1.462	1.029	2.076	**3.399E-02**				
MSSmut/MSSwt	2.586	1.262	5.296	**9.408E-03**	1.709	0.741	3.945	2.090E-01
MSIwt/MSSwt	0.338	0.138	0.827	**1.746E-02**	0.426	0.173	1.049	6.340E-02
MSImut/MSSwt	0.396	0.146	1.073	6.858E-02	0.377	0.137	1.039	5.934E-02
MSIwt/MSSmut	0.134	0.044	0.411	**4.454E-04**	0.156	0.039	0.616	**8.069E-03**
MSImut/MSSmut	0.158	0.047	0.525	**2.631E-03**	0.193	0.051	0.737	**1.612E-02**
MSImut/MSIwt	1.199	0.322	4.467	7.868E-01	1.705	0.406	7.154	4.658E-01
MSI and *BRAF* mutation interaction				5.440E-02				5.380E-02
OS								
Sex (female/male)	0.823	0.598	1.131	2.296E-01				
Age (≥60/<60 years)	1.320	0.906	1.923	1.476E-01				
TNM stage (III–IV/I–II)	1.776	1.293	2.439	**3.900E-04**				
Tumor location (proximal/distal)	0.958	0.697	1.317	7.909E-01				
Adjuvant chemotherapy (yes/no)	0.787	0.562	1.102	1.630E-01				
MSSmut/MSSwt	3.050	1.548	6.007	**1.264E-03**	2.600	1.313	5.150	**6.132E-03**
MSIwt/MSSwt	0.751	0.394	1.429	3.824E-01	0.837	0.438	1.599	5.906E-01
MSImut/MSSwt	0.753	0.351	1.612	4.646E-01	0.728	0.340	1.559	4.132E-01
MSIwt/MSSmut	0.268	0.108	0.660	**4.199E-03**	0.315	0.115	0.863	**2.461E-02**
MSImut/MSSmut	0.264	0.098	0.710	**8.322E-03**	0.287	0.104	0.793	**1.604E-02**
MSImut/MSIwt	0.986	0.375	2.593	9.777E-01	0.936	0.344	2.545	6.850E-01
MSI and *BRAF* mutation interaction				4.317E-01				3.694E-01

Note: For RFS, TNM stage and adjuvant chemotherapy were adjusted in multivariate analysis; for OS, TNM stage was adjusted in multivariate analysis; Bold, statistically significant.
Abbreviations: LCI, lower 95% confidence interval of HR; MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival; RFS, recurrence-free survival; UCI, upper 95% confidence interval of HR.

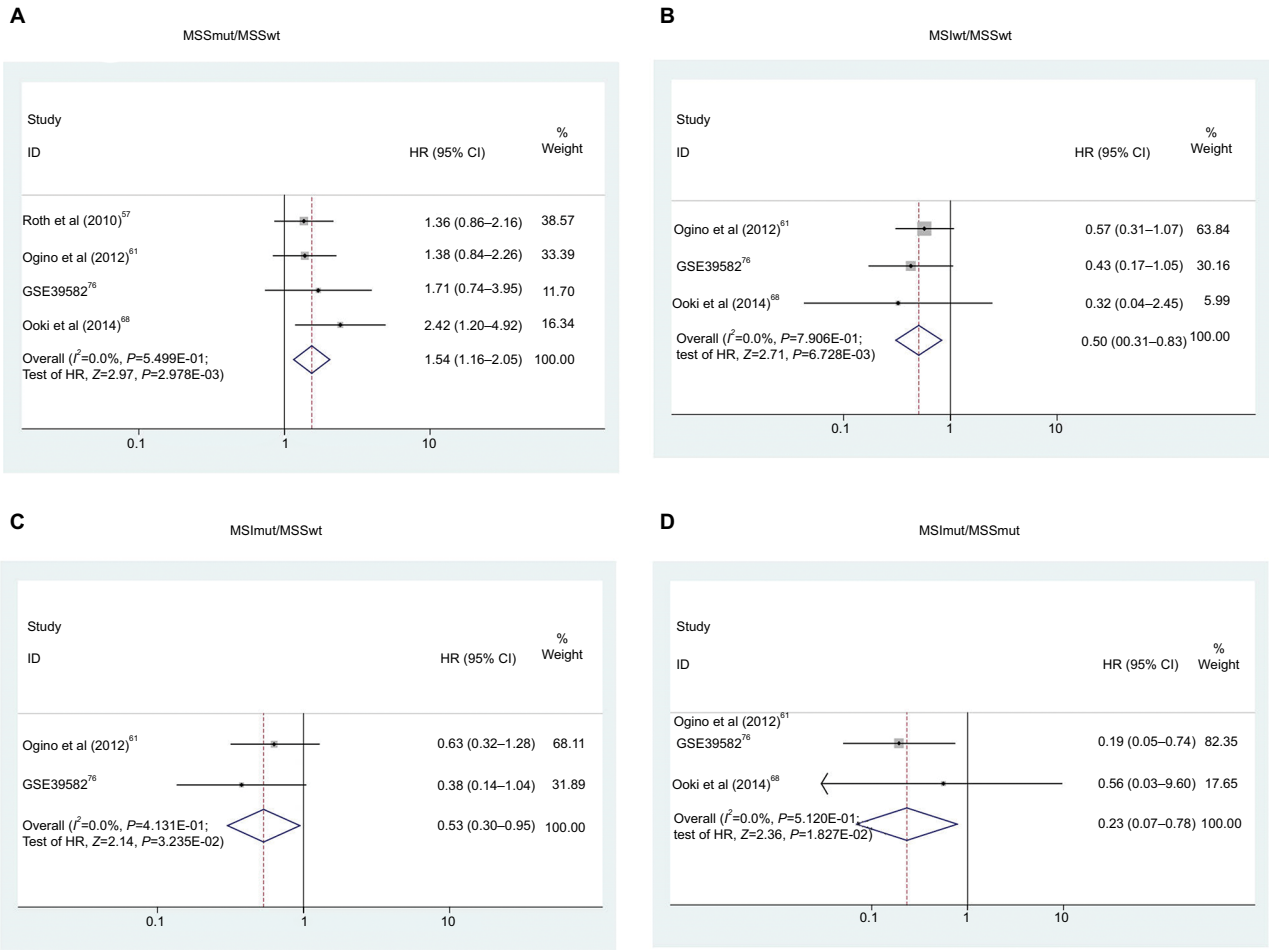

Figure 3 Forest plot for MSI/*BRAF* mutation status and RFS in colorectal cancer.

Notes: The colorectal cancer was divided into four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut. RFS was compared between MSSmut and MSSwt (**A**), MSIwt and MSSwt (**B**), MSImut and MSSwt (**C**), and MSImut and MSSmut (**D**) by pooling the previous studies.

Abbreviations: MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; RFS, recurrence-free survival.

Table 3 Meta-analysis results for associations of MSI/*BRAF* mutation status with RFS/PFS in colorectal cancer

Groups	N	HR	HRlci	HRuci	P_{HR}	Model	I^2 (%)	P_{heter}	P_{Begg}	P_{Egger}
RFS										
MSSmut/MSSwt	4	1.543	1.159	2.053	**2.978E-03**	F	0.0	5.499E-01	3.077E-01	2.388E-01
MSIwt/MSSwt	3	0.505	0.308	0.828	**6.728E-03**	F	0.0	7.906E-01	2.983E-01	3.043E-01
MSImut/MSSwt	2	0.535	0.302	0.943	**3.235E-02**	F	0.0	4.131E-01		
MSImut/MSSmut	2	0.233	0.069	0.783	**1.827E-02**	F	0.0	5.120E-01		
PFS										
MSSmut/MSSwt	4	1.452	1.020	2.067	**3.845E-02**	R	60.4	5.554E-02	3.077E-01	4.938E-01
MSIwt/MSSwt	4	1.453	0.920	2.294	1.096E-01	R	58.3	6.579E-02	7.339E-01	9.647E-01
MSImut/MSSwt	1	1.380	1.070	1.770	**1.207E-02**					
MSImut/MSIwt	4	1.061	0.505	2.232	8.729E-01	R	52.5	9.704E-02	7.339E-01	8.668E-01
MSIwt/MSSmut	1	0.990	0.790	1.240	9.283E-01					
MSImut/MSSmut	4	0.958	0.618	1.486	8.493E-01	F	33.7	2.096E-01	7.339E-01	7.859E-01

Note: Bold, statistically significant.

Abbreviations: HRlci, lower 95% CI of HR; HRuci, upper 95% CI of HR; MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; R, random-effects model; F, fixed-effects model; PFS, progression-free survival; RFS, recurrence-free survival.

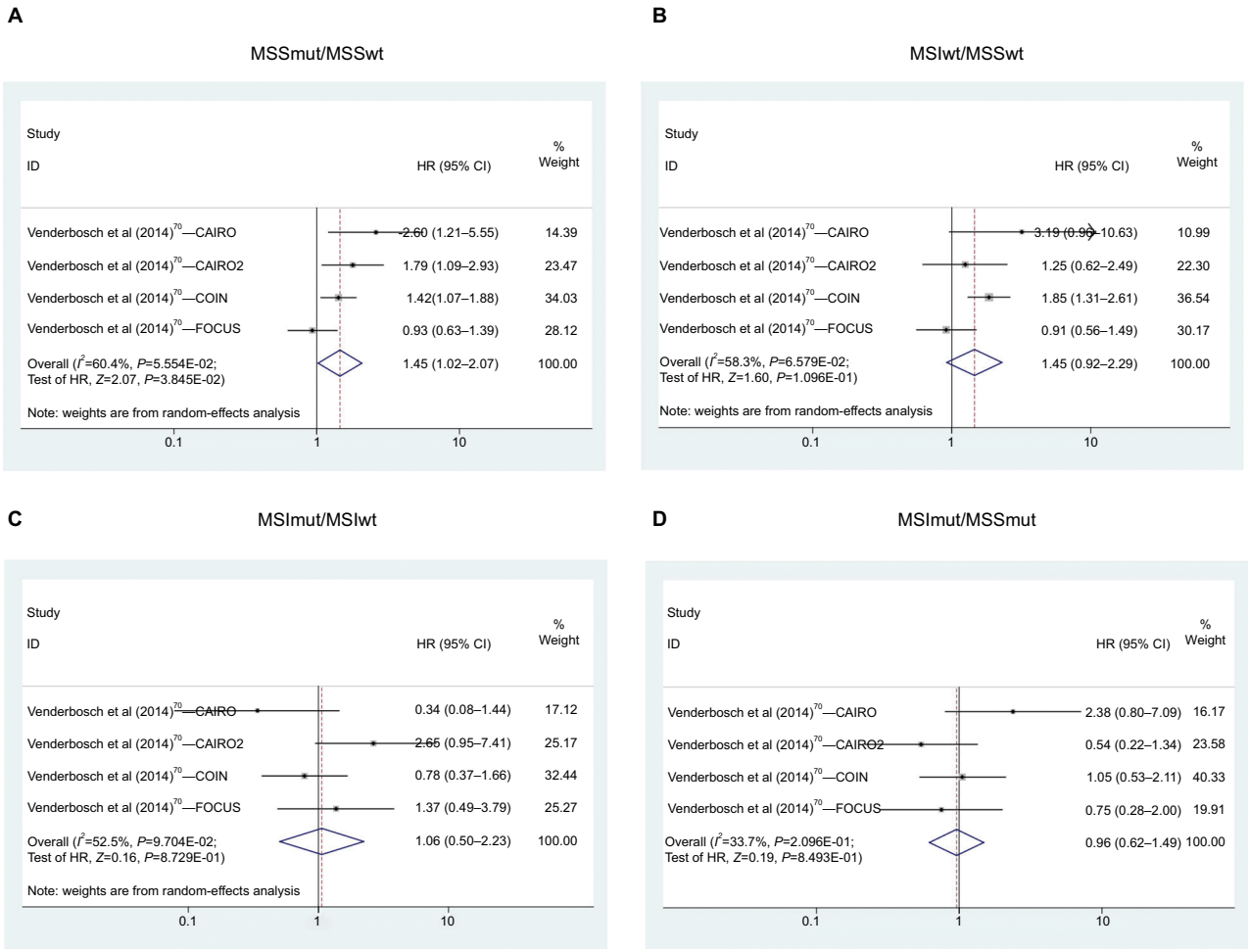

Figure 4 Forest plot for MSI/*BRAF* mutation status and PFS in colorectal cancer.
Notes: The colorectal cancer was divided into four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut. PFS was compared between MSSmut and MSSwt (**A**), MSIwt and MSSwt (**B**), MSImut and MSIwt (**C**), and MSImut and MSSmut (**D**) by pooling the previous studies.
Abbreviations: MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; PFS, progression-free survival.

level (MSI/*BRAF*mut vs MSS/*BRAF*wt: N=13, HR = 1.324, 95% CI = 0.938–1.868, *P*=1.096E-01; Figure 5E). HR of the patients with MSI/*BRAF*wt was 0.560 (N=11, 95% CI = 0.433–0.725, *P*=1.034E-05, Figure 5F) compared to MSS/*BRAF*mut. Subgroup analyses were also performed according to the cancer type, tumor stage, therapeutic strategy, sample size, and methods for obtaining HR. Associations of MSI/*BRAF* mutation status with OS in CRC in subgroups were similar with the results overall except the subgroup of stage IV cancers and first-line chemotherapy–treated stage IV cancers. In the subgroup of stage IV CRC, *BRAF* mutation was a poor factor for OS in both MSS (MSS/*BRAF*mut vs MSS/*BRAF*wt: N=8, HR = 2.180, 95% CI = 1.844–2.578, *P*=0.000E+00) and MSI patients (MSI/*BRAF*mut vs MSI/*BRAF*wt: N=6, HR = 1.865, 95% CI = 1.205–2.887, *P*=5.110E-03; Table 4). However, unlike the subgroup of stage I–III, whose results were similar with the overall results, MSI showed poor effects on OS in *BRAF* wild-type patients (MSI/*BRAF*wt vs MSS/*BRAF*wt: N=6, HR = 1.493, 95% CI = 1.187–1.879, *P*=6.262E-04), and no significant effects in *BRAF*-mutated patients (MSI/*BRAF*mut vs MSS/*BRAF*mut: N=5, HR = 1.143, 95% CI = 0.789–1.655, *P*=4.839E-01; Table 4). In the subgroup of first-line chemotherapy–treated stage IV CRCs, *BRAF* mutation was a poor factor for OS in MSS patients (MSS/*BRAF*mut vs MSS/*BRAF*wt: N=4, HR = 1.981, 95% CI = 1.606–2.444, *P*=9.963E-10) and MSI had no prognostic effects on OS in *BRAF*-mutated or *BRAF* wild-type patients (Table 4). As both MSI and *BRAF* mutation were correlated with proximal/right-sided CC compared with other CRCs, subgroup analysis according to the tumor location was necessary. However, the data from the included studies were not sufficient to support the subgroup analysis. Instead, we

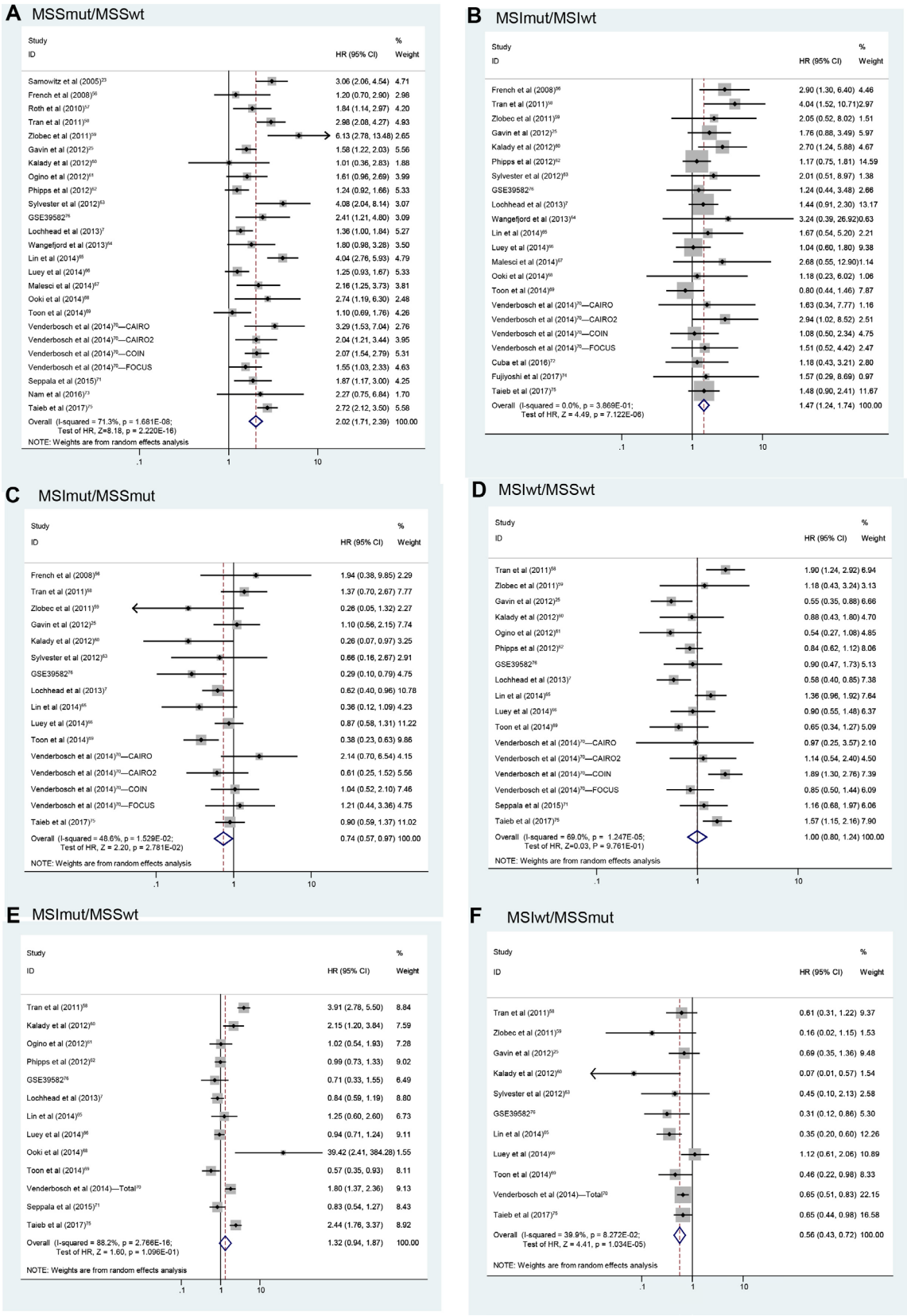

Figure 5 Forest plot for MSI/*BRAF* mutation status and OS in colorectal cancer.

Notes: The colorectal cancer was divided into four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut. OS was compared between MSSmut and MSSwt (**A**), MSImut and MSIwt (**B**), MSImut and MSSmut (**C**), MSIwt and MSSwt (**D**), MSImut and MSSwt (**E**), and MSIwt and MSSmut (**F**) by pooling the previous studies.

Abbreviations: MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival.

Table 4 Meta-analysis results for associations of MSI/*BRAF* mutation status with OS in colorectal cancer

Groups	N	HR	HRlci	HRuci	P_{HR}	Model	I^2 (%)	P_{heter}	P_{Begg}	P_{Egger}
MSSmut/MSSwt										
Overall	25	2.018	1.706	2.388	**2.220E-16**	R	71.3	**1.681E-08**	2.937E-01	3.369E-01
Cancer type										
CC	10	2.104	1.584	2.795	**2.747E-07**	R	77.6	**6.932E-06**		
MIX	17	1.913	1.561	2.344	**3.850E-10**	R	66.7	**4.565E-05**		
Stage										
I–III	12	2.047	1.623	2.582	**1.448E-09**	R	61.9	**2.352E-03**		
IV	8	2.180	1.844	2.578	**0.000**E+00	F	0.0	4.341E-01		
Therapy										
S + AC	7	2.000	1.488	2.687	**4.225E-06**	R	63.3	**1.200E-02**		
S ± AC	21	2.112	1.701	2.624	**1.380E-11**	R	73.5	**2.391E-08**		
Chemotherapy	4	1.981	1.606	2.444	**9.963E-10**	F	5.7%	3.640E-01		
Sample size										
<500	9	2.515	1.805	3.505	**5.037E-08**	R	51.2%	**3.713E-02**		
500–1,000	8	1.856	1.415	2.432	**7.464E-06**	R	56.5%	**2.431E-02**		
>1,000	9	1.933	1.472	2.540	**2.137E-06**	R	82.9%	**1.681E-07**		
Data resource										
KM	6	3.213	2.728	3.783	**0.000**E+00	F	20.5%	2.790E-01		
M	14	1.668	1.389	2.002	**4.253E-08**	R	49.2%	**1.923E-02**		
U	7	1.702	1.489	1.946	**6.217E-15**	F	15.2%	3.144E-01		
MSIwt/MSSwt										
Overall	17	0.996	0.801	1.24	9.761E-01	R	69.0	**1.247E-05**	9.045E-01	3.712E-01
Cancer type										
CC	6	0.999	0.677	1.475	9.970E-01	R	74.9	**1.272E-03**		
MIX	12	1.018	0.785	1.322	8.887E-01	R	65.4	**8.364E-04**		
Stage										
I–III	6	0.912	0.589	1.412	6.818E-01	R	73.2	**2.214E-03**		
IV	6	1.493	1.187	1.879	**6.262E-04**	F	40.5	1.350E-01		
Therapy										
S + AC	5	0.912	0.511	1.628	7.566E-01	R	77.5	**1.369E-03**		
S ± AC	11	0.979	0.763	1.255	8.650E-01	R	57.2	**9.493E-03**		
Chemotherapy	4	1.242	0.779	1.979	3.628E-01	R	53.3	9.246E-02		
Sample size										
<500	5	1.321	0.981	1.779	6.577E-02	F	28.4	2.319E-01		
500–1,000	4	0.904	0.669	1.223	5.157E-01	F	11.5	3.353E-01		
>1,000	8	1.023	0.719	1.456	8.966E-01	R	81.4	**3.425E-06**		
Data resource										
KM	7	1.388	1.168	1.650	**1.992E-04**	F	17.4	2.966E-01		
M	7	0.878	0.614	1.256	4.777E-01	R	75.2	**4.737E-04**		
U	4	0.742	0.546	1.008	5.613E-02	F	9.2	3.476E-01		
MSImut/MSSwt										
Overall	13	1.324	0.938	1.868	1.096E-01	R	88.2	**2.766E-16**	8.572E-01	9.922E-01
Cancer type										
CC	5	1.289	0.810	2.051	2.846E-01	R	72.8	**5.389E-03**		
MIX	9	1.346	0.878	2.064	1.738E-01	R	90.6	**5.235E-15**		
Stage										
I–III	6	1.623	0.999	2.637	**5.000E-02**	R	74.1	**1.697E-03**		
IV	2	2.634	1.232	5.633	**1.242E-02**	R	91.8	**4.909E-04**		
Therapy										
S + AC	3	1.669	0.919	3.031	9.296E-02	R	66.4	5.079E-02		
S ± AC	10	1.196	0.829	1.727	3.371E-01	R	80.5	**5.600E-07**		
Chemotherapy	1	1.800	1.371	2.362	**2.235E-05**					
Sample size										
<500	4	2.551	1.009	6.447	**4.770E-02**	R	85.4	**1.305E-04**		
500–1,000	3	1.185	0.684	2.055	5.485E-01	R	79.9	**6.874E-03**		
>1,000	6	1.090	0.715	1.663	6.892E-01	R	85.1	**2.782E-06**		
Data resource										
KM	8	1.907	1.255	2.898	**2.528E-03**	R	87.1	**1.916E-09**		
M	6	0.849	0.713	1.010	6.431E-02	F	0.0	5.365E-01		

(*Continued*)

Table 4 (*Continued*)

Groups	N	HR	HRlci	HRuci	P_{HR}	Model	I^2 (%)	P_{heter}	P_{Begg}	P_{Egger}
MSImut/MSIwt										
Overall	22	1.470	1.243	1.740	**7.122E-06**	F	0.0	3.869E-01	1.010E-01	8.788E-02
Cancer type										
CC	7	1.554	1.167	2.070	**2.612E-03**	F	0.0	6.929E-01		
MIX	16	1.401	1.146	1.712	**1.002E-03**	F	9.7	3.427E-01		
Stage										
I–III	8	1.599	1.217	2.101	**7.517E-04**	F	0.3	4.268E-01		
IV	6	1.865	1.205	2.887	**5.110E-03**	F	4.9	3.850E-01		
Therapy										
S + AC	4	1.777	1.252	2.521	**1.282E-03**	F	0.0	5.766E-01		
S ± AC	16	1.439	1.165	1.779	**7.517E-04**	F	0.0	5.722E-01		
Chemotherapy	4	1.534	0.921	2.553	1.010E-01	F	0.0	5.241E-01		
Sample size										
<500	10	2.207	1.556	3.129	**8.996E-06**	F	0.0	8.056E-01		
500–1,000	4	2.013	1.074	3.770	**2.926E-02**	F	0.0	7.291E-01		
>1,000	8	1.247	1.003	1.550	**4.659E-02**	F	0.0	6.770E-01		
Data resource										
KM	8	1.188	0.915	1.543	1.971E-01	F	0.0	7.553E-01		
M	7	1.548	1.186	2.022	**1.327E-03**	F	25.6	2.330E-01		
U	8	1.844	1.303	2.611	**5.606E-04**	F	0.0	7.506E-01		
MSIwt/MSSmut										
Overall	11	0.560	0.433	0.725	**1.034E-05**	R	39.90	8.272E-02	5.900E-02	1.060E-01
Cancer type										
CC	5	0.511	0.390	0.670	**1.174E-06**	F	28.7	2.302E-01		
MIX	7	0.641	0.524	0.785	**1.708E-05**	F	43.60	1.002E-01		
Stage										
I–III	6	0.605	0.467	0.785	**1.568E-04**	F	19.0	2.891E-01		
IV	3	0.640	0.510	0.805	**1.281E-04**	F	0.0	8.106E-01		
Therapy										
S + AC	4	0.624	0.449	0.867	**4.954E-03**	F	0.0	6.662E-01		
S ± AC	10	0.490	0.343	0.699	**8.148E-05**	R	46.5	5.145E-02		
Chemotherapy	1	0.650	0.510	0.829	**5.205E-04**					
Sample size										
<500	5	0.406	0.247	0.669	**4.001E-04**	F	25.0	2.551E-01		
>1,000	6	0.573	0.4	0.822	**2.446E-03**	R	49.40%	7.841E-02		
Data resource										
KM	10	0.572	0.483	0.679	**1.455E-10**	F	25.70	2.072E-01		
M	2	0.635	0.184	2.185	4.715E-01	R	77.6	**3.470E-02**		
MSImut/MSSmut										
Overall	16	0.741	0.567	0.968	**2.781E-02**	R	48.6	**1.529E-02**	8.181E-01	7.608E-01
Cancer type										
CC	6	0.640	0.385	1.063	**8.543E-02**	R	56.1	**4.400E-02**		
MIX	11	0.743	0.534	1.033	**7.673E-02**	R	51.6	**2.359E-02**		
Stage										
I–III	7	0.651	0.444	0.954	**2.781E-02**	R	48.9	6.827E-02		
IV	5	1.143	0.789	1.655	4.839E-01	F	0.0	4.886E-01		
Therapy										
S + AC	4	0.918	0.656	1.284	6.171E-01	F	1.1	3.870E-01		
S ± AC	11	0.579	0.408	0.822	**2.213E-03**	R	53.1	**1.903E-02**		
Chemotherapy	4	1.054	0.676	1.645	8.181E-01	F	0.9	3.870E-01		
Sample size										
<500	7	0.718	0.353	1.460	3.628E-01	R	60.5	**1.883E-02**		
500–1,000	2	0.825	0.420	1.620	5.755E-01	F	0.0	3.222E-01		
>1,000	8	0.677	0.503	0.912	**1.017E-02**	R	54.1	**3.293E-02**		
Data resource										
KM	10	0.633	0.420	0.955	**2.926E-02**	R	58.2	**1.057E-02**		
M	3	0.635	0.399	1.012	**5.613E-02**	R	53.9	1.142E-01		
U	5	0.855	0.523	1.397	5.287E-01	R	53.9	6.990E-02		

Note: Bold, statistically significant.

Abbreviations: AC, adjuvant chemotherapy; CC, colon cancer; M, HR was obtained from multivariate analysis; MIX, the studies included both CC and rectal cancer and results of which could not be separated; KM, HR was obtained indirectly from Kaplan–Meier plot curves; MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; RFS, recurrence free survival; S, surgery; U, HR was obtained from univariate analysis; R, random-effects model; F, fixed-effects model.

analyzed the influence of prevalence of proximal/right-sided cancer on the associations in comparisons of different MSI/*BRAF* mutation subtypes by meta-analysis regression and no significant result was found (Table S1).

Heterogeneity, publication bias, and sensitivity analysis

For RFS, no heterogeneity was found by all comparisons (Table 3). For PFS, heterogeneity was found in comparing MSS/*BRAF*wt with MSI/*BRAF*wt and MSI/*BRAF*mut and in comparing MSI/*BRAF*wt with MSI/*BRAF*mut. When evaluating the associations of OS with MSI/*BRAF* mutation status, heterogeneity existed in all analyses overall except for the comparison of MSI/*BRAF*mut with MSI/*BRAF*wt (Table 4). During comparison of MSS/*BRAF*mut with MSS/*BRAF*wt, heterogeneity reduced to no significance in subgroups of stage IV, KM, and U. During comparison of MSI/*BRAF*wt with MSS/*BRAF*wt, heterogeneity reduced to no significance in subgroups of stage IV, <500 cases, 500–1000 cases, KM, and U. During comparison of MSI/*BRAF*mut with MSS/*BRAF*wt, heterogeneity reduced to no significance in subgroups of M. For MSI/*BRAF*wt vs MSS/*BRAF*mut, heterogeneity decreased in subgroups of CC, stage 1–III and IV, surgery plus adjuvant chemotherapy, <500 cases, and KM. For MSI/*BRAF*mut vs MSS/*BRAF*mut, heterogeneity reduced in subgroups of stage IV, surgery plus adjuvant chemotherapy, chemotherapy, and 500–1000 cases. Potential publication bias was first evaluated by Begg's funnel plots. Symmetrical funnel plots were obtained in all analyses (Figure 6). The Egger's test was also performed to provide the statistical evidence of publication bias, and the results did not show publication bias in all the pooling analyses (Tables 3 and 4). Finally, the sensitivity analysis was performed to examine the influence set by the individual study on the pooled HRs by deleting each study once. All the results were almost same with the overall results in all comparisons (Figure 7).

Interaction of MSI and *BRAF* mutation

In the included studies, the prevalence of *BRAF* mutation in MSI cases was much higher than that in MSS cases (45.5% vs 7.1%, pooled OR = 11.247, 95% CI = 8.734–14.482, *P*=0.000E+00), suggesting a significant association of MSI with *BRAF* mutation in CRC, which promoted us to investigate the interaction of MSI and *BRAF* mutation when evaluating the associations of MSI/*BRAF* mutation status with OS in CRC. Meta-analysis regression suggested that MSI did not influence the association of *BRAF* mutation with OS in CRC (coefficient = −0.041, *Z*=−0.290, *P*=7.718E-01; Table 5), and *BRAF* mutation did not influence the association of MSI with OS in CRC (coefficient = −0.239, *Z*=−0.770, *P*=4.413E-01; Table 5). Test of interaction was performed according to the method suggested in the previous report,[31] and no interaction of MSI with *BRAF* mutation was observed.

Discussion

CRC is a heterogeneous disease and presents significant difference in clinical presentation, prognosis, and treatment response, even at the same pathological stage. Additional molecular biomarkers such as MSI and *BRAF* mutation have been mostly investigated to classify CRC into intrinsic subtypes and predict the therapy response and prognosis.[6–9] However, due to the opposing effects between MSI and *BRAF* mutation in general, whether the concomitant evaluation of both MSI and *BRAF* status provides an additive or subtractive effect on patient outcome is not conclusive and consistent.

The previous studies suggested that the frequencies of MSI and *BRAF* mutation in CRC were ~15% and 14%, respectively.[14,15,77] In the present study, of the 24,067 cases, 11.9% cases were with MSI phenotype and 11.6% cases with *BRAF* mutation, a little smaller than that in the previously reported study.[21] The prevalence of *BRAF* mutation in MSI cases was much higher than that in MSS cases. However, the frequency of MSI or *BRAF* mutation in individual studies ranged drastically (MSI: 1.5%–22.6%; *BRAF* mutation: 3.0%–24.3%), which might be due to the diversity of the included CRC patients and different detection methods for the molecular biomarkers.

Currently, several meta-analyses studies have been performed to investigate the prognostic value of MSI or *BRAF* mutation in CRC. Gkekas et al[78] have pooled 19 studies containing 5,998 cases and found no significant association of MSI with OS (HR = 0.73, 95% CI = 0.33–1.65) and disease-free survival (DFS; HR = 0.60, 95% CI = 0.27–1.32) in stage II CC. In 2012, Safaee Ardekani et al[79] combined 26 studies including 11,773 patients and found that *BRAF* mutation was significantly associated with OS in CRC (HR = 2.25, 95% CI = 1.82–2.83). Multiple meta-analyses were performed in mCRC to explore the associations of *BRAF* mutation with prognosis and achieved consistent results that *BRAF* mutation was significantly associated with shorter PFS and OS.[80–84] In stage II–III CRC treated with surgery and adjuvant therapy, *BRAF* mutation was also found to be associated with shorter OS and DFS.[85] However, there was no meta-analysis study summarizing the prognosis of the CRC patients with different subtypes defined by MSI and *BRAF* mutation, ie, MSS/*BRAF*wt, MSS/*BRAF*mut, MSI/

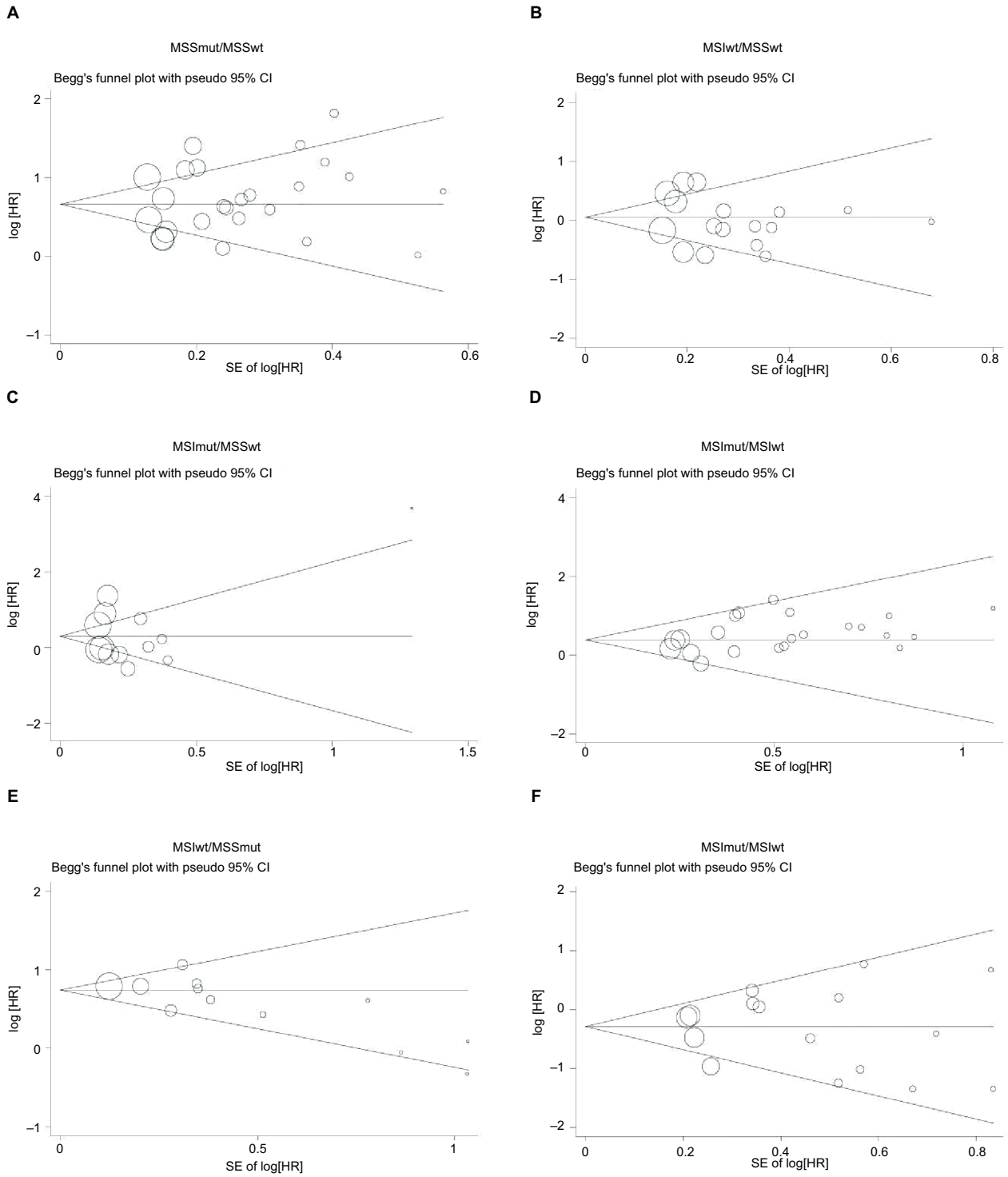

Figure 6 Begg's funnel plot for the publication bias analysis for associations of MSI/*BRAF* mutation status with OS in colorectal cancer.

Notes: The colorectal cancer was divided into four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut. Potential publication bias was explored by Begg's funnel plots for OS comparison between MSSmut and MSSwt (**A**), MSIwt and MSSwt (**B**), MSImut and MSSwt (**C**), MSImut and MSIwt (**D**), MSIwt and MSSmut (**E**), and MSImut and MSSmut (**F**).

Abbreviations: MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival.

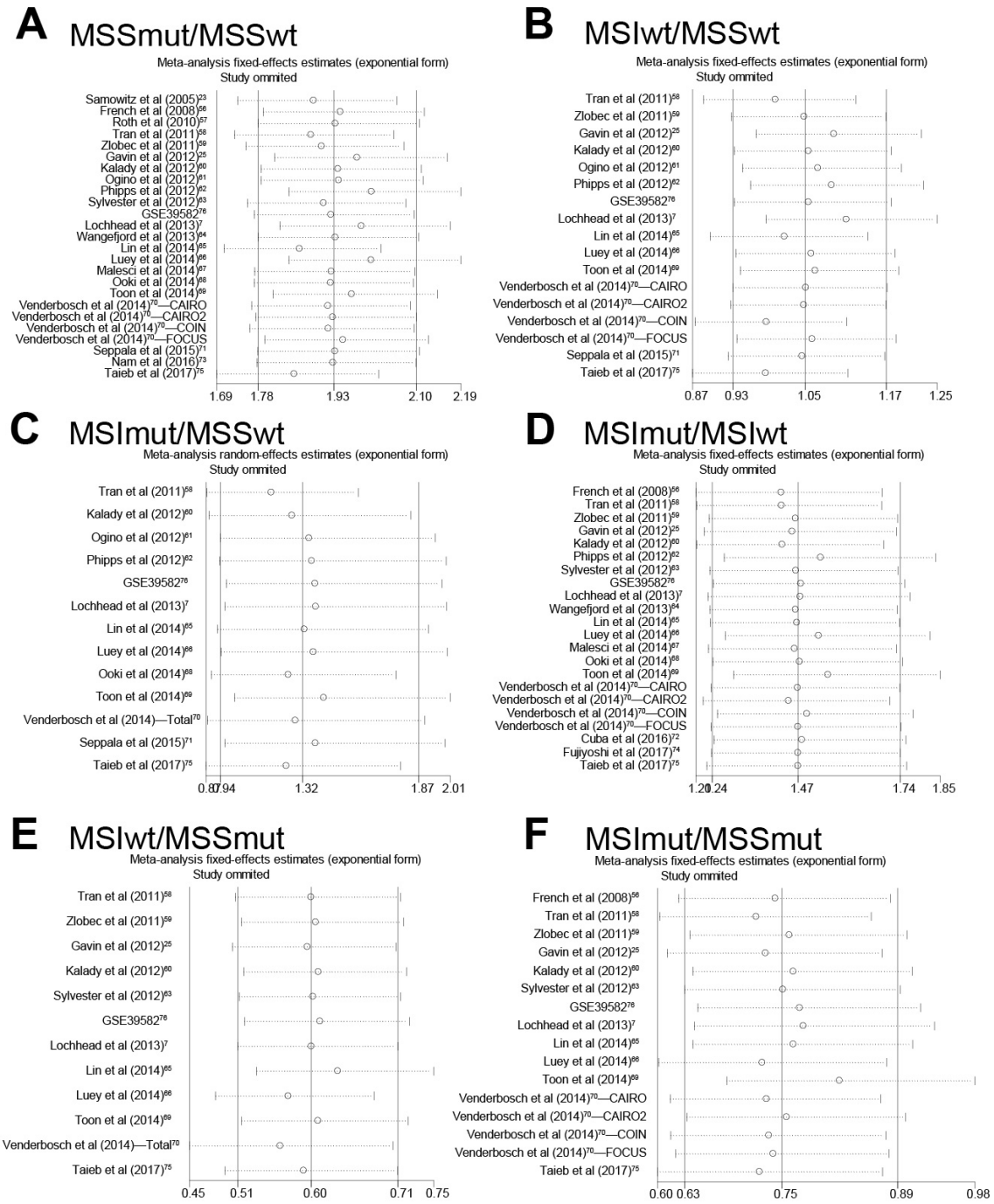

Figure 7 Sensitivity analyses for publication bias analysis for associations of MSI/*BRAF* mutation status with OS in colorectal cancer.

Notes: The colorectal cancer was divided into four subtypes according to the combination of MSI and *BRAF* mutation, ie, MSSwt, MSSmut, MSIwt, and MSImut. Sensitivity analyses were performed for OS comparison between MSSmut and MSSwt (**A**), MSIwt and MSSwt (**B**), MSImut and MSSwt (**C**), MSImut and MSIwt (**D**), MSIwt and MSSmut (**E**), and MSImut and MSSmut (**F**).

Abbreviations: MSI, microsatellite instability; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival.

Table 5 Influence of MSI on the association of *BRAF* mutation with OS in colorectal cancer was analyzed by meta-analysis regression and test of interaction

Comparison	Covariate/ stratification	Meta-analysis regression			Test of interaction	
		Coef	Z	*P*	T	*P*
*BRAF*mut/wt	MSI	–0.041	–0.290	7.718E-01	–0.304	7.639E-01
MSI/MSS	*BRAF* mutation	–0.239	–0.770	4.413E-01	–0.725	4.850E-01

Note: The test of interaction was performed according to the report by Altman and Bland.[31]

Abbreviations: Coef, coefficient; MSI, microsatellite instability; MSS, microsatellite stable; OS, overall survival; Z and T, statistics from Z test and t test. respectively; P, P values.

*BRAF*wt, and MSI/*BRAF*mut. In the present study, our results suggested that *BRAF* mutation was associated with shorter OS in both MSS CRC patients and MSI CRC patients overall and in all subgroups stratified by cancer type, tumor stage, and treatment, which were similar with the prognostic value of *BRAF* mutation in entire CRC and mCRC.[79–84] Similar to the previous results, we also found the poor prognostic effects of *BRAF* mutation in both MSS and MSI CRC treated with surgery and adjuvant therapy, and MSI did not change the poor effect of *BRAF* mutation.[85] Our results also revealed that MSI was not associated with OS in *BRAF*wt patents with CRC overall or in stage I–III CRC, similar to the study by Gkekas et al[78] performed in stage II CC regardless of *BRAF* mutation. In contrast, MSI was a favorable factor for OS in *BRAF*-mutated CRC patients. On the basis of the previous reports and our results, we speculated that the effect of *BRAF* mutation was bigger than that of MSI. To predict prognosis, CRC should be stratified by *BRAF* mutation first (Figure 8A). MSS/*BRAF*mut was a poor prognostic factor, while MSS/*BRAF*wt and MSI/*BRAF*wt were comparable and favorable

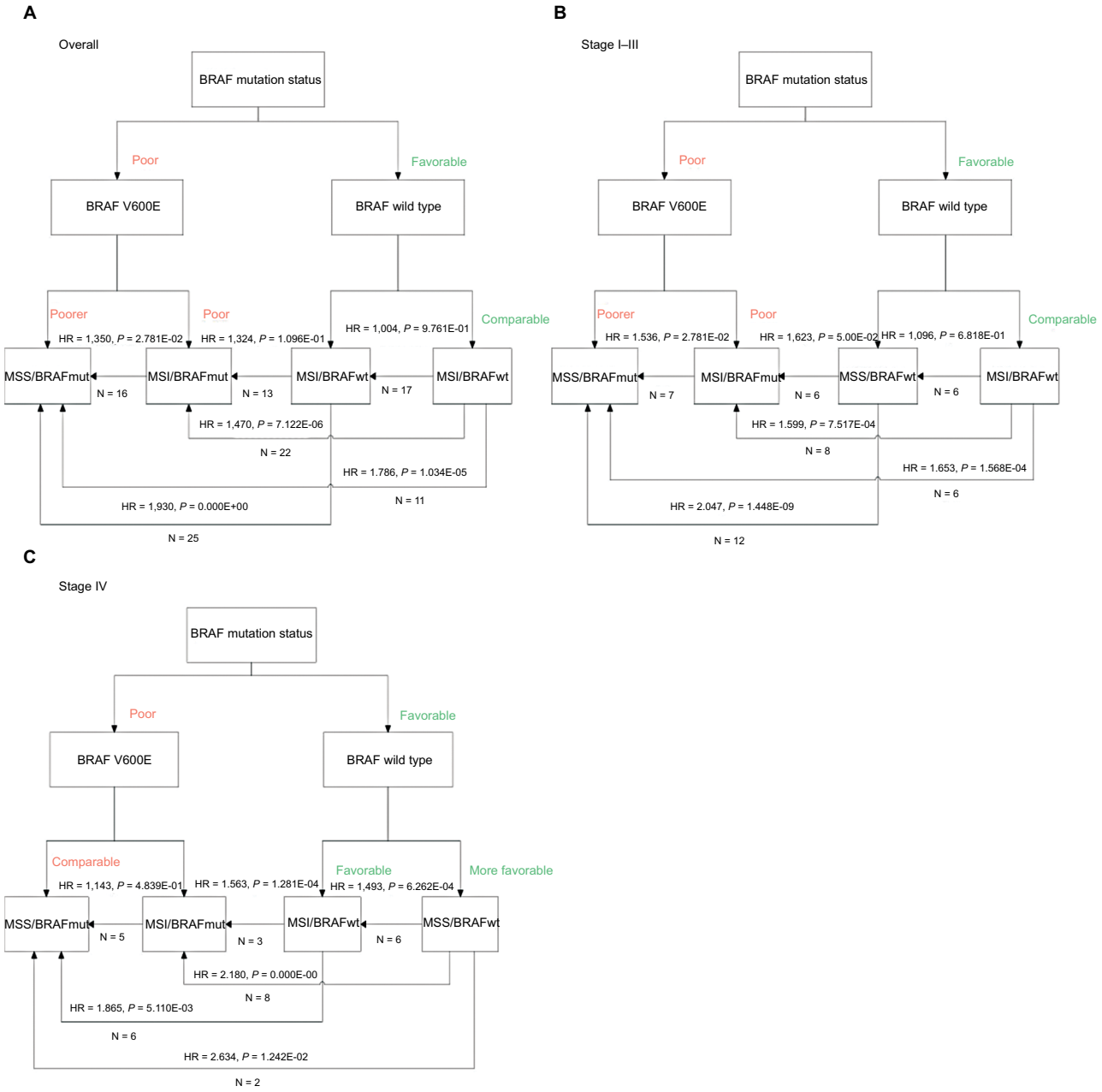

Figure 8 CRC stratification and prognosis comparison according to MSI and *BRAF* mutation status.
Note: (**A**) Overall, (**B**) Stage I–III CRC, and (**C**) Stage IV CRC.
Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; MSI/*BRAF*mut, microsatellite instability and *BRAF* mutation; MSI/*BRAF*wt, microsatellite instability and *BRAF* wild type; MSS/*BRAF*mut, microsatellite stable and *BRAF* mutation; MSS/*BRAF*wt, microsatellite stable and *BRAF* wild type.

and MSI/*BRAF*mut was moderate in CRC. Almost the same results were observed in all subgroup analyses except in the subgroup of stage IV CRC (Figure 8B and C and Table 4). In the subgroup of stage IV, unlike the subgroup of stage I–III, MSI showed poor effects on OS in *BRAF* wild-type patients (Figure 8C and Table 4). This might be not consistent with the current knowledge that MSI status did not affect the prognosis in mCRC. Due to the limited included studies of mCRC, the results for MSI might be not stable. Thus, we screened the published literature reporting the prognostic value of MSI status in mCRC regardless of *BRAF* mutations. After pooling the crude HRs from 20 primary studies, we did not find significant associations of MSI with OS in CRC. However, when stratifying by the frequency of MSI, we found that MSI was a poor factor for OS in the subgroup with low frequency of MSI, while MSI was a favorable factor for OS in the subgroup with high frequency of MSI (data not shown). We speculated that the prognostic value of MSI in mCRC was affected by the detected frequency of MSI.

Although we pooled all the potential studies with big sample size and high methodological quality, some conclusions in the present studies should be treated carefully. First, although we performed subgroup analysis according to the cancer type, stage, and therapy, other tumor characteristics such as vascular invasion, tumor differentiation, and tumor budding in localized CRC and ECOG performance status and number of organs in mCRC were not analyzed due to the rare original data in the primary studies. Second, crude HRs were obtained by different methods, such as univariate and multivariate analyses and Kaplan–Meier plot curve, especially for HRs calculated from Kaplan–Meier plot curve, which might deviate from the "real" HRs derived from original data. All these differences might bring unreliability to the conclusions of the present meta-analysis. Thus, further well-designed studies with larger sample size and detailed clinical characteristics and treatments should be conducted to confirm the results and avoid potential biases.

In summary, we got a comprehensive result from the current meta-analysis that among the four subtypes according to MSI and *BRAF* mutation in CRC, MSS/*BRAF*mut was a poor prognostic factor, while MSS/*BRAF*wt and MSI/*BRAF*wt were comparable and favorable and MSI/*BRAF*mut was moderate in overall and stage I–III CRC. In stage IV CRC, especially those with *BRAF* wt, MSI played an unfavorable role in prognosis. The results suggested that the combination of MSI and *BRAF* could predict prognosis in CRC and facilitated the planning of individualized treatment strategies, thereby improving the prognosis in the CRC patients with more aggressive genetic background.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Influence of tumor location on the associations of different MSI/*BRAF* mutation genotypes with OS in colorectal cancer analyzed by meta-analysis regression

Comparison	Meta-analysis regression		
	Coef	*Z*	*P*-value
MSImut/MSSmut	–2.712615	–1.410	1.585E-01
MSIwt/MSSwt	–4.083789	–1.320	1.868E-01
MSImut/MSIwt	0.0270588	0.030	9.761E-01
MSSmut/MSSwt	0.9104029	0.950	3.421E-01

Note: The difference in tumor location in individual included studies was reflected by the ratio of proximal or right colon cancer to the total colorectal cancers and used as the covariate in meta-analysis regression.

Abbreviations: Coef, coefficient; MSImut, microsatellite instability and *BRAF* mutation; MSIwt, microsatellite instability and *BRAF* wild type; MSSmut, microsatellite stable and *BRAF* mutation; MSSwt, microsatellite stable and *BRAF* wild type; OS, overall survival.