Research Paper

Clinicopathological Significance of *BRAF^V600E* Mutation in Colorectal Cancer: An Updated Meta-Analysis

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Abstract

Background and Aims: Numerous studies have identified *BRAF^V600E* mutation as a predictive factor of anti-EGFR antibodies in colorectal cancer (CRC). However, the association between *BRAF^V600E* mutation and clinicopathological features remains unclear. Therefore, we aimed to conduct an updated and comprehensive meta-analysis to evaluate the above issues.

Methods: We performed a systematic literature search from PubMed, Web of Science, Embase, and PMC database examining the association between *BRAF^V600E* mutation and clinicopathological features in CRC patients. Odds ratio with 95% confidence interval were used to estimate the effects of *BRAF^V600E* mutation on each clinicopathological parameter with fixed-effect model or random-effect model.

Results: Sixty-one studies published, including 32407 CRC patients from multiple countries, were included in the meta-analysis. The overall *BRAF^V600E* mutation rate was 11.38%, and *BRAF^V600E* mutation was positively related to high disease stage (OR=0.81; 95% CI=0.72–0.92; *P*=0.001), high T stage (OR=0.51; 95% CI=0.40–0.65; *P*<0.00001), proximal colon (OR=4.76; 95% CI=3.81–5.96; *P*<0.00001) or right colon (OR=5.15; 95% CI=4.35–6.10, *P*<0.00001) tumor location, poor tumor differentiation (OR=0.27; 95% CI=0.21–0.34; *P*<0.00001), mucinous histology (OR=2.97; 95% CI=2.37–3.72; *P*<0.00001), K-ras-wild type (OR=0.04; 95% CI=0.02–0.07; *P*<0.00001), TP53-wild type (OR=0.50; 95% CI=0.31–0.78; *P*=0.003), deficient DNA mismatch repair (OR=2.93; 95% CI=1.78–4.82; *P*<0.00001), high microsatellite instability (OR=11.15; 95% CI=8.51–14.61; *P*<0.00001) and high CpG island methylator phenotype (OR=0.04; 95% CI=0.03–0.08; *P*<0.00001).

Conclusions: Our updated meta-analysis demonstrated that *BRAF^V600E* mutation was related to poor prognosis of CRC and associated with the distinct molecular phenotypes.

Key words: colorectal cancer, *BRAF* mutation, prognosis, meta-analysis

Introduction

Colorectal cancer (CRC), the third most common cancer, causes the fourth most frequent cancer-related deaths worldwide [1]. It has been widely recognized that constitutive activation of the RAS-RAF-MEK-ERK (MAPK) pathway plays a critical roles in CRC development and progression [2]. Gain-of-function mutations of the key protein *BRAF* in this pathway will constitutively activate this pathway, suggesting the crucial role of *BRAF* mutation in CRC [3]. The *BRAF^V600E* mutation, inducing the substitution of valine for glutamate at position 600 of the b-raf protein, accounts for approximately 90% of BRAF mutations and has more important significance compared to other BRAF mutation types in CRC, and

about 10% of CRC patients harbor the $BRAF^{V600E}$ mutation [3]. Increasing studies have discussed the relationship between $BRAF^{V600E}$ mutation and the effect of anti-EGFR inhibitors in CRC, but the effects of $BRAF^{V600E}$ mutation on the clinicopathological characteristics of CRC remains limited. Therefore, in this article we comprehensively estimate the association between $BRAF^{V600E}$ mutation and clinicopathological characteristics of CRC patients.

Methods

Literature search strategy

We searched PubMed, Web of Science, Embase, and PMC database for relevant publications with the following search terms: ("colorectal cancer" or "rectal cancer" or "colon cancer") and ("$BRAF$ mutation" or $BRAF^{V600E}$). Original articles about human studies written in English published before June 18, 2018 were included.

Inclusion criteria

The studies were gone through in accordance to the predetermined selection. The inclusion criteria were: (1) the association between $BRAF^{V600E}$ mutation and clinicopathological characteristics was studied; (2) sufficient published data for calculating an odds ratio (OR) and 95% confidence interval (CI) were reported; (3) the most appropriate article was selected when multiple articles associated with the same patient population were published. The exclusion criteria were: (1) review articles; (2) articles without enough data to analyzed; and (3) single case reports. The quality of each study was assessed using the Newcastle-Ottawa Scale (NOS).

Data extraction

For every appropriate study, the relevant data were extracted, including name of the first author, publication year, country where the study was conducted, follow-up time, number of patients with $BRAF^{V600E}$ mutation, total number of patients, patient demographics (age and gender); clinicopathological characteristics including tumor site, disease stage, T stage, N stage, metastasis status, tumor size, tumor differentiation and mucinous histology; molecular characteristics including KRAS mutation status, CpG island methylator phenotype (CIMP), TP53 mutation status, DNA mismatch repair (MMR) status and microsatellite instability (MSI) status).

Statistical analysis

Meta-analysis was performed using RevMan (Cochrane Collaboration, Oxford, UK). The strength of the association between the $BRAF^{V600E}$ mutation and clinicopathological parameters was assessed by odds ratio (OR) with the corresponding 95% confidence interval (CI). In the course of data pooling, statistical heterogeneity was defined by using chi-square-based Q-test. The I^2 value indicates the degree of heterogeneity. A P-value<0.10 and/or I^2>50% are considered significant heterogeneity, and then a random-effect model is used. Otherwise, a fixed-effect model is used.

Results

Characteristics of eligible literatures

According to the search terms, a total of 1332 eligible citations were obtained. After screening the abstract, 1228 citations were excluded. Among the remaining 104 citations, 43 citations were excluded because of the reasons shown in Figure 1. Finally, 61 studies published from 2006 to 2018 were included in the meta-analysis (**Figure 1**). A total of 32407 CRC patients from China, Japan, South Korea, India, French, Sweden, Greece, American, Netherlands, Italy, Germany, Australia, and so on were included, and among these patients, 3688 patients were with $BRAF^{V600E}$ mutation (11.38%). The study sample sizes ranged from 69 to 1980 cases. $BRAF^{V600E}$ mutation rate among all studies ranged from 3.14% to 23.14%, which was consistent with the results in the previous study [4]. All specimens were derived from CRC tissues by either biopsy or surgical resection, and were detected for $BRAF$ mutation status mainly by direct sequencing, pyrosequencing, allele-specific PCR and immunohistochemistry (IHC) method.

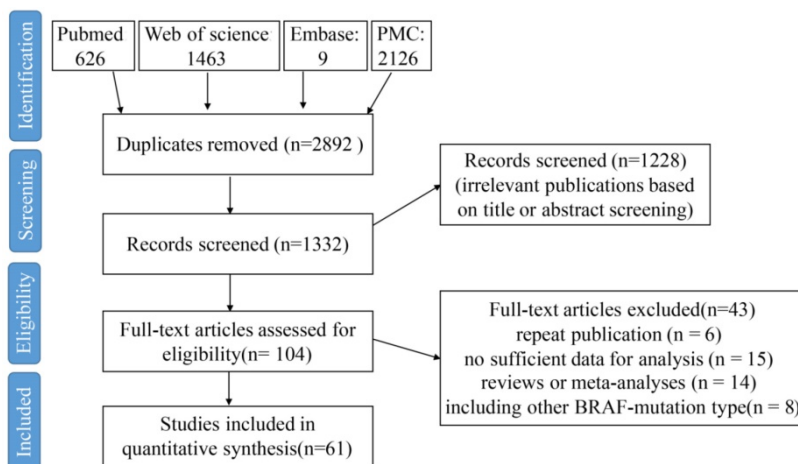The basic characters of the 61 eligible studies were summarized in

Figure 1. A flow chart of the study selection process.

Supplementary Table 1. Thirty-five studies are with sample size below 500 [5-37, 64], whereas twenty-six studies are with sample size over 500 [38-63]. The earliest study was published in July 2005 [51], and the latest study was published in August 2017 [49]. Most of these studies involved patients with stage I-IV CRC [5, 6, 8, 10, 12, 14, 15, 19, 21, 22, 24, 26, 27, 31, 32, 34, 38, 39, 42, 44-47, 51, 52, 54, 55, 57-60, 64], and six studies only involved patients with stage IV CRC [23, 29, 33, 35, 43, 56]. All the studies have a NOS score of ≥5, and 18 studies have a NOS score of ≥6 (**Supplementary Table 1**).

Correlation of *BRAF^V600E* mutation with clinicopathological characteristics of CRC patients

Demographic characteristics (Age and Gender)

A total of 14 studies investigated the association between *BRAF^V600E* mutation and age. Of 2434 patients younger than 60 years, 182 (7.47%) patients were *BRAF^V600E* mutation positive, and 551 (14.26%) of 3864 patients 60 year or older were *BRAF^V600E* mutation positive. The association between *BRAF^V600E* mutation and age did not reach statistical significance (OR=0.66; 95% CI=0.43–1.00; *P*=0.05) (**Figure 2A**, **Table 1**). Fifty-six studies analyzed the association between *BRAF^V600E* mutation and gender. Of 14453 male patients, 1214 (8.40%) CRC patients were with *BRAF^V600E* mutation, and 1822 (15.04%) of 12048 female patients were with *BRAF^V600E* mutation. There was a significantly negative association between *BRAF^V600E* mutation and male gender (OR=0.53; 95% CI=0.49–0.57; *P*<0.00001) (**Figure 2B**, **Table 1**).

Table 1. Overall analysis of the association between *BRAF^V600E* mutation and clinicopathological features in CRC patients.

Clinicopathological features	OR	95% CI	*P* value
Demographic characteristics			
age (<60 years)	0.66	0.43–1.00	0.05
gender (male)	0.53	0.49–0.57	<0.00001
Clinical factures			
disease stage (stage I-II)	0.81	0.72–0.92	0.001
tumor size (<5cm)	0.83	0.45–1.55	0.56
T stage (T1-2)	0.51	0.40–0.65	<0.00001
N stage (N0)	0.85	0.73–1.00	0.05
metastasis (yes)	1.30	0.90–1.88	0.16
tumor location (proximal colon)	4.76	3.81–5.96	<0.00001
tumor location (right colon)	5.15	4.35–6.10	<0.00001
tumor differentiation (well/moderate)	0.27	0.21–0.34	<0.00001
mucinous histology (mucinous)	2.97	2.37–3.72	<0.00001
Molecular features			
K-ras mutation status (mutation)	0.04	0.02–0.07	<0.00001
TP53 mutation status (mutation)	0.50	0.31–0.78	0.003
MMR status (dMMR)	2.93	1.78–4.82	<0.00001
MSI status (MSI high)	11.15	8.51–14.61	<0.00001
CIMP phenotype (CIMP low/negative)	0.04	0.03–0.08	<0.00001

Clinical Factures (Disease stage, T stage, N stage, tumor size, metastasis status, tumor site, tumor differentiation, and mucinous histology)

Twenty-six studies analyzed the association between disease stage and *BRAF^V600E* mutation. Of 5457 patients with stage I or II, 528 (9.68%) patients were *BRAF^V600E* mutation positive, and 733 (11.65%) patients was *BRAF^V600E* mutation positive from 6290 patients diagnosed with stage III or IV disease. Stage I or II were negatively related to *BRAF^V600E* mutation (OR=0.81; 95% CI=0.72–0.92; *P*=0.001), indicating that CRC patients with *BRAF^V600E* mutation trend to have more advanced disease stage (**Figure 3A**, **Table 1**). Furthermore, patients with *BRAF^V600E* mutation were also negatively associated with low T stage (OR=0.51; 95% CI=0.40–0.65; *P*<0.00001) (**Figure 3C**, **Table 1**). However, the overall analysis showed *BRAF^V600E* mutation did not statistically significant correlated with tumor size (OR=0.83; 95% CI=0.45–1.55; *P*=0.56) (**Figure 3B**, **Table 1**), N stage (OR=0.85; 95% CI=0.73–1.00; *P*=0.05) (**Figure 3D**, **Table 1**) and metastasis status (OR=1.30; 95% CI=0.90–1.88; *P*=0.16) (**Figure 3E**, **Table 1**).

In total, forty-five studies investigated the relationship between *BRAF^V600E* mutation and tumor site. And among these studies, twenty-four studies categorized tumors as proximal colon, distal colon or rectal tumor, and another twenty-one studies classified the tumor as right colon, left colon or rectal tumor. The final results showed that *BRAF^V600E* mutation was significantly associated with proximal colon tumor location (OR=4.76; 95% CI=3.81–5.96; *P*<0.00001) or right colon tumor location (OR=5.15; 95% CI=4.35–6.10; *P*<0.00001) (**Figure 4A-B**, **Table 1**).

Twenty studies assessed the association between *BRAF^V600E* mutation and tumor differentiation. 592 (7.81%) patients were with *BRAF^V600E* mutation of 7579 patients with well or moderate differentiation, and 310 (26.34%) patients were with *BRAF^V600E* mutation of 1177 patients with poor differentiation. It was obvious that *BRAF^V600E* mutation was negatively associated with well or moderate differentiation, indicating that CRC patients with *BRAF^V600E* mutation trend to have aggressive tumor phenotype (OR=0.27; 95% CI=0.21–0.34; *P*<0.00001) (**Figure 4C**, **Table 1**). Besides, *BRAF^V600E* mutation was also strikingly related to mucinous histology (OR=2.97; 95% CI=2.37–3.72; *P*<0.00001) (**Figure 4D**, **Table 1**).

Molecular Features (K-ras mutation status, TP53 mutation status, MMR capacity, MSI status, and CIMP)

Twelve studies analyzed the association between *BRAF^V600E* mutation and K-ras mutation status. Notably, K-ras mutation and *BRAF^V600E* mutation were negatively related (OR=0.04; 95% CI=0.02–0.07;

P<0.00001) (**Figure 5A**, **Table 1**). Of 1616 K-ras-mutated patients, only nine (0.56%) ones were *BRAF^V600E* mutated, while 468 (15.38%) patients of 3043 K-ras-wild patients were *BRAF^V600E* mutated.

Interestingly, *BRAF^V600E* mutation was also negatively associated with TP53 mutation (OR=0.50; 95% CI=0.31–0.78; *P*=0.003) (**Figure 5B** and **Table 1**).
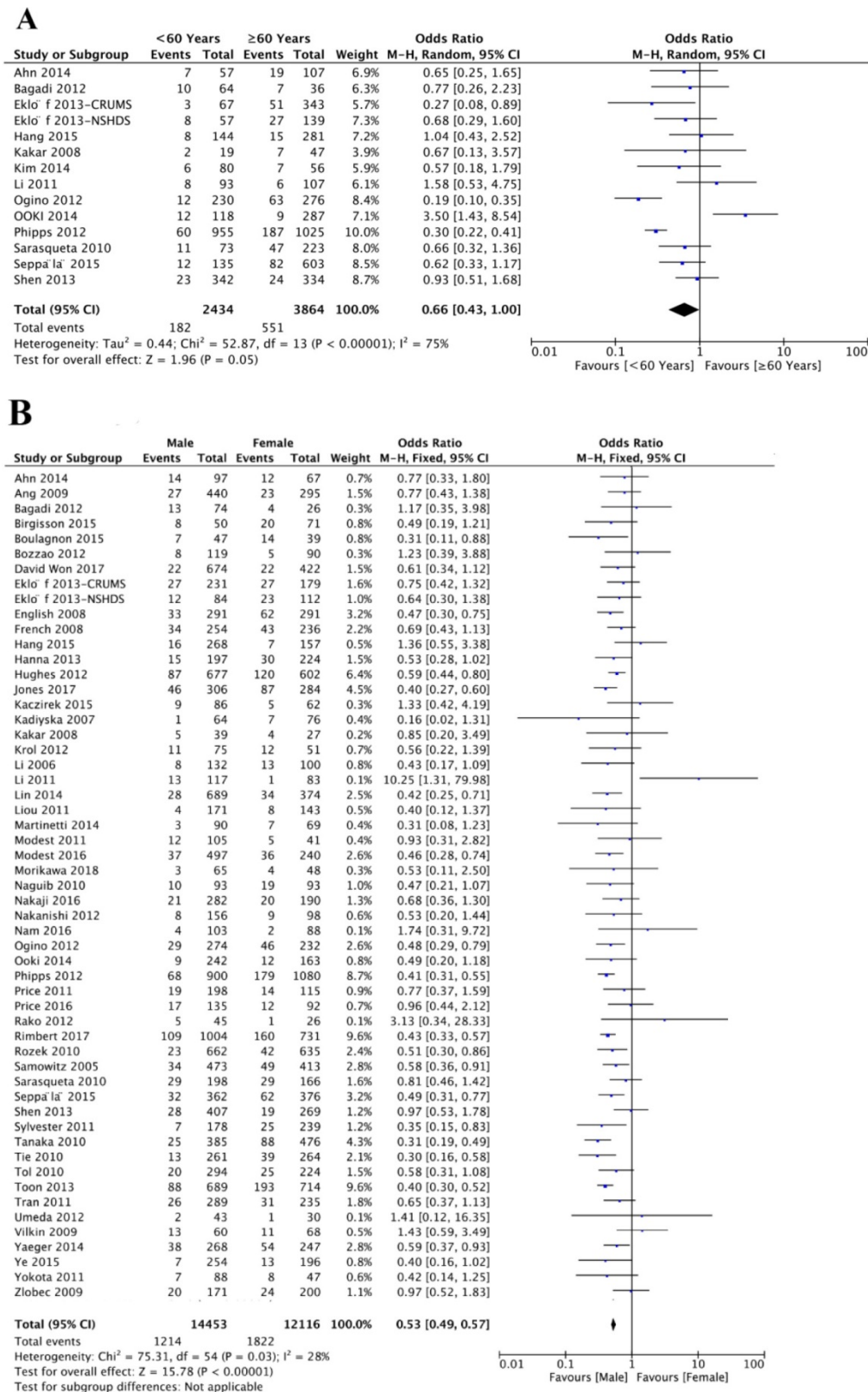

Figure 2. The association of *BRAF^V600E* mutation with demographics, including age (A) and gender (B).

A

Study or Subgroup	Stage I–II Events	Total	Stage III–IV Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Ahn 2014	14	83	12	81	1.8%	1.17 [0.50, 2.70]
Ang 2009	7	271	28	283	4.9%	0.24 [0.10, 0.56]
Bagadi 2012	3	18	14	62	1.0%	0.69 [0.17, 2.71]
Birgisson 2015	12	40	16	81	1.3%	1.74 [0.73, 4.15]
Boulagnon 2015	11	30	10	56	0.8%	2.66 [0.97, 7.31]
Bozzao 2012	1	33	12	167	0.7%	0.40 [0.05, 3.22]
David Won 2017	17	545	27	523	4.9%	0.59 [0.32, 1.10]
Eklof 2013–CRUMS	31	223	23	179	4.0%	1.10 [0.61, 1.95]
Eklof 2013–NSHDS	14	105	21	90	3.6%	0.51 [0.24, 1.06]
French 2008	19	133	58	357	4.9%	0.86 [0.49, 1.51]
Hang 2015	8	215	15	210	2.7%	0.50 [0.21, 1.21]
Hanna 2013	2	89	37	296	3.0%	0.16 [0.04, 0.68]
Kakar 2008	4	41	6	28	1.2%	0.40 [0.10, 1.56]
Krol 2012	12	69	11	56	1.8%	0.86 [0.35, 2.13]
Lin 2014	32	542	30	521	5.2%	1.03 [0.61, 1.72]
Liou 2011	8	162	4	152	0.7%	1.92 [0.57, 6.52]
Martinetti 2014	5	60	5	99	0.6%	1.71 [0.47, 6.17]
Nakaji 2016	16	235	25	237	4.2%	0.62 [0.32, 1.19]
Nakanishi 2012	10	123	7	131	1.1%	1.57 [0.58, 4.26]
Nam 2016	1	21	5	170	0.2%	1.65 [0.18, 14.84]
Seppala 2015	44	406	50	322	9.0%	0.66 [0.43, 1.02]
Shen 2013	22	330	25	343	4.2%	0.91 [0.50, 1.65]
Sylvester 2011	17	201	15	210	2.4%	1.20 [0.58, 2.47]
Tanaka 2010	61	441	47	323	8.5%	0.94 [0.63, 1.42]
Toon 2013	139	755	137	642	22.0%	0.83 [0.64, 1.08]
Yaeger 2014	8	68	83	443	3.5%	0.58 [0.27, 1.26]
Ye 2015	10	218	10	228	1.7%	1.05 [0.43, 2.57]
Total (95% CI)		**5457**		**6290**	**100.0%**	**0.81 [0.72, 0.92]**
Total events	528		733			

Heterogeneity: Chi² = 38.48, df = 26 (P = 0.05); I² = 32%
Test for overall effect: Z = 3.27 (P = 0.001)

Favours [Stage I–II]   Favours [Stage III–IV]

B

Study or Subgroup	< 5cm Events	Total	≥ 5cm Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Birgisson 2015	7	38	20	82	47.2%	0.70 [0.27, 1.83]
OOKI 2014	15	269	6	135	34.4%	1.27 [0.48, 3.35]
RAKO 2012	3	50	3	20	18.4%	0.36 [0.07, 1.97]
Total (95% CI)		**357**		**237**	**100.0%**	**0.83 [0.45, 1.55]**
Total events	25		29			

Heterogeneity: Chi² = 1.78, df = 2 (P = 0.41); I² = 0%
Test for overall effect: Z = 0.58 (P = 0.56)

Favours [<5 cm]   Favours [≥5 cm]

C

Study or Subgroup	T 1–2 Events	Total	T 3–4 Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Ahn 2014	6	38	20	126	3.5%	0.99 [0.37, 2.69]
David Won 2017	7	273	37	796	8.3%	0.54 [0.24, 1.23]
Hang 2015	7	141	16	284	4.6%	0.88 [0.35, 2.18]
Liou 2011	4	71	8	243	1.5%	1.75 [0.51, 6.00]
Nakanishi 2012	1	47	16	207	2.6%	0.26 [0.03, 2.01]
Ogino 2012	8	58	66	442	6.0%	0.91 [0.41, 2.01]
OOKI 2014	1	73	20	332	3.2%	0.22 [0.03, 1.64]
Sarasqueta 2010	4	21	55	276	2.8%	0.95 [0.31, 2.92]
Seppala 2015	9	181	85	557	17.9%	0.29 [0.14, 0.59]
Shen 2013	5	123	42	551	6.7%	0.51 [0.20, 1.33]
Toon 2013	33	305	243	1092	42.8%	0.42 [0.29, 0.63]
Zlobec 2009	7	79	37	0		Not estimable
Total (95% CI)		**1410**		**4906**	**100.0%**	**0.51 [0.40, 0.65]**
Total events	92		645			

Heterogeneity: Chi² = 14.58, df = 10 (P = 0.15); I² = 31%
Test for overall effect: Z = 5.51 (P < 0.00001)

Favours [T 1–2]   Favours [T 3–4]

D

Study or Subgroup	N0 Events	Total	≥ N1 Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Ahn 2014	14	86	12	78	3.3%	1.07 [0.46, 2.48]
David Won 2017	17	570	27	524	8.5%	0.57 [0.30, 1.05]
Li 2006	13	128	4	70	1.5%	1.87 [0.58, 5.95]
Nakaji 2016	19	250	22	222	6.7%	0.75 [0.39, 1.42]
Nakanishi 2012	11	130	6	124	1.8%	1.82 [0.65, 5.08]
Sarasqueta 2010	21	94	38	201	5.9%	1.23 [0.68, 2.25]
Seppala 2015	50	432	88	592	20.5%	0.75 [0.52, 1.09]
Shen 2013	23	342	24	332	7.1%	0.93 [0.51, 1.67]
Toon 2013	137	764	138	633	38.7%	0.78 [0.60, 1.02]
Umeda 2012	3	49	0	24	0.2%	3.69 [0.18, 74.32]
Zlobec 2009	22	185	21	174	6.0%	0.98 [0.52, 1.86]
Total (95% CI)		**3030**		**2974**	**100.0%**	**0.85 [0.73, 1.00]**
Total events	330		380			

Heterogeneity: Chi² = 9.45, df = 10 (P = 0.49); I² = 0%
Test for overall effect: Z = 1.93 (P = 0.05)

Favours [N0]   Favours [≥N1]

E

Study or Subgroup	Metastasis–Y Events	Total	Metastasis–N Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Ahn 2014	1	7	25	157	3.9%	0.88 [0.10, 7.63]
Bozzao 2012	3	96	4	24	13.3%	0.16 [0.03, 0.78]
Seppala 2015	18	98	76	630	36.0%	1.64 [0.93, 2.88]
Shen 2013	6	55	41	619	12.8%	1.73 [0.70, 4.27]
Toon 2013	13	55	262	1342	33.9%	1.28 [0.68, 2.41]
Total (95% CI)		**311**		**2772**	**100.0%**	**1.30 [0.90, 1.88]**
Total events	41		408			

Heterogeneity: Chi² = 7.92, df = 4 (P = 0.09); I² = 49%
Test for overall effect: Z = 1.39 (P = 0.16)

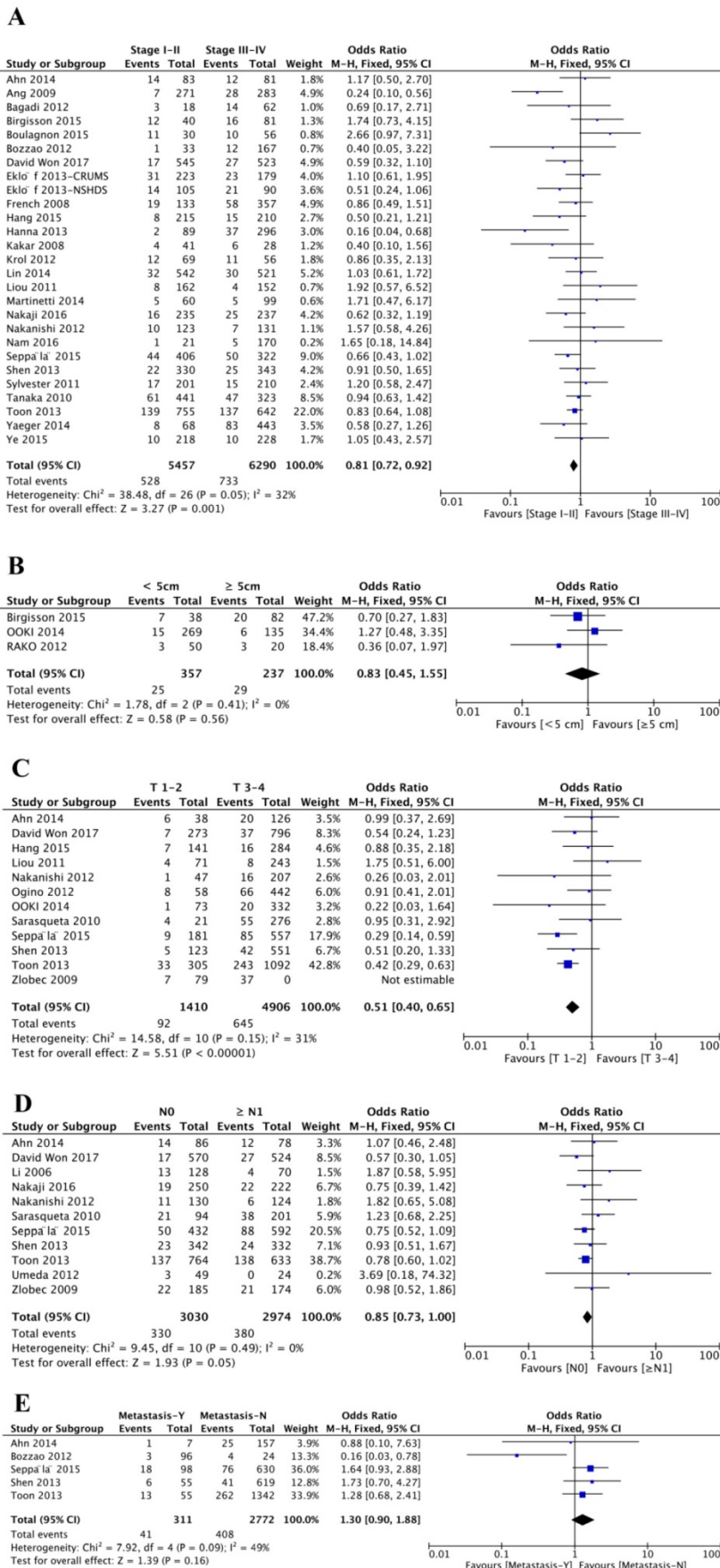Favours [Metastasis–Y]   Favours [Metastasis–N]

Figure 3. Meta-analysis of association between *BRAF^V600E* mutation and clinical features, including disease stage (A), tumor size (B), T stage (C), N stage (D) and metastasis status (E).
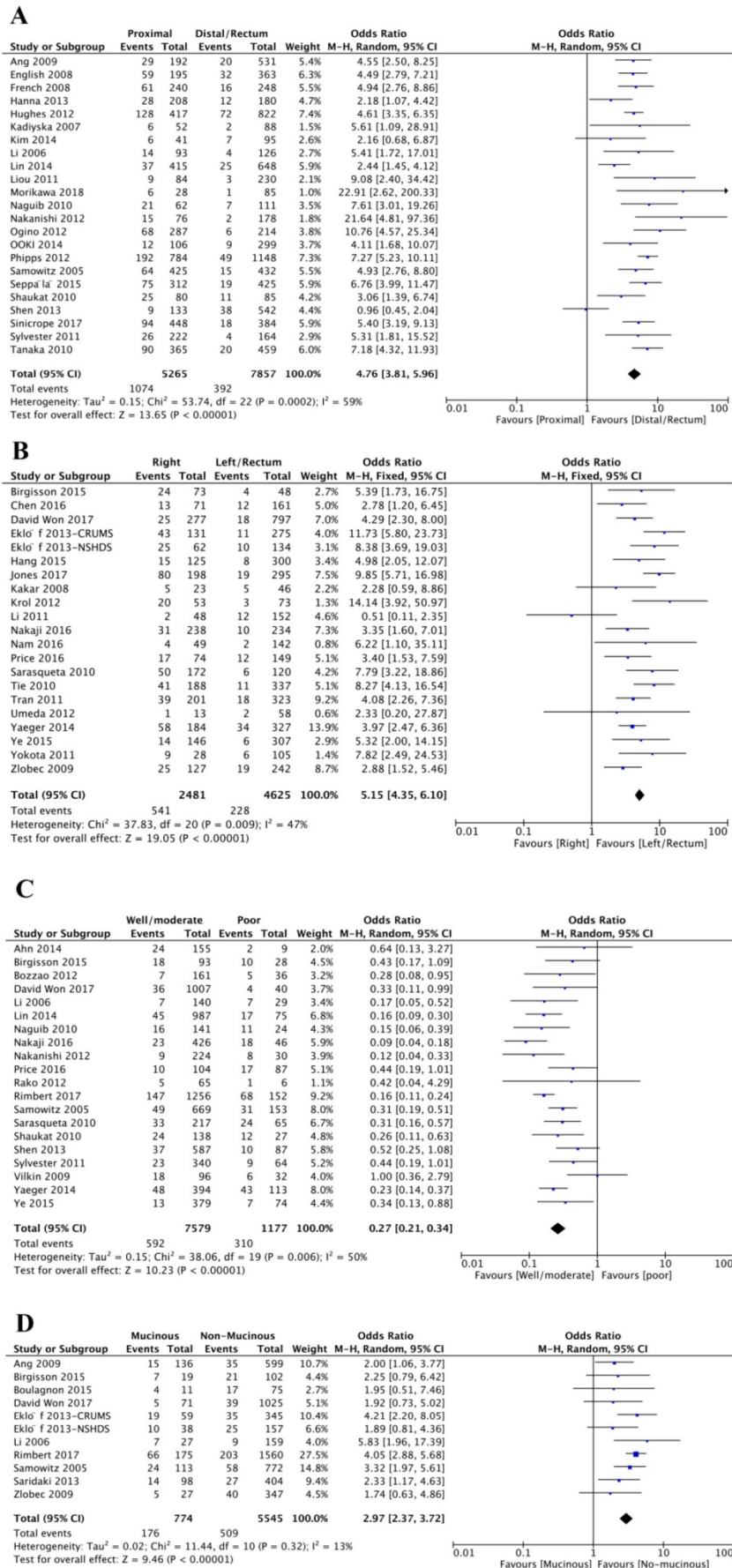
A

Study or Subgroup	Proximal Events	Total	Distal/Rectum Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ang 2009	29	192	20	531	5.4%	4.55 [2.50, 8.25]
English 2008	59	195	32	363	6.3%	4.49 [2.79, 7.21]
French 2008	61	240	16	248	5.5%	4.94 [2.76, 8.86]
Hanna 2013	28	208	12	180	4.7%	2.18 [1.07, 4.42]
Hughes 2012	128	417	72	822	7.4%	4.61 [3.35, 6.35]
Kadiyska 2007	6	52	2	88	1.5%	5.61 [1.09, 28.91]
Kim 2014	6	41	7	95	2.6%	2.16 [0.68, 6.87]
Li 2006	14	93	4	126	2.7%	5.41 [1.72, 17.01]
Lin 2014	37	415	25	648	5.9%	2.44 [1.45, 4.12]
Liou 2011	9	84	3	230	2.1%	9.08 [2.40, 34.42]
Morikawa 2018	6	28	1	85	1.0%	22.91 [2.62, 200.33]
Naguib 2010	21	62	7	111	3.5%	7.61 [3.01, 19.26]
Nakanishi 2012	15	76	2	178	1.8%	21.64 [4.81, 97.36]
Ogino 2012	68	287	6	214	3.8%	10.76 [4.57, 25.34]
OOKI 2014	12	106	9	299	3.7%	4.11 [1.68, 10.07]
Phipps 2012	192	784	49	1148	7.3%	7.27 [5.23, 10.11]
Samowitz 2005	64	425	15	432	5.5%	4.93 [2.76, 8.80]
Seppälä 2015	75	312	19	425	5.9%	6.76 [3.99, 11.47]
Shaukat 2010	25	80	11	85	4.2%	3.06 [1.39, 6.74]
Shen 2013	9	133	38	542	4.4%	0.96 [0.45, 2.04]
Sinicrope 2017	94	448	18	384	5.9%	5.40 [3.19, 9.13]
Sylvester 2011	26	222	4	164	2.9%	5.31 [1.81, 15.52]
Tanaka 2010	90	365	20	459	6.0%	7.18 [4.32, 11.93]
Total (95% CI)		**5265**		**7857**	**100.0%**	**4.76 [3.81, 5.96]**
Total events	1074		392			

Heterogeneity: Tau² = 0.15; Chi² = 53.74, df = 22 (P = 0.0002); I² = 59%
Test for overall effect: Z = 13.65 (P < 0.00001)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Favours [Proximal] Favours [Distal/Rectum]

B

Study or Subgroup	Right Events	Total	Left/Rectum Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Birgisson 2015	24	73	4	48	2.7%	5.39 [1.73, 16.75]
Chen 2016	13	71	12	161	5.0%	2.78 [1.20, 6.45]
David Won 2017	25	277	18	797	7.0%	4.29 [2.30, 8.00]
Eklöf 2013-CRUMS	43	131	11	275	4.0%	11.73 [5.80, 23.73]
Eklöf 2013-NSHDS	25	62	10	134	3.1%	8.38 [3.69, 19.03]
Hang 2015	15	125	8	300	3.4%	4.98 [2.05, 12.07]
Jones 2017	80	198	19	295	7.5%	9.85 [5.71, 16.98]
Kakar 2008	5	23	5	46	2.2%	2.28 [0.59, 8.86]
Krol 2012	20	53	3	73	1.3%	14.14 [3.92, 50.97]
Li 2011	2	48	12	152	4.6%	0.51 [0.11, 2.35]
Nakaji 2016	31	238	10	234	7.3%	3.35 [1.60, 7.01]
Nam 2016	4	49	2	142	0.8%	6.22 [1.10, 35.11]
Price 2016	17	74	12	149	5.1%	3.40 [1.53, 7.59]
Sarasqueta 2010	50	172	6	120	4.2%	7.79 [3.22, 18.86]
Tie 2010	41	188	11	337	5.1%	8.27 [4.13, 16.54]
Tran 2011	39	201	18	323	9.2%	4.08 [2.26, 7.36]
Umeda 2012	1	13	2	58	0.6%	2.33 [0.20, 27.87]
Yaeger 2014	58	184	34	327	13.9%	3.97 [2.47, 6.36]
Ye 2015	14	146	6	307	2.9%	5.32 [2.00, 14.15]
Yokota 2011	9	28	6	105	1.4%	7.82 [2.49, 24.53]
Zlobec 2009	25	127	19	242	8.7%	2.88 [1.52, 5.46]
Total (95% CI)		**2481**		**4625**	**100.0%**	**5.15 [4.35, 6.10]**
Total events	541		228			

Heterogeneity: Chi² = 37.83, df = 20 (P = 0.009); I² = 47%
Test for overall effect: Z = 19.05 (P < 0.00001)

Odds Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [Right] Favours [Left/Rectum]

C

Study or Subgroup	Well/moderate Events	Total	Poor Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ahn 2014	24	155	2	9	2.0%	0.64 [0.13, 3.27]
Birgisson 2015	18	93	10	28	4.5%	0.43 [0.17, 1.09]
Bozzao 2012	7	161	5	36	3.2%	0.28 [0.08, 0.95]
David Won 2017	36	1007	4	40	3.7%	0.33 [0.11, 0.99]
Li 2006	7	140	7	29	3.4%	0.17 [0.05, 0.52]
Lin 2014	45	987	17	75	6.8%	0.16 [0.09, 0.30]
Naguib 2010	16	141	11	24	4.3%	0.15 [0.06, 0.39]
Nakaji 2016	23	426	18	46	5.9%	0.09 [0.04, 0.18]
Nakanishi 2012	9	224	8	30	3.9%	0.12 [0.04, 0.33]
Price 2016	10	104	17	87	5.1%	0.44 [0.19, 1.01]
Rako 2012	5	65	1	6	1.1%	0.42 [0.04, 4.29]
Rimbert 2017	147	1256	68	152	9.2%	0.16 [0.11, 0.24]
Samowitz 2005	49	669	31	153	8.0%	0.31 [0.19, 0.51]
Sarasqueta 2010	33	217	24	65	6.7%	0.31 [0.16, 0.57]
Shaukat 2010	24	138	12	27	4.8%	0.26 [0.11, 0.63]
Shen 2013	37	587	10	87	5.8%	0.52 [0.25, 1.08]
Sylvester 2011	23	340	9	64	5.2%	0.44 [0.19, 1.01]
Vilkin 2009	18	96	6	32	4.0%	1.00 [0.36, 2.79]
Yaeger 2014	48	394	43	113	8.0%	0.23 [0.14, 0.37]
Ye 2015	13	379	7	74	4.4%	0.34 [0.13, 0.88]
Total (95% CI)		**7579**		**1177**	**100.0%**	**0.27 [0.21, 0.34]**
Total events	592		310			

Heterogeneity: Tau² = 0.15; Chi² = 38.06, df = 19 (P = 0.006); I² = 50%
Test for overall effect: Z = 10.23 (P < 0.00001)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Favours [Well/moderate] Favours [poor]

D

Study or Subgroup	Mucinous Events	Total	Non-Mucinous Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ang 2009	15	136	35	599	10.7%	2.00 [1.06, 3.77]
Birgisson 2015	7	19	21	102	4.4%	2.25 [0.79, 6.42]
Boulagnon 2015	4	11	17	75	2.7%	1.95 [0.51, 7.46]
David Won 2017	5	71	39	1025	5.1%	1.92 [0.73, 5.02]
Eklöf 2013-CRUMS	19	59	35	345	10.4%	4.21 [2.20, 8.05]
Eklöf 2013-NSHDS	10	38	25	157	6.6%	1.89 [0.81, 4.36]
Li 2006	7	27	9	159	4.0%	5.83 [1.96, 17.39]
Rimbert 2017	66	175	203	1560	27.5%	4.05 [2.88, 5.68]
Samowitz 2005	24	113	58	772	14.8%	3.32 [1.97, 5.61]
Saridaki 2013	14	98	27	404	9.4%	2.33 [1.17, 4.63]
Zlobec 2009	5	27	40	347	4.5%	1.74 [0.63, 4.86]
Total (95% CI)		**774**		**5545**	**100.0%**	**2.97 [2.37, 3.72]**
Total events	176		509			

Heterogeneity: Tau² = 0.02; Chi² = 11.44, df = 10 (P = 0.32); I² = 13%
Test for overall effect: Z = 9.46 (P < 0.00001)

Odds Ratio M-H, Random, 95% CI
0.01 0.1 1 10 100
Favours [Mucinous] Favours [No-mucinous]

Figure 4. The association of *BRAF^V600E* mutation with tumor characteristics, including tumor site (A and B), tumor differentiation (C) and mucinous histology (B).

Only two studies investigated the relationship between $BRAF^{V600E}$ mutation and mismatch repair (MMR) capacity. The results showed that 24 (18.75%) patients were $BRAF^{V600E}$ mutation positive from 128 patients with deficient MMR (dMMR) capacity, and 90 (7.51%) patients were $BRAF^{V600E}$ mutation positive from 1198 patients with proficient MMR (pMMR) capacity. $BRAF^{V600E}$ mutation was significantly related to dMMR (OR=2.93; 95% CI=1.78–4.82; P<0.00001) (**Figure 5C**, **Table 1**). Twenty-seven studies investigated the $BRAF^{V600E}$ mutation and microsatellite instability (MSI). Of 1872 patients with high microsatellite instability (MSI-High), 864 (46.15%) patients were $BRAF^{V600E}$ mutated, and of 11668 patients with low microsatellite instability (MSI-low) or microsatellite stable (MSS), 810 (6.94%) patients were $BRAF^{V600E}$ mutated. There was a significant association between $BRAF^{V600E}$ mutation and MSI-high (OR=11.15; 95% CI=8.51–14.61; P<0.00001) (**Figure 5D**, **Table 1**). Ten studies were analyzed for CpG island methylator phenotype (CIMP) and $BRAF^{V600E}$ mutation. Of 4112 patients with low or negative CIMP, 179 (4.35%) patients were with $BRAF^{V600E}$ mutation, and of 834 patients with high CIMP, 359 (43.05%) patients were with $BRAF^{V600E}$ mutation. According to the result, $BRAF^{V600E}$ mutation was negatively associated with high CMIP (OR=0.04; 95% CI=0.03–0.08; P<0.00001) (**Figure 5E** and **Table 1**).

Additional analyses

A funnel plot of effects calculated from individual studies examining the association between $BRAF^{V600E}$ mutation and disease stage was conducted to estimate the presence of publication bias. Because there are small studies with negative results in the literature, no strong indication of publication bias exist among the series of studies included in this meta-analysis.

Discussion

$BRAF^{V600E}$ mutation was an important molecular alternation in CRC patients. In our study, the highest $BRAF^{V600E}$ mutation rate reached to 23.14% and the average $BRAF^{V600E}$ mutation rate was 11.35% among all the involved studies, similar to other reports [4]. Clinicopathological parameters have crucial roles in predicting the prognosis of cancer patients. It is necessary to clarify the relationship between $BRAF^{V600E}$ mutation and clinicopathological parameters in CRC patients [65]. Our meta-analysis indicated that $BRAF^{V600E}$ mutation was significantly associated with female, advanced disease stage, high T stage, proximal or right tumor location, poor tissue differentiation and mucinous phenotype. As high disease stage, high T stage, poor tissue differentiation

and mucinous histology were the multiple risk factors of the prognosis in CRC patients, it may be deemed that $BRAF^{V600E}$ mutation was a poor predictive indicator [20, 51, 65]. Our study also showed that $BRAF^{V600E}$ mutation had a crucial association with disease stage, T stage, N stage and tissue differentiation, which demonstrated the important role of $BRAF^{V600E}$ mutation in occurrence and development of CRC.

Intriguingly, tumors located in the proximal colon were 4.76-fold more likely to have $BRAF^{V600E}$ mutation than tumor located in the distal or rectal colon. Moreover, the $BRAF^{V600E}$ mutation was 5.15-fold more frequent in tumors located in the right colon than tumors located in the left or rectal colon. The association between $BRAF^{V600E}$ mutation and tumor sites was very strong and the reason of the association has not been clarified clearly. Previous studies have indicated that colorectal tumors located in different sites have totally different outcomes and specific biomolecular characteristics [66]. Our study also demonstrated that the difference in regard to $BRAF^{V600E}$ mutation in different tumor location. Moreover, the different $BRAF^{V600E}$ mutation rates among different tumor sites might be useful for formulating treatment therapy for CRC located in different tumor sites [67, 68].

Nowadays, in addition to clinicopathologic stage and histological morphology, molecular markers play increasing roles in making therapeutical decision for cancer patients. Melanoma with $BRAF^{V600E}$ mutation is more sensitive to immunotherapy [69]. Deficient MMR status has been demonstrated to predict the response of PD-1 blockade in metastatic CRC [70], and MSI-High also has been recognized as a predictive factor of programmed death ligand-1 inhibitor pembrolizumab in metastatic/refractory CRC [71]. Our results revealed that $BRAF^{V600E}$ mutation was significantly related to K-ras-wild type, TP53-wild type, deficient MMR, high MSI and high CIMP. This association between $BRAF^{V600E}$ mutation and other molecular features cloud be important to understand the molecular distinction between CRC patients with or without $BRAF^{V600E}$ mutation. However, the association between $BRAF^{V600E}$ mutation and the therapeutical response in CRC needs more prospective investigations.

In this article, although we conducted comprehensive and detailed meta-analysis, there are still some limitations. Firstly, with regard to some clinicopathologic characteristics, the number of the involved patients was limited. Small studies are prone to introduce unstable results and related to publication bias. Secondly, most of these studies were retrospective or observational studies (data not

shown), which might induce heterogeneity. Thirdly, the mutation detection assays were different among these studies. The most two commonly used methods are direct sequencing and pyrosequencing. Different *BRAF^V600E* mutation assays also affected the accuracy and precision of the pooled estimates.
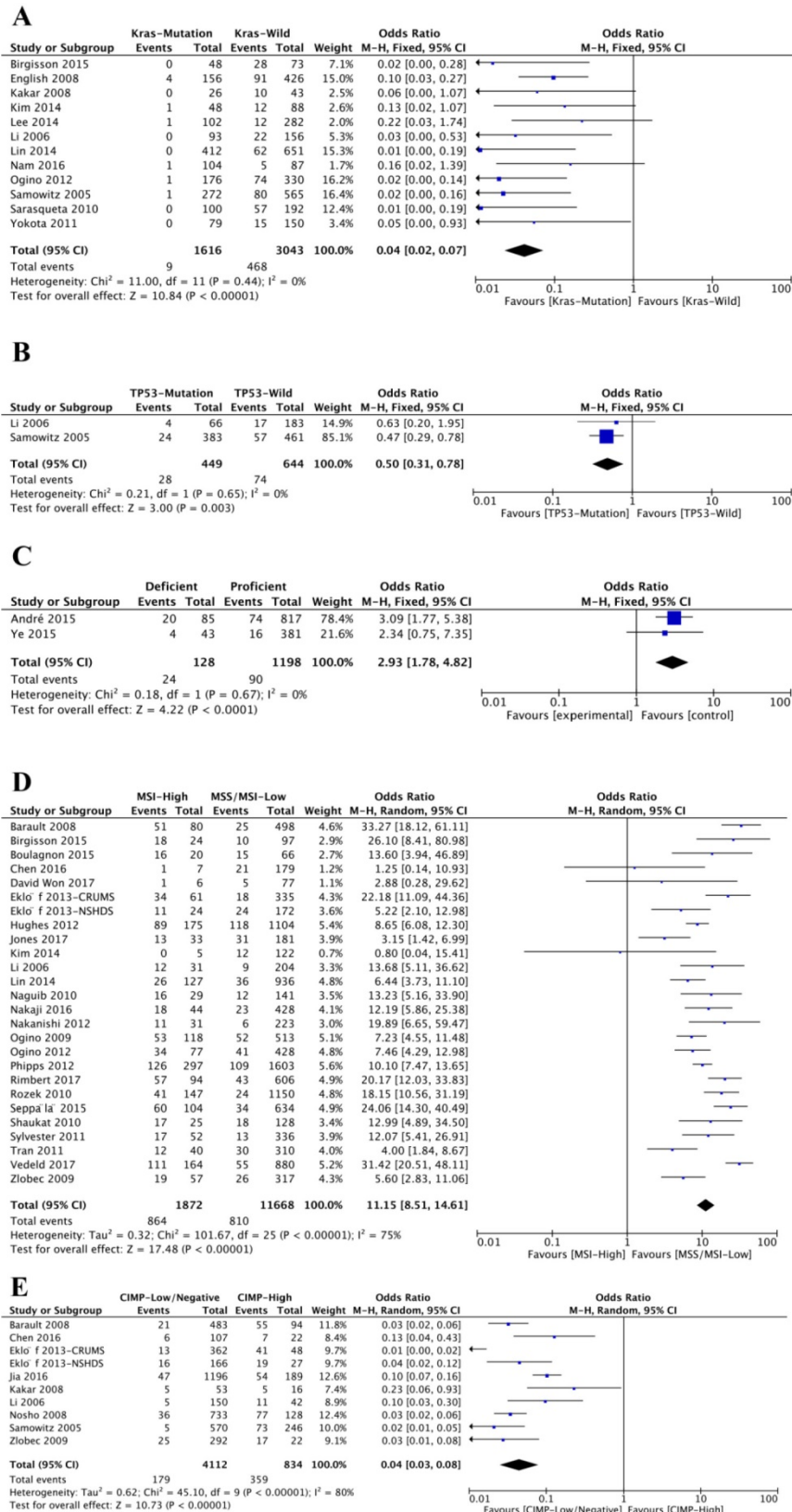

Figure 5. The association of *BRAF^V600E* mutation with molecular features, including Kras mutation status (A), TP53 mutation status (B), MMR capacity (C), MSI status (D) and CIMP phenotype (E).

In conclusion, our updated and comprehensive meta-analysis based on a large number of clinical data demonstrated that $BRAF^{V600E}$ mutation is a biological predictor for poor prognosis in CRC patients, which helps to elucidate the mechanisms of progression and metastasis of CRC and to develop novel therapeutic strategies for CRC.

Supplementary Material

Supplementary tables.
http://www.jcancer.org/v10p2332s1.pdf

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Competing Interests

The authors have declared that no competing interest exists.

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