KRAS/NRAS/BRAF mutational profile and association with clinicopathological characteristics in patients with metastatic colorectal cancer

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Abstract. Colorectal cancer (CRC) is increasingly prevalent in Jordan and poses a significant public health challenge. The presence of Kirsten rat sarcoma viral oncogene homolog (KRAS), neuroblastoma RAS viral oncogene homolog (NRAS) and v-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF) mutations is key in CRC diagnostics, as these mutations are associated with resistance to monoclonal antibodies targeting the epidermal growth factor receptor. The present study aimed to identify these mutations in patients with CRC and assess their associations with clinicopathological characteristics. A retrospective analysis was conducted using data from 262 patients with metastatic CRC (mCRC) at the Jordanian Military Cancer Center-Royal Medical Services (Amman, Jordan). Variables such as age, sex, tumor differentiation and the mutational status of KRAS, NRAS and BRAF, along with tumor location, were analyzed statistically to explore associations between mutations and tumor characteristics. Among the included patients, 48.5% had KRAS mutations, 3.8% had NRAS mutations and 0.8% had BRAF mutations. The majority of KRAS mutations were in exon 2 at codons 12 and 13, with the highest mutational rate at 45.8%. In the univariate model, NRAS mutations were significantly associated with moderately differentiated tumors and the multivariate hierarchical regression analysis established that KRAS mutations were significantly associated with histological subtypes [mucinous adenocarcinoma, tubular adenocarcinoma, signet adenocarcinoma and adenocarcinoma (not specified)]. These results highlighted the molecular profiles and clinicopathological characteristics of patients with mCRC, which demonstrated the associations between mutational status and the varying clinicopathological aspects based on the type of RAS mutation. Thus, these specific traits (patient's age, sex, CRC site, histological subtypes and tumor grade) may be taken into account when evaluating the predictive significance of RAS and BRAF status in CRC and tailored treatment strategies.

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Abbreviations: CRC, colorectal cancer; mCRC, metastatic CRC; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B

Key words: KRAS, NRAS, BRAF, CRC

Introduction

According to the World Health Organization (WHO), colorectal cancer (CRC) is a malignant neoplasm that develops when normal epithelial cells lining the large intestine undergo malignant transformation and give rise to adenocarcinoma (1). CRC is the third leading cause of cancer-related mortality (1) and accounted for 576,858 deaths and 1.15 million new cases in 2020, based on the report of GLOBOCAN 2020 database for 185 countries and 36 cancer types (2). The number of new cases of CRC is projected to rise to 1.92 million by 2040 (3).

According to previous statistics, CRC has a mortality rate of 10.5% in men and 9% in women, making it the second leading cause of cancer-related death in both sexes in Jordan (4), and the 5-year survival rate is 22.6% (5). Thus, to improve survival in patients with advanced-stage disease, a further understanding of the molecular mechanisms underlying CRC pathogenesis and the application of targeted therapies in clinical practice is required.

The pathophysiology of CRC is complex and multifaceted, leading to the widespread acceptance that the causes are

heterogeneous (6). Changes at the genomic, transcriptomic, epigenomic and metabolomic levels all serve key roles in the etiology and progression of CRC, and our understanding of this disease remains limited (7). Different molecular subtypes have distinct clinical and pathological characteristics, and varying responses to cytotoxic and targeted therapies. In metastatic CRC (mCRC), it is currently advised that thorough RAS and v-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF) mutation testing should be performed before treatment (8). RAS proteins are GTPases and there are three subtypes: Kirsten rat sarcoma viral oncogene homolog (KRAS) and Harvey rat sarcoma viral oncogene homolog were discovered in 1982 (9), whereas neuroblastoma RAS viral oncogene homolog (NRAS) was not discovered until 1983 (10). NRAS mutations are infrequent, occurring in ~4% of CRC cases (11). NRAS mutant tumors are predominantly situated in the proximal colon and are more prevalent among older patients (11). KRAS mutations are linked to right-sided primary CRC, whereas NRAS mutations are more prevalent in left-sided CRC, particularly in women (12). The prognosis for patients with RAS mutations is worse compared with that of patients with a wild-type RAS genotype. KRAS and NRAS mutations exert differing effects on survival in mCRC; the prognosis for the aggressive NRAS mutant molecular subgroup is unfavorable (13).

KRAS is a component located downstream of the epidermal growth factor receptor (EGFR) signaling pathway. KRAS operates as an intracellular signal transducer by linking signals from cell surface receptors to intracellular targets that control important functions for tumor progression, including proliferation, differentiation and apoptosis (Fig. 1) (14).

The development of CRC is associated with the presence of activating mutations in oncogenes; mutant RAS proteins elicit downstream oncogenic signaling, resulting in tumor cells acquiring aggressive characteristics such as increased cell mitosis (15). Activating mutations in the KRAS gene are associated with ~45% of colorectal malignancies, and a large proportion of mutations are found in codons 12 (30%) and 13 (8%) of exon 2 (16). The presence of these somatic mutations leads to continuous activation of the EGFR pathway, resulting in the acquisition of resistance to anti-EGFR therapies, including panitumumab or cetuximab (17). Previous research has demonstrated that in mCRC, KRAS mutations in exon 2 were not associated with any therapeutic benefit when using anti-EGFR monoclonal antibodies (18). Consequently, the use of cetuximab and panitumumab as anti-EGFR monoclonal antibodies is restricted to patients with wild-type RAS mCRC and therefore, this mandates that RAS mutation screening should be conducted to determine whether anti-EGFR monoclonal antibodies should be recommended. It has been demonstrated that KRAS mutations predict a worse prognosis for patients with CRC (19). The FOCUS trial reported that the presence of activating mutations in the KRAS and BRAF oncogenes is associated with shorter overall survival. Patients with a KRAS mutation had significantly worse overall survival compared with patients with KRAS wild-type tumors [hazard ratio (HR), 1.24; 95% confidence interval (CI), 1.06-1.46; P=0.008]. However, these KRAS mutations did not significantly affect progression-free survival (HR, 1.14; 95% CI, 0.98-1.33; P=0.09) (20). The Raf protein has also been extensively studied and shown to be involved in signal transduction, cellular proliferation and carcinogenesis (Fig. 1); ~8% of CRC tumors exhibit activating mutations in BRAF, which predominantly impacts codon 600 (21). The presence of BRAF mutations in mCRC is widely recognized to have a notable negative prognostic effect (22). These mutations require an alternative therapeutic strategy since conventional anti-EGFR treatments are frequently ineffective (23). However, numerous targeted methods are now being investigated for various types of cancer with BRAF mutations, owing to the potential significance of BRAF mutations as a driving signal in tumor growth (24). Previous studies have shown that KRAS mutations, as well as BRAF mutations, have distinguished pathological and clinical characteristics (25,26). For example, CRC with KRAS exon 2 mutations are more common in elderly individuals, particularly males, and are commonly seen in the proximal colon when compared with the wild-type exon (27). Mutations in the BRAF gene are strongly associated with females, the elderly, mucinous differentiation, low histological grade and tumors located in the proximal region of the colon (28). Thus, the molecular variations emphasize the significance of tailored treatment strategies in mCRC.

Although routine testing for RAS and BRAF mutations is conducted on patients with mCRC in Jordan before recommending anti-EGFR therapy, to the best of our knowledge, no previous studies have reported on the possible association between clinicopathological features and complete RAS and BRAF status, which could help researchers identify other factors that influence therapeutic response to anti-EGFR antibodies. Therefore, the aim of the present study was to identify mutations in the RAS and BRAF genes in patients with sporadic CRC and investigate their associations with clinicopathological characteristics.

Materials and methods

Study design. The present study retrospectively analyzed patients' electronic medical records from a single center, the Jordanian Military Cancer Center-Royal Medical Services (Amman, Jordan). The present study included 262 patients diagnosed with mCRC between January 2020 and January 2022. Data regarding age, sex, RAS and BRAF status, primary tumor site, histological subtypes and grade were acquired. The inclusion criteria for patients were as follows: Age ≥18 years old and diagnosis of adenocarcinoma of the colon or rectum shown histologically. The exclusion criteria were as follows: Patients with tumor types other than CRC, patients in which RAS and BRAF mutations could not be determined (wild-type or mutant), and cases where data was missing from the patients' electronic medical records. The primary tumor sites were categorized as follows: Tumors on the left side of the body, which are more likely to develop in the distal one-third transverse colon, splenic flexure, descending colon, sigmoid colon and rectum, and tumors on the right side of the body, which are more likely to develop in the cecum, ascending colon, hepatic flexure and proximal two-thirds of the transverse colon (29). Transverse colon cancer is characterized by tumors situated between the hepatic and splenic flexures, and is extremely uncommon, comprising 10% of all colon malignancies (30).

Histological subtypes were classified as classical adenocarcinoma, mucinous adenocarcinoma, tubuvillous adenoma,

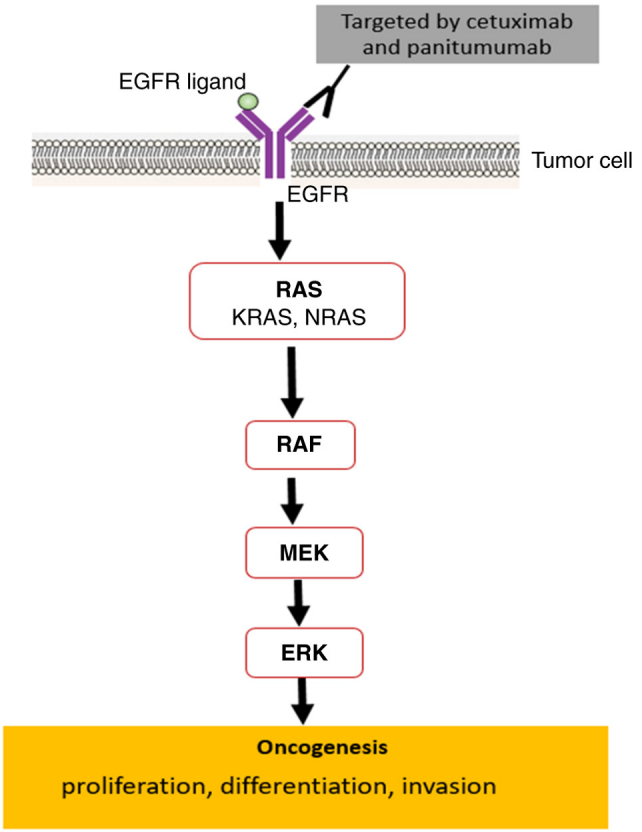

Figure 1. EGFR and the RAS-RAF signaling pathway in tumor cells. The EGFR ligand activates the EGFR receptor, resulting in the activation of the RAS-RAF-MEK-ERK pathway, promoting tumor proliferation, differentiation and invasion. Cetuximab and panitumumab are anti-EGFR monoclonal antibodies that prevent ligand-induced activation of the downstream signaling. However, cetuximab and panitumumab often lack efficacy in patients with CRC with RAS or BRAF mutations. EGFR, epidermal growth factor receptor; RAS, rat sarcoma virus; RAF, rapidly accelerated fibrosarcoma; ERK, extracellular signal-regulated kinase; MEK, MAP-ERK kinase.

Table I. Characteristics of patients with metastatic CRC (n=262).

Characteristics	Patients, n (%)
Sex	
Female	109 (41.6)
Male	153 (58.4)
Age[a], years	
≤50	92 (35.1)
>50	170 (64.9)
CRC site	
Right	30 (11.5)
Left	224 (85.5)
Transverse	6 (2.3)
Unknown	2 (0.8)
Histological subtype	
Mucinous adenocarcinoma	11 (4.2)
Tubular adenocarcinoma	3 (1.1)
Signet adenocarcinoma	2 (0.8)
Adenocarcinoma (not specified)	246 (93.9)
Tumor grade	
Poorly differentiated	23 (8.8)
Moderately differentiated	193 (73.7)
Well-differentiated	11 (4.2)
Not determined	35 (13.4)
Mutation status	
Wild-type	123 (46.9)
Mutated	139 (53.1)

[a]Mean ± SD, 55.91±12.806 years; range, 19-85 years. CRC, colorectal cancer.

invasive adenocarcinoma, medullary adenocarcinoma, adenosquamous carcinoma or undifferentiated carcinoma. Additionally, the histological grade was categorized as well-differentiated, moderately differentiated or poorly differentiated.

Tumor molecular analysis. The KRAS, NRAS and BRAF mutation status of patients with CRC were extracted retrospectively from the electronic medical records of patients at the Jordanian Military Cancer Center-Royal Medical Services. However, all patients underwent tumor tissue biopsies to ascertain RAS and BRAF mutation status using quantitative polymerase chain reaction and reverse hybridization in an accredited diagnostic laboratory at Jordan University Hospital (Amman, Jordan) and only the final results were sent to the Royal Medical Services.

Statistical analysis. Statistical analysis was performed using SPSS (version 23.0; IBM Corp.). Categorical variables are presented as the frequency (n) and percentage. A χ^2 test or Fisher's exact test was used to determine the associations between variables. The φ factor was used to examine the strength of association (as a measure of effect size) as follows: 0, no association; 0.1, small association; 0.3, medium association; 0.5, strong association; and 1, complete association. Univariate analysis was used to assess these associations, utilizing statistical tests such as the χ^2 or Fisher's exact test when appropriate. In addition, a multivariate hierarchical regression analysis was performed to examine the impact of covariates on gene mutations. The regression analysis consisted of three models, the first model included mutant or wild-type as predictors, the second model included mutant or wild-type status, age and sex as predictors, and the third model included histological subtypes and tumor grade as predictors alongside mutant or wild-type status, age and sex. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sample characteristics. A total of 262 patient records of mCRC were analyzed (Table I). The cohort's mean age was 55.91±12.81 years, with an age range of 19-85 years, and patients >50 years old accounted for 64.9% of the cases (n=170). Of the 262 patients, 153 (58.4%) were male and 109 (41.6%) were female. Regarding the tumor site, 224 (85.5%) samples were left-site tumors, which was more frequent

Table II. Mutational status and detailed mutation classes found in patients (n=262).

Mutational status	Mutations, n (%)	Exon	Codon
A, KRAS	130[a] (49.6)		
KRAS G12A	20 (7.6)	2	12,13
KRAS G12D	32 (12.2)	2	12,13
KRAS G12V	40 (15.3)	2	12,13
KRAS G12S	5 (1.9)	2	12,13
KRAS G12C	4 (1.5)	2	12,13
KRAS G13A	8 (3.1)	2	12,13
KRAS G13D	11 (4.2)	2	12,13
KRAS Q61x	4 (1.5)	3	61
KRAS K117x	2 (0.8)	4	117
KRAS A146x	4 (1.5)	4	146
B, NRAS	10 (3.8)		
NRAS G12x-G13x	5 (1.9)	2	12,13
NRAS Q61K	2 (0.8)	3	61
NRAS Q61L	2 (0.8)	3	61
NRAS A146x	1 (0.4)	4	146
C, BRAF	2 (0.76)		
BRAF V600E	2 (0.76)	15	600
Total number of KRAS/NRAS/BRAF mutations	142 (54.2)	-	-

[a]Total number of patients with KRAS mutations is 130, as 4 patients have 2 concurrent KRAS mutations and 1 had data missing; -, not applicable; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

compared with right-site tumors (n=30; 11.5%) or transverse site tumors (n=6; 2.3%). More than one-half of tumor cases (n=139; 53%) had a mutation in the RAS gene and BRAF, whereas 46.9% (n=123) of the cases had wild-type RAS gene and BRAF. In terms of histological subtypes, a large proportion of cases were adenocarcinoma (not specified; n=246; 93.9%), followed by mucinous adenocarcinoma (n=11; 4.2%), then tubular adenocarcinoma (n=3; 1.1%) and signet adenocarcinoma (n=2; 0.8%).

The characteristics of patients with mCRC are shown in Table I. According to the WHO criteria (31), tumor histology was graded as poorly differentiated, moderately differentiated and well-differentiated at 8.8 (n=23), 73.7 (n=193) and 4.2% (n=11), respectively; the histological grade was not determined in 13.4% (n=35) of patients.

Mutational status and detailed mutation. Out of 139 patients with mCRC carrying a mutation, 127 patients (48.5%) had KRAS mutations and 120 mutations (45.8%) were detected in exon 2, with 10 mutations occurring outside of exon 2 (exon 3 or exon 4). The most prevalent KRAS exon 2 mutations were G12V (n=40; 15.3%), followed by G12D (n=32; 12.2%), while KRAS K117x was the least common KRAS mutation (n=2; 0.8%). Notably, 4 patients with KRAS mutation exhibited 2 mutations each: 2 with G12C and G12V mutations, 1 with G12V and G12A mutations, and 1 with G13D and KRAS G12S mutations. Moreover, there is missing data regarding detailed mutation for one patient thus, the total KRAS mutation count was 130 (49.6%).

Regarding NRAS, 10 patients possessed a mutation of this gene. Half of the cases demonstrated a substitution of glycine to any amino acid mutation (G12x-G13x) accounting for 1.9% (n=5) of mutated cases. Moreover, Q61K and Q61L mutations were present at the same frequency and percentage (both n=2; 0.8%), and the A164x mutation was the least common among all RAS mutations accounting for 0.4% (n=1). For BRAF mutations, 2 cases of V600E were observed (0.8%). Table II illustrates all the mutations and the detailed classes. Moreover, none of the patients carrying a mutation with the KRAS or NRAS genotype had a simultaneous BRAF mutation (Fig. 2).

Association between RAS mutations, wild-type status and the patients' clinicopathological features. The associations between the mutational status of the RAS gene and various clinicopathological characteristics were assessed using univariate analyses, such as the χ^2 or Fisher's exact test, as appropriate. RAS mutations were more prevalent in males (n=78; 56.9%), in patients >50 years old (n=94; 68.6%) and in tumors located in the left side of the colon (n=115; 83.9%). Additionally, RAS mutations were more frequently observed, albeit not significantly, with the adenocarcinoma (not specified) histological subtype (n=129; 94.2%) and moderately differentiated tumor grade (n=102; 74.5%). No other clinicopathological features had notable associations with RAS mutations (Table III).

Table III. Univariate analysis of the association between RAS mutation, wild-type status and clinicopathological features.

Characteristics	RAS status			
	Mutated, n (%)	Wild-type, n (%)	P-value	Association strength (φ)
Sex				
Female	59 (43.1)	50 (40)	0.615	-0.031
Male	78 (56.9)	75 (60)		
Age, years				
≤50	43 (31.4)	49 (39.2)	0.186	0.082
>50	94 (68.6)	76 (60.8)		
CRC site				
Right	17 (12.4)	13 (10.4)	0.687[a]	0.091
Left	115 (83.9)	109 (87.2)		
Transverse	3 (2.2)	3 (2.4)		
Unknown	2 (1.5)	-		
Histological subtype				
Mucinous adenocarcinoma	5 (3.6)	6 (4.8)	0.953[a]	0.042
Tubular adenocarcinoma	2 (1.5)	1 (0.8)		
Signet adenocarcinoma	1 (0.7)	1 (0.8)		
Adenocarcinoma (not specified)	129 (94.2)	117 (93.6)		
Tumor grade				
Poorly differentiated	17 (12.4)	6 (4.8)	0.056	0.170
Moderately differentiated	102 (74.5)	91 (72.8)		
Well-differentiated	4 (2.9)	7 (5.6)		
Not determined	14 (10.2)	21 (16.8)		

[a]Fisher's exact test; -, not applicable; CRC, colorectal cancer.

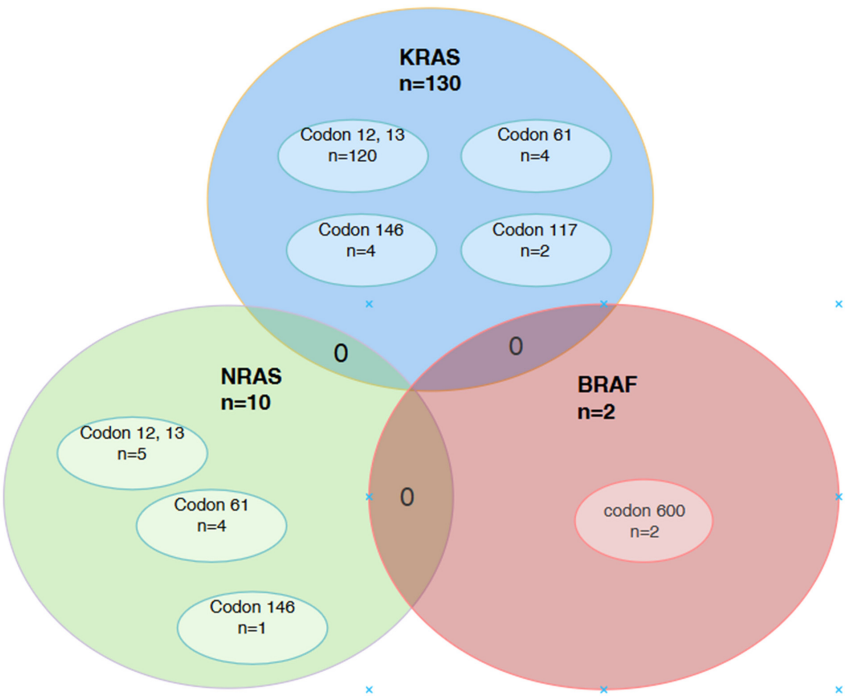

Figure 2. Distribution of single and concomitant mutations in the KRAS, NRAS and BRAF genes. In the present study, 120 KRAS mutations were distributed across codons 12 and 13, codons 61 and 146, and codon 117. There were 10 NRAS mutations distributed across codons 12 and 13, codon 61 and codon 146. There were 2 BRAF mutations in codon 600. Although overlapping mutations between KRAS, NRAS and BRAF were not detected, 4 patients did possess two KRAS mutations each, which made the total number of KRAS mutations detected 130. KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table IV. Univariate analysis of the association between KRAS, NRAS, BRAF mutations, wild-type status and clinicopathological features.

A, KRAS status

Characteristics	Mutated, n (%)	Wild-type, n (%)	P-value	Association strength (φ)
Sex				
Female	54 (42.5)	55 (40.7)	0.868[a]	-0.018
Male	73 (57.5)	80 (59.3)		
Age, years				
≤50	41 (32.3)	51 (37.8)	0.352	0.058
>50	86 (67.7)	84 (62.2)		
CRC site				
Right	17 (13.4)	13 (9.6)	0.354[b]	0.117
Left	106 (83.5)	118 (87.4)		
Transverse	2 (1.6)	4 (3.0)		
Unknown	2 (1.6)	-		
Histological subtype				
Mucinous adenocarcinoma	5 (3.9)	6 (4.4)	0.682[b]	0.094
Tubular adenocarcinoma	2 (1.6)	1 (0.7)		
Signet adenocarcinoma	-	2 (1.5)		
Adenocarcinoma (not specified)	120 (94.5)	126 (93.3)		
Tumor grade				
Poorly differentiated	13 (10.2)	10 (7.4)	0.499	0.095
Moderately differentiated	96 (75.6)	97 (71.9)		
Well-differentiated	4 (3.1)	7 (5.2)		
Not determined	14 (11.0)	21 (15.6)		

B, NRAS status

Characteristics	Mutated, n (%)	Wild-type, n (%)	P-value	Association strength (φ)
Sex				
Female	5 (50)	104 (41.3)	0.746[b]	-0.034
Male	5 (50.0)	148 (58.7)		
Age, years				
≤50	2 (20.0)	90 (35.7)	0.307	0.063
>50	8 (80.0)	162 (64.3)		
CRC site				
Right	-	30 (11.9)	0.215[b]	0.123
Left	9 (90.0)	215 (85.3)		
Transverse	1 (10.0)	5 (2.0)		
Unknown	-	2 (0.8)		
Histological subtype				
Mucinous adenocarcinoma	-	11 (4.4)	0.116[b]	0.216
Tubular adenocarcinoma	-	3 (1.2)		
Signet adenocarcinoma	1 (10.0)	1 (0.4)		
Adenocarcinoma (not specified)	9 (90.0)	237 (94.0)		
Tumor grade				
Poorly differentiated	4 (40.0)	19 (7.5)	0.02[b,c]	0.228
Moderately differentiated	6 (60.0)	187 (74.2)		
Well-differentiated	-	11 (4.4)		
Not determined	-	35 (13.9)		

Table IV. Continued.

C, BRAF status

Characteristics	Mutated, n (%)	Wild-type, n (%)	P-value	Association strength (φ)
Sex				
Female	1 (50.0)	108 (41.5)	>0.999[b]	-0.015
Male	1 (50.0)	152 (58.5)		
Age, years				
≤50	-	92 (35.4)	0.543[b]	0.065
>50	2 (100.0)	168 (64.6)		
CRC site				
Right	-	30 (11.5)	>0.999[b]	0.036
Left	2 (0.9)	222 (85.4)		
Transverse	-	6 (2.3)		
Unknown	-	2 (0.8)		
Histological subtype				
Mucinous adenocarcinoma	1 (50)	10 (3.8)	0.119[b]	0.20
Tubular adenocarcinoma	-	3 (1.2)		
Signet adenocarcinoma	-	2 (0.8)		
Adenocarcinoma (not specified)	1 (50)	245 (94.2)		
Tumor grade				
Poorly differentiated	-	23 (8.8)	0.458[b]	0.097
Moderately differentiated	1 (50)	192 (73.8)		
Well-differentiated	-	11 (4.2)		
Not determined	1 (50)	34 (13.1)		

[a]Continuity correction; [b]Fisher's exact test; [c]P<0.05; -, not applicable; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma RAS viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CRC, colorectal cancer.

Association between KRAS, NRAS, BRAF mutations and wild-type status with the patients' clinicopathological features. Univariate analysis was performed to investigate the association between different subtypes of RAS mutations (KRAS and NRAS) and BRAF mutations with various clinicopathological characteristics (Table IV). Both KRAS (n=86; 67.7%) and NRAS n=8; 80%) mutations were more frequent in patients >50 years old and in tumors located in the left side of the colon (KRAS n=106; 83.5%), (NRAS n=9; 90%) frequently, as well as both mutations (KRAS n=120; 94.5%, and NRAS n=9; 90%) were more prevalent in patients with adenocarcinoma (not specified); however, these findings were not statistically significant. By contrast, NRAS mutations were significantly associated with moderately differentiated tumors (n=6; 60%, P<0.05). Similar to KRAS mutations, BRAF mutations were more common in patients >50 years old (n=2; 100%) and in tumors located in the left side of the colon (n=2; 0.9%), although the association was not significant (Table IV).

A multivariate hierarchical regression analysis was performed to examine the impact of covariates on gene mutations. For the KRAS gene, in the first model the predictor was the mutant or wild-type. The result showed that the mutant or wild-type is a significant predictor (B=0.914; β=0.912; P<0.001). The model accounted for 83.2% of the variance in KRAS (R^2=0.832; adjusted R^2=0.832), with a model significance (F=1,291.813; P<0.001) (Table V).

The second model included mutant or wild-type status, age and sex as predictors. Mutant or wild-type status remained a significant predictor (B=0.917; β=0.916; P<0.001), with only a slight increase in its effect size compared to model 1. Age (B=-0.031; β=-0.030; P>0.05) and sex (B=-0.017; β=-0.017; P>0.05) were not significant predictors. Model 2 accounted for 83.2% of the variance in KRAS, as seen in model 1 (R^2=0.832; adjusted R^2=0.832), but there was no significant increase in R^2 compared to model 1 (F-change=0.795; P>0.05).

The third model included histological subtypes and tumor grade as predictors alongside mutant or wild-type status, age and sex. Mutant or wild-type status remained a significant predictor (B=0.915; β=0.914; P<0.001). Age (B=-0.037; β=-0.037; P>0.05) and sex (B=-0.013; β=-0.013; P>0.05) were not statistically significant predictors. Histological subtype was a significant predictor (B=-0.074; β=-0.057; P<0.001); however, the tumor grade was not significant (B=0.014; β=0.022; P>0.05). The model accounted for 83.6% of the variance in KRAS (R^2=0.836; adjusted R^2=0.836), with a small but significant increase in explanatory power compared with model 2 (F-change=3.251; P<0.05). One-way ANOVA as the out-put from multivariate hierarchical regression confirmed

Table V. A multivariate hierarchical regression analysis of the KRAS gene.

A, Model 1

Variable	B	SE.B	β
Mutant or wild-type	0.914	0.025	0.912[a]
Age			
Sex			
Histological subtype			
Tumor grade			
R^2	0.832		
Adjusted R^2		0.832	
F change in R^2			1,291.813[a]

B, Model 2

Variable	B	SE.B	β
Mutant or wild-type	0.917	0.026	0.916[a]
Age	-0.031	0.027	-0.030
Sex	-0.017	0.026	-0.017
Histological subtypes			
Tumor grade			
R^2	0.832		
Adjusted R^2		0.832	
F change in R^2			0.795

C, Model 3

Variable	B	SE.B	β
Mutant or wild-type	0.915	0.025	0.914[a]
Age	-0.037	-0.037	-0.037
Sex	-0.013	0.026	-0.013
Histological subtypes	-0.074	0.033	-0.057[a]
Tumor grade	0.014	0.017	0.022
R^2	0.836		
Adjusted R^2		0.836	
F change in R^2			3.251[b]

[a]P<0.001 and [b]P<0.05. KRAS, Kirsten rat sarcoma viral oncogene homolog; SE. B, Standard Error of B; β, standardized coefficient; B, unstandardized coefficient.

the statistical significance of all three models: Model 1, $F_{(1, 261)}$=1,291.813, P<0.001; model 2, $F_{(3, 259)}$=430.455, P<0.001; and model 3, $F_{(6, 256)}$=222.487, P<0.001 (numbers in brackets represent degree of freedom and sample number) (Table VI).

For the NRAS gene, in the first model, the mutant or wild-type status was a significant predictor (B=0.72; β=0.187; P<0.05). The model accounted for 3.5% of the variance in NRAS (R^2=0.035; adjusted R^2=0.031; F=9.462; P<0.05), which indicated a significant contribution of mutant or wild-type (Table VII).

The second model incorporated age and sex as predictors alongside the mutant or wild-type variable. Mutant or wild-type status was a significant predictor of NRAS (B=0.07; β=0.182; P<0.05), with only a slight decrease in its effect size compared with model 1. Age (B=0.02; β=0.05; P>0.05) and sex (B=0.013; β=0.034; P>0.05) were not significant predictors of NRAS. Model 2 accounted for 3.8% of the variance in NRAS (R^2=0.038; adjusted R^2=0.027), with a non-significant overall F-statistic (F=0.435; P>0.05).

The third model included histological subtypes and tumor grade as additional predictors. Mutant or wild-type status remained a significant predictor of NRAS (B=0.072; β=0.186; P<0.05). Age (B=0.023; β=0.057; P>0.05) and sex (B=0.010; β=0.026; P>0.05) were not significant predictors. Similarly, histological subtypes (B=0.054; β=0.112; P>0.05) and tumor grade (B=0.053; β=0.107; P>0.05) were not significantly associated with NRAS. Model 3 accounted for 6.3% of the variance in NRAS (R^2=0.063; adjusted R^2=0.031), with a significant F-statistic (F=2.253; P<0.05). However, the increase in variance accounted for model 3 compared with the model 2 was a modest increase (ΔR^2=0.025).

ANOVA confirmed that the three models were statistically significant: Model 1, $F_{(1, 261)}$=9.462; P<0.05; model 2, $F_{(3, 259)}$=3.431, P<0.05; model 3, $F_{(6, 256)}$=2.867; P<0.05. These results demonstrate that each model represented a statistically significant improvement over the null model (Table VIII).

Discussion

CRC is a highly prevalent malignancy with highly variable occurrence and fatality rates globally (2). In 2023, an estimated 153,020 individuals received a diagnosis of CRC, with 52,550 fatalities attributed to the disease, encompassing 19,550 cases and 3,750 deaths among those <50 years of age (32).

The impact of the RAS mutation pattern on anticancer therapy orientation has been extensively documented (33). Patients with CRC tumors mutations in exon 2 of the KRAS gene (specifically in codons 12 and 13) do not experience a clinical response from anti-EGFR-based therapies (34). Notably, some patients with CRC with wild-type KRAS exon 2 do not exhibit a favorable response to anti-EGFR therapy, indicating that the presence of other RAS mutations (namely, KRAS exons 3 and 4 or NRAS exons 2, 3 and 4) and BRAF or PIK3CA mutations can serve as a negative indicator for the effectiveness of anti-EGFR treatment (23). A case study on brain metastasis in a patient with KRAS wild-type CRC treated with cetuximab alongside chemotherapy (capecitabine and oxaliplatin) demonstrated a substantial reduction in brain metastases with the cetuximab and chemotherapy regimen before any radiation intervention (35). However, to the best of our knowledge, no real-world data has been published for patients with CRC receiving anti-EGFR treatment and cetuximab.

In the present retrospective study, the RAS and BRAF gene mutation rates in patients with mCRC were investigated. In addition, the associations between these genetic alterations and clinicopathological characteristics were examined. Results from the present study were consistent with previous studies where >50% of cases had CRC mutations (36). The prevalence of KRAS mutations in patients with mutations

Table VI. ANOVA results for predictive models of KRAS mutation variance based on genetic, demographic and histological factors.

Model	Sum of squares	df	Mean square	F	P-value
1	54.475	1	54.475	1,291.813	<0.001
2	54.542	3	18.181	430.455	<0.001
3	54.943	6	9.157	222.487	<0.001

df, degrees of freedom; KRAS, Kirsten rat sarcoma viral oncogene homolog.

Table VII. A multivariate hierarchical regression analysis of the NRAS gene.

A, Model 1

Variable	B	SE.B	β
Mutant or wild-type	0.72	0.023	0.187[a]
Age			
Sex			
Histological subtypes			
Tumor grade			
R^2	0.035		
Adjusted R^2		0.031	
F change in R^2			9.462[a]

B, Model 2

Variable	B	SE.B	β
Mutant or wild-type	0.07	0.024	0.182[a]
Age	0.02	0.025	0.05
Sex	0.013	0.024	0.034
Histological subtypes			
Tumor grade			
R^2	0.038		
Adjusted R^2		0.027	
F change in R^2			0.435

C, Model 3

Variable	B	SE.B	β
Mutant or wild-type	0.072	0.023	0.186[a]
Age	0.023	0.025	0.057
Sex	0.010	0.024	0.026
Histological subtypes	0.054	0.030	0.112
Tumor grade	0.053	0.030	0.107
R^2	0.063		
Adjusted R^2		0.031	
F change in R^2			2.253[a]

[a]P<0.05. NRAS, neuroblastoma RAS viral oncogene homolog; SE. B, Standard Error of B; β, standardized coefficient; B, unstandardized coefficient.

was 48.5%, close to a results of a previous study conducted in Jordan (44%) (37). For comparison, Western Europe has a KRAS mutation rate of 44.7% (38), Indonesia has a rate of 41% (39) and China has a rate of 36.1% (40).

In contrast to KRAS, NRAS changes are infrequent and there is currently limited evidence on the incidence of these mutations (41). In the present study, the NRAS mutation rate was 3.82%. Zhang *et al* (42) reported a rate of 3.69% in a Chinese study, and studies on Italian and Indian patients indicated frequencies of 6 and 6.3%, respectively (43). By contrast, Greek and Romanian patients had a higher incidence rate of 9.57% (44). According to the present study, BRAF mutations were detected in 0.76% of Jordanian patients, which is lower compared with the percentage for Western countries (9.2%) and Asian countries (4.9%) (45). The KRAS, NRAS and BRAF frequency variations between studies are likely due to several factors, including ethnicity, geographical dispersion and utilization of distinct methods/assays to evaluate the presence of these mutations.

The present study examined KRAS mutations in patients with mCRC, namely those located in exon 2 and those outside of exon 2. The prevalence of KRAS mutations was ~45.8%, consistent with findings from a prior study in Jordan (37). Another previous study reported mutational frequencies in this exon ranging from 15-46% based on country (46). According to the present study, among the KRAS mutations, G12V has the highest prevalence, followed by G12D, G12A, G13D, G13A, G12S and G12C. The findings indicate minor variations compared with research conducted on Western populations, implying that ethnicity may influence the patterns of KRAS mutations (47). In the present study, among the NRAS mutations (3.9%), 1.9% of the samples had a mutation in exon 2 (codon 12 or 13), which is comparable to the Chinese population (32). The prevalence of BRAF mutations ranges from 1.1 to 25% globally (41). Notably, the frequency of the V600E mutation in the present study (0.76%) was detected in exon 15, which is lower than the frequency seen in previous Asian studies (48,49). The clinical significance of the KRAS mutations, except those of codons 12 and 13, remains unclear. Loupakis *et al* (50) reported that a patient with mCRC and KRAS A146 mutation was resistant to cetuximab. In another study, it was found that NRAS mutation carriers showed a significantly lower response rate compared with patients with wild-type KRAS when treated with cetuximab (23). Nevertheless, there is an ongoing debate over the association between BRAF mutations and the effectiveness of anti-EGFR treatment (51). For example, a previous study reported that

Table VIII. ANOVA results for predictive models of NRAS mutation variance using genetic, demographic and histological predictors.

Model	Sum of squares	Degree of freedom (df)	Mean square	F	P-value
1	0.338	1	0.338	9.462	<0.05
2	0.369	3	0.123	3.431	<0.05
3	0.608	6	0.101	2.867	<0.05

df, degrees of freedom; NRAS, neuroblastoma RAS viral oncogene homolog.

RAS status was fully determined and a thorough analysis of the association between clinicopathological and molecular features indicated no statistically significant association (52). Secondly, the association between RAS subtypes and BRAF with patients' clinicopathologicalfeatures was analyzed and the findings were contradictory. Previous findings have indicated that KRAS mutations are associated with different clinicopathological criteria, although this association is not universally observed in all studies (36,41,53).

In the present study, a multivariate analysis demonstrated a significant association between KRAS mutations and histological subtypes (41). Additionally, there are numerous discrepancies and variations regarding KRAS mutations and tumor locations or histological subtypes (45). For example, correlations were found between KRAS mutation and tumors in the right colon (54) or rectal tumors (28), whereas other studies reported an association between KRAS mutations and a patient's age (55), tumor site and differentiation (55). The association between NRAS mutations and clinicopathological features was investigated in the present study. The results showed an association with tumor grades. NRAS mutations were more frequent in moderately differentiated tumors compared with poorly differentiated tumors. However, a previous study established a significant association between NRAS mutations and a patient's age (56). Another study found an association between NRAS mutations and the initial phases of cancer and the lack of lymph node metastases (55). While other study found that NRAS mutations are more commonly found in left-sided malignancies and women (57), whereas Chang *et al* (58) identified a link between NRAS mutations and men. In addition, Shen *et al* (59) reported that these mutations occurred more frequently in distant metastatic tumors and their occurrence varied depending on the different phases of the tumor. Another study did not identify any associations (60). Notably, previous research conducted on Western populations reported an association between BRAF-mutant CRC and the female sex (61). However, the present study did not find a statistically significant association between BRAF mutations and sex. In contrast to the study by Zhang *et al* (41), which demonstrated a significant correlation between BRAF mutations and right-sided colon cancer, the present study did not demonstrate any significant association between BRAF mutations and tumor site or any other clinicopathological features. However, it is important to note that the findings of the present study are constrained by the limited number of patients with a BRAF mutation, which amounted to only 2 patients.

The present study has certain limitations, including the limited sample size and single-centered nature of the present study, which made it difficult to draw broader conclusions. The association between KRAS, NRAS and BRAF mutations and the clinicopathological features of patients with CRC may be further understood in future investigations with larger patient groups.

The retrospective nature of the present study was designed to demonstrate the association between RAS mutations and clinicopathological variables regardless of the possible survival benefits, and may help improve the life expectancy of patients receiving anti-EGFR treatment with a wild-type RAS genotype. It can be considered that the samples being studied inherently exhibit variability in response to treatment. The therapeutic regimens differed across the patients, which led to heterogeneity and may have impacted the median overall survival, particularly in patients with wild-type KRAS. This is because not all patients received cetuximab and panitumumab as their initial treatment due to limited availability and were thus treated with other chemotherapeutic regimens. However, future work will focus on treatment efficacy and overall patient survival, with predictive significance in selecting patients for anti-EGFR therapy.

In conclusion, the present study examined the association between clinicopathological characteristics and the mutational landscape of mCRC in a cohort of patients. The results emphasize the diversity of CRC and the need for individualized treatment approaches, and highlight novel research opportunities, particularly in comprehending the distinctive characteristics of CRC in particular communities. Studies such as these serve a key role in influencing clinical practice, determining future research paths, and developing improved and personalized treatments for CRC based on individual patient characteristics as a key objective in the field of oncology. Furthermore, multicenter studies are currently in the design phase to validate the generalizability in a wide range of patients. These studies will additionally determine the validity on patients of differing demographics, improving reliability and accuracy in clinical settings worldwide. The research may then be linked with clinical decision-making to help drive personalized medicine and evidence-based therapeutics. Future research is warranted to define the predictive significance of the RAS and BRAF mutation status, and to discover any variations that may benefit from different treatments based on patients' prognostic values.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

RawA and RazA conceptualized the present study, contributed to the drafting of the manuscript, and revised it critically for important intellectual content. RoaA conducted the formal analysis, contributed substantially to the acquisition and interpretation of data, wrote parts of the manuscript, and participated in revising the manuscript critically for important intellectual content. AAl was involved in the design of the study, validated the experimental procedures, contributed to the drafting of the manuscript, and revised it critically for important intellectual content. EQ used the SPSS software, entered the data for analysis, and assisted in interpreting the data outcomes as part of the manuscript's drafting and critical revision process. RawA validated the study, curated the data, wrote the original draft, and revised the manuscript critically. MO and AAb were involved in the conceptualization and design of the study, critically evaluated and interpreted the experimental data, contributed to the drafting and critical revision of the manuscript for important intellectual content, ensured the integrity and accuracy of the work, and wrote, reviewed and edited the manuscript, ensuring the interpretation and application of data were appropriately conducted. AAb and RazA confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present investigation is characterized as an observational retrospective study. All patients were treated according to standard clinical protocols. The present study received approval from the Institutional Review Board (IRB) of The Hashemite University (Zarqa, Jordan; approval no. 13/9/2021/2022) and Jordanian Royal Medical Services (Amman, Jordan). The requirement for informed consent was waived by the Jordanian Royal Medical Services (Amman, Jordan; IRB approval no. 2/2024, dated 12/2/2024).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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