Original Article

Molecular spectrum of *KRAS, NRAS, BRAF, PIK3CA, TP53,* and *APC* somatic gene mutations in Arab patients with colorectal cancer: determination of frequency and distribution pattern

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Background: The frequency rates of mutations such as *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* in colorectal cancer (CRC) differ among populations. The aim of this study was to assess mutation frequencies in the Arab population and determine their correlations with certain clinicopathological features.

Methods: Arab patients from the Arab Gulf region and a population of age- and sex-matched Western patients with CRC whose tumors were evaluated with next-generation sequencing (NGS) were identified and retrospectively reviewed. The mutation rates of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53*, and *APC* were recorded, along with clinicopathological features. Other somatic mutation and their rates were also identified. Fisher's exact test was used to determine the association between mutation status and clinical features.

Results: A total of 198 cases were identified; 99 Arab patients and 99 Western patients. Fifty-two point seven percent of Arab patients had stage IV disease at initial presentation, 74.2% had left-sided tumors. Eighty-nine point two percent had tubular adenocarcinoma and 10.8% had mucinous adenocarcinoma. The prevalence rates of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53*, *APC*, *SMAD*, *FBXW7* mutations in Arab population were 44.4%, 4%, 4%, 13.1%, 52.5%, 27.3%, 2% and 3% respectively. Compared to 48.4%, 4%, 4%, 12.1%, 47.5%, 24.2%, 11.1% and 0% respectively in matched Western population. Associations between these mutations and patient clinicopathological features were not statistically significant.

Conclusions: This is the first study to report comprehensive hotspot mutations using NGS in Arab patients with CRC. The frequency of *KRAS*, *NRAS*, *BRAF*, *TP53*, *APC* and *PIK3CA* mutations were similar to reported frequencies in Western population except SMAD4 that had a lower frequency and higher frequency of *FBXW7* mutation.

Keywords: Somatic mutations; colorectal cancer (CRC); next-generation sequencing (NGS); Arab population

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second most common in females, worldwide (1). CRC incidence has been increasing in Arab countries such as Kuwait and Saudi Arabia (2,3). In Saudi Arabia, the incidence of CRC accounted for 10.4% of all cancers in 2010; it was the most common cancer in males and the third most common in females, after breast and thyroid cancers (4). In the Kuwaiti population, CRC was the most common cancer in males (11.3%) and the second most common in females (9.1%) in 2000–2009 (5). Gene mutation and defective cell regulation are important processes in the development of CRC (6). Accumulation of these mutations, including mutations in *KRAS*, *NRAS*, *BRAF* and *PIK3CA*, activate multiple signaling pathways, such as *RAS-RAF-MAPK* and *PI3K-PTEN-AKT*, that play a major role in regulating cell proliferation, angiogenesis, cell motility and apoptosis (7-9).

Assessment of genetic mutations is an essential element in the modern era of personalized cancer treatment. In the past years, our understanding of some of these mutations and their predictive and prognostic potential has revolutionized the treatment for various malignancies, with improved outcome and patient care [e.g., targeting wild-type *RAS* in metastatic colon cancer (10), targeting *HER2* in gastric adenocarcinoma] (11).

Anti-*EGFR* medications such as cetuximab and panitumumab are used for treatment of wild-type *RAS* metastatic CRC, but patients with mutations in the extended *RAS* family are resistant to these medications. Similarly, the patients with *BRAF* and the *PIK3CA* mutation have shown negative response to treatment with *EGFR* inhibitors (12-18).

The frequency rates of these mutations in CRC differ between populations. Zhang *et al.* have reported differences in the genetic profiles of *KRAS*, *NRAS*, *PIK3CA*, and *BRAF* at mutation hotspots between CRC patients from China and those from Western countries. The rate of these mutations in Arab patients with CRC is not well defined (9). The evaluations of the rates of these mutations in Arab population with CRC have been limited to few mutations including *KRAS* and *BRAF* (19,20).

The standard definition of the Arab world comprises the 22 countries and territories of the Arab League. The Arab Gulf countries which are also part of the Arab League are: Saudi Arabia, United Arab Emirates, Kuwait, Qatar, Bahrain and Oman.

The rate of some mutations of CRC in the Arab population from the Arabian Peninsula has been reported previously. A study by Siraj *et al.* reported a *BRAF* mutation rate of 2.5% in a Saudi Arabian population (19).

The rate of *KRAS* in Arab population from outside the Arab Gulf population has been reported, Elbjeirami *et al.* reported a *KRAS* mutation rate of 44% in a Jordanian population (20). The ratio of patients with mutated versus wild-type *KRAS* in the Jordanian study was similar to that reported in Western countries. Studies from Egypt showed high proportion (35%) of young onset CRC in patients under age 40. The studies also showed distinct *KRAS* and microsatellite instability (MSI) profiles between young and old CRC patients in Egypt (21,22). DNA methylation was also different in tumors of CRC patients from Egypt, Jordan, and Turkey (23).

The largest study, which included 500 patients from Saudi Arabia, assessed *KRAS* and *BRAF* using polymerase chain reaction (PCR) and DNA sequencing; the reported frequency rates were 30.1% and 2.4%, respectively (24). However, no studies have utilized next-generation sequencing (NGS) to assess in-depth mutations in Arab patients with CRC.

In the present study, we aimed to evaluate hotspot mutations by NGS in an Arab population from the Gulf countries with CRC and explored correlations of the mutations with clinicopathological features in this under-studied population.

Methods

Objectives

The primary objective of the study was to determine the frequencies of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53*, and *APC* mutations, as well as other somatic mutations, in CRC tumors from 99 Arab patients from the Gulf countries and to compare the results with 99 Western matched patients from our database at MD Anderson Cancer Center and with the frequencies among other populations. The secondary objective was to determine the relationships between these mutations and clinicopathological features of these patients.

Study design

We conducted a retrospective case-case study of Arab-patients from the Gulf countries who were treated in the U.S. at MD Anderson Cancer Center and the Mayo Clinic in Rochester, Minnesota. The electronic databases at both institutions were searched for all patients with a diagnosis

of CRC from 2010 to 2014 who had standardized hotspot mutation testing using a 46- or 50-gene multiplex platform by NGS. The electronic records then were searched manually with various criteria to identify patients from the Arabian Peninsula using country of origin, primary language (Arabic), and sponsoring country that covering the medical expenses for the patient and matched Western patients that were treated at MD Anderson Cancer Center. We identified 99 Arab patients with CRC and there were matched (age, sex and type of testing 46- or 50-gene, see below for testing details) with 99 Western patients who had the same testing during the same period. The study was approved by institutional ethics board of MD Anderson Cancer Center (NCT01772771) and Mayo Clinic (15-000563).

Clinicopathological information was abstracted from the medical records for the following variables: age (≤50 or >50 years), sex (male or female), tumor site (right colon, left colon, or rectum), histological type, differentiation, MSI, and TNM stage.

We also conducted a comprehensive literature search for all studies that reported mutation rates in CRC tumors in populations around the world (Middle Eastern, Western, and Asian population). The method of detecting these mutations and the result for each population were recorded. The results were then pooled by region and compared with those from other regions and from our current study population.

Mutations assessments

All the samples (60% primary tumor and 40% other metastatic lesions) were originally evaluated using hematoxylin and eosin staining for tumor cellularity. DNA was extracted, purified, and quantified after hematoxylin and eosin staining. Genomic analysis samples were evaluated using NGS using the Ion AmpliSeq Cancer Panel (Life Technologies, Grand Island, NY, USA) test to assess hotspot mutations in 46 genes (38 patients). Testing later expanded to 50 genes by adding *EZH2*, *IDH2*, *GNA11*, and *GNAQ* (61 patients). *Table S1* lists all tested genes.

Statistical analysis

Fisher's exact test was used to determine the association between mutation status and clinical factors, as well as the association between markers.

The analysis determined the association between mutation status of each marker (i.e., *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53*, *APC*) with clinicopathological features, especially histopathological type, tumor differentiation, tumor site, patients' age, and sex and the association between markers. For all statistical analysis, we used IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA), and the P value was considered to be significant if it was less than 0.05.

Results

Study population

A total of 198 cases were identified; 99 Arab patients and 99 age- and sex-matched Western patients. Of the 99 Arab patients, 74 (74.7%) patients were from MD Anderson and 25 (25.3%) were from the Mayo Clinic. All of Western patients were treated at MD Anderson Cancer Center. The majority of Arab patients were from Saudi Arabia (38.3%) and the United Arab Emirates (34.3%). The major ethnicity of Western patients were White (79%) followed by Black Afro-American (11%) and Hispanic (10%).

Clinicopathological features

The demographic characteristics and clinicopathological variables for the Arab and Western population are given in *Table 1*. The mean age of Arab and Western patients was 50.8 and 48.03 years respectively. Of the 99 Arab patients identified, 52.7% had stage IV disease at their initial presentation. Seventy-four point two percent had left-sided tumors including rectum, sigmoid colon and splenic flexure compared to 25.8% had right sided tumors including cecum, hepatic flexure, and transverse colon. Furthermore, the histology of tubular adenocarcinoma (89.2%) was higher than mucinous adenocarcinoma (10.8%). In addition, the percentage of patients with moderately differentiated histology and poorly differentiated histology were 71% and 23.7%, respectively. The clinicopathological variables of the Western population are given in *Table 1*.

Distribution of KRAS, NRAS, TP53, BRAF, PIK3CA, and APC in the 99 Arab patients with CRC

The rate of mutations in the 99 Arab patients and 99 Western matched patients with CRC using 46-gene (38 patients) and 50-gene (61 patients) panels in each cohort are given in *Table 2*.

Of the 4% of Arab patients with *NRAS* mutation, none

Table 1 Demographic characteristics and clinicopathological variables of 99 Arab patients with colorectal cancer a matched 99 Western patients

Characteristic	Arab patients (N=99) (%)	Western patients (N=99) (%)
Sex (%)		
Male	60 (60.6)	61 (61.6)
Female	39 (39.4)	38 (38.4)
Testing type (%)		
46 genes	38 (38.4)	38 (38.4)
50 genes	61 (61.6)	61 (61.6)
Age at diagnosis (mean ± SD) [range]		
All	50.80±13.62 [20–77]	48.03±12.50 [20–79]
Male	52.70±14.60	50.10±13.30
Female	47.80±11.50	44.60±10.30
Age* (years) (%)		
≤50	47 (47.5)	47 (47.5)
>50	52 (52.5)	52 (52.5)
The distribution of patients from the six Arab Gulf countries (%)		
Saudi Arabia	39	–
United Arab Emirates	34	–
Kuwait	11	–
Qatar	7	–
Bahrain	2	–
Oman	2	–
Not available	5	–
Race among Western patients (%)		
White	–	79 (79.8)
Black African American	–	11 (11.1)
Hispanic	–	9 (9.1)
Primary tumor site (side of body)** (%)		
Left sided	69 (69.7)	64 (64.6)
Right sided	24 (24.2)	35 (35.4)
Unknown (missing data)	6 (6.1)	–
Primary tumor site (specific location)** (%)		
Ascending colon	9 (9.1)	7 (7.1)
Cecum	7 (7.1)	18 (18.2)
Hepatic flexure	1 (1.0)	4 (4.0)
Splenic flexure	1 (1.0)	2 (2.0)
Transverse colon	5 (5.0)	4 (4.0)
Descending colon	6 (6.0)	15 (15.2)
Sigmoid colon	27 (27.3)	18 (18.2)
Rectum	37 (37.4)	31 (31.3)
Unknown (missing data)	6 (6.1)	–
Histological type** (%)		
Tubular adenocarcinoma	83 (83.8)	93 (94.0)
Mucinous adenocarcinoma	10 (10.1)	6 (6.0)
Unknown (missing data)	6 (6.1)	–
Tumor differentiation** (%)		
Well	5 (5.1)	2 (2.1)
Moderate	66 (66.6)	79 (84.1)
Poor	22 (22.2)	13 (13.8)
Unknown (missing data)	6 (6.1)	–
TNM stage** (%)		
I	0	3 (3.1)
II	3 (3.0)	6 (6.1)
III	41 (41.4)	15 (15.3)
IV	49 (49.5)	74 (75.5)
Unknown (missing data)	6 (6.1)	–
KRAS mutation (%)		
Positive	44 (44.4)	48 (48.4)
Negative	55 (55.6)	51 (51.6)
NRAS mutation (%)		
Positive	4 (4.0)	4 (4.0)
Negative	95 (96.0)	95 (96.0)
BRAF mutation (%)		
Positive	4 (4.0)	4 (4.0)
Negative	95 (96.0)	95 (96.0)
TP53 mutation (%)		
Positive	52 (52.5)	47 (47.5)
Negative	47 (47.5)	52 (52.5)
APC mutation (%)		
Positive	27 (27.3)	24 (24.2)
Negative	72 (72.7)	75 (75.8)

*, for 1 patient (1%), age of diagnosis was not available; **, for 6 patients (6.1%), data were not available for the primary tumor site, histological type, tumor differentiation, and TNM stage.

had *KRAS* mutation, in keeping with previous reports that these mutations are mutually exclusive (25). *BRAF* mutation was found in four Arab patients and was mutually exclusive of *KRAS* or *NRAS* mutations. Eight tumors of Arab patients had both *KRAS* and *PIK3CA* mutations. *PIK3CA* mutations were present in 8 (8.1%) Arab patients with *KRAS* mutations, compared with only 5 Arab patients (5%) with

Table 2 The rate of mutations in 99 Arab patients and matched 99 Western cohort with CRC using 46 genes (38 patients) and 50 genes (61 patients) in each cohort

CRC somatic mutation	No. of Arab patients with the mutation (%)	No. of Western matched patients with the mutation (%)
KRAS	44 (44.4)	48 (48.4)
NRAS	4 (4.0)	4 (4.0)
BRAF	4 (4.0)	4 (4.0)
PIK3CA	13 (13.1)	12 (12.1)
TP53	52 (52.5)	47 (47.5)
APC	27 (27.3)	24 (24.2)
FBXW7	3 (3.0)	0
SMAD4	2 (2.0)	11 (11.1)
GNAS	2 (2.0)	1 (1.0)
AKT1	2 (2.0)	2 (2.0)
PDGFRA	2 (2.0)	1 (1.0)
ATM	3 (3.0)	1 (1.0)
KIT1	2 (2.0)	3 (3.3)

CRC, colorectal cancer.

wild-type *KRAS*. This finding suggests that *PIK3CA* and *KRAS* gene mutations represent overlapping subgroups in CRC.

Correlation of gene mutations with clinicopathological findings

A summary of the relationships among the gene mutations and clinicopathological features in Arab CRC patients is provided in *Tables 3,4*.

The associations between *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53* and *APC* mutations and Arab population clinicopathological features such as age, gender, family history, personal history of familial adenomatous polyposis (FAP), tumor site, tumor histology, differentiation, and stage were not statistically significant. An exception was a statistically significant association of *TP53* mutation with age >50 years (P=0.009). PIK3CA and *TP53* were statistically significantly associated with absence of an *APC* gene mutation (P=0.039 and P=0.04), respectively.

Discussion

Here we present a large retrospective, two-center study that evaluated the frequencies of *KRAS*, *NRAS*, *BRAF*, *TP53*, *APC*, and *PIK3CA* somatic mutations in a cohort of 99 Arab CRC patients. This is the first study that comprehensively evaluated hotspot somatic mutations in Arab patients with CRC.

The majority of the population from the current study was from Saudi Arabia and the United Arab Emirates, however, all the gulf countries share common tribal genetic origin of the population from the Arabian peninsula (26).

Our study utilized comprehensive NGS platform to analyze the mutational profile of Arab CRC patients and assess the frequency. The frequencies of *KRAS*, *NRAS*, *BRAF*, *TP53*, and *APC* mutations. We were able to demonstrate similar mutational frequencies to those in most target genes compared with the Western population with the exception; however, *PIK3CA* which occurred at a lower frequency in the Arab population patients than in Western patients.

We performed a comprehensive literature review for somatic mutations testing in patients with CRC. The total number of cases were 2,981 in Middle Eastern countries (16 studies), 22,441 cases in Western countries (43 studies), and 8,053 in Asian countries (27 studies). Rate of mutations in each study, method of testing, pooled mutation rates based on geographical distribution and total pooled mutation rates from all reported studies to date are summarized in *Table 5*.

Wild-type *KRAS* and *NRAS* oncogenes encode a family of small proteins with homology to G-proteins that regulate cellular signal transduction (106). The *KRAS* mutation frequency rate differs throughout the world. Soliman *et al.* reported that mutation of the *KRAS* gene was uncommon in Egyptian CRCs in general (11% of patients), in contrast to Western cases (28% in sporadic CRCs), and was only identified in patients older than 40 years (21). The study by Elbjeirami *et al.* reported *KRAS* mutation (44%) in a Jordanian population (20). The ratio of patients with mutated versus wild-type *KRAS* in our current study was similar to that reported in Western countries but differed from Egypt (107) ,which is a neighboring Middle Eastern country but similar to the Jordanian study (20). Other studies from Saudi Arabia reported rates of *KRAS* mutation to be 42.2% (108), 28.6% (19), 56% (33), and 30.1% (109). The results of our study are in line with all of the other Arab studies. The data from our study did not show statistical significance between *KRAS* gene mutation in the Arab population and any covariate such as age or gender, which is consistent with the results of a similar study in a Western population (110). Unlike *KRAS* mutation frequency rates,

Table 3 Correlation between *KRAS*, *NRAS*, and *BRAF* mutation status and clinicopathological features in Arab CRC patients

Clinicopathological features	*KRAS* status			*NRAS* status			*BRAF* status		
	Wild type (%)	Mutant type (%)	P	Wild type (%)	Mutant type (%)	P	Wild type (%)	Mutant type (%)	P
Age (years)			0.8			0.3			1
>50	28 (53.8)	24 (46.2)		51 (98.1)	1 (1.9)		50 (96.2)	2 (3.8)	
≤50	26 (56.5)	20 (43.5)		43 (93.5)	3 (6.5)		44 (95.7)	2 (4.3)	
Sex			0.5			1			0.6
Female	20 (51.3)	19 (48.7)		38 (97.4)	1 (2.6)		37 (94.9)	2 (5.1)	
Male	35 (58.3)	25 (41.7)		57 (95.0)	3 (5.0)		58 (96.7)	2 (3.3)	
Family history			0.7038			0.3			1
No	46 (53.5)	40 (46.5)		83 (96.5)	3 (3.5)		83 (96.5)	3 (3.5)	
Yes	3 (42.9)	4 (57.1)		6 (85.7)	1 (14.3)		7 (100.0)	–	
Personal history of FAP			1			1			1
No	48 (52.2)	44 (47.8)		88 (95.7)	4 (4.3)		89 (96.7)	3 (3.3)	
Yes	1 (100.0)	–		1 (100.0)	–		1 (100.0)	–	
Primary tumor site			1			0.5693			1
Left sided	36 (52.2)	33 (47.8)		65 (94.2)	4 (5.8)		67 (97.1)	2 (2.9)	
Right sided	13 (54.2)	11 (45.8)		24 (100.0)	–		23 (95.8)	1 (4.2)	
Tumor differentiation			0.5			0.2			0.2
Well	4 (80.0)	1 (20.0)		4 (80.0)	1 (20.0)		4 (80.0)	1 (20.0)	
Moderate	33 (50.0)	33 (50.0)		64 (97.0)	2 (3.0)		64 (97.0)	2 (3.0)	
Poor	12 (54.5)	10 (45.5)		21 (95.5)	1 (4.5)		22 (100.0)	–	
MSI			0.09			1			0.6
High	–	1 (100.0)		1 (100.0)	–		1 (100.0)	–	
Intact	4 (100.0)	–		4 (100.0)	–		4 (100.0)	–	
Stable	10 (43.5)	13 (56.5)		22 (95.7)	1 (4.3)		23 (100.0)	–	
Unknown	20 (40.0)	30 (60.0)		47 (94.0)	3 (6.0)		47 (94.0)	–	
Histological type			0.3			1			1
Tubular adenocarcinoma	42 (50.6)	41 (49.4)		79 (95.2)	4 (4.8)		80 (96.4)	3 (3.6)	
Mucinous adenocarcinoma	7 (70.0)	3 (30.0)		10 (100.0)	–		10 (100.0)	–	
TNM stage at diagnosis			0.1			0.2			0.6
I	1 (100.0)	–		1 (100.0)	–		1 (100.0)	–	
II	1 (50.0)	1 (50.0)		2 (100.0)	–		2 (100.0)	–	
III	26 (63.4)	15 (36.6)		41 (100.0)	–		39 (95.1)	–	
IV	21 (42.9)	28 (57.1)		45 (91.8)	4 (8.2)		48 (98.0)	–	
Clinical status			0.2			0.07			0.2
Alive	20 (55.6)	16 (44.4)		36 (100.0)	–		36 (100.0)	–	
Dead	16 (42.1)	22 (57.9)		34 (89.5)	4 (10.5)		35 (92.1)	3 (7.9)	
Unknown	13 (68.4)	6 (31.6)		19 (100.0)	–		19 (100.0)	–	

FAP, familial adenomatous polyposis; MSI, microsatellite instability; CRC, colorectal cancer.

Table 4 Correlation between *PIK3CA*, *TP53*, and *APC* mutation status and clinicopathological features in Arab CRC patients

Clinicopathological features	*PIK3CA* status			*TP53* status			*APC* status		
	Wild type (%)	Mutant type (%)	P	Wild type (%)	Mutant type (%)	P	Wild type (%)	Mutant type (%)	P
Age (years)			0.08			0.009			0.5
>50	42 (80.8)	10 (19.2)		31 (59.6)	21 (40.4)		36 (69.2)	16 (30.8)	
<50	43 (93.5)	3 (6.5)		15 (32.6)	31 (67.4)		35 (76.1)	11 (23.9)	
Sex			1			0.07			1
Female	34 (87.2)	5 (12.8)		14 (35.9)	25 (64.1)		28 (71.8)	11 (28.2)	
Male	52 (86.7)	8 (13.3)		33 (55.0)	27 (45.0)		44 (73.3)	16 (26.7)	
Family history			1			0.3			1
No	74 (86.0)	12 (14.0)		40 (46.5)	46 (53.5)		65 (75.6)	21 (24.4)	
Yes	6 (85.7)	1 (14.3)		5 (71.4)	2 (28.6)		5 (71.4)	2 (28.6)	
Personal history of FAP			1			1			0.2
No	79 (85.9)	13 (14.1)		45 (48.9)	47 (51.1)		70 (76.1)	22 (23.9)	
Yes	1 (100.0)	–		–	1 (100.0)		–	1 (100.0)	
Tumor site			1			0.3			0.2
Left	80 (86.0)	13 (14.0)		31 (44.9)	38 (55.1)		49 (71.0)	20 (29.0)	
Right	21 (87.5)	3 (12.5)		14 (58.3)	10 (41.7)		21 (87.5)	3 (12.5)	
Differentiation			1			0.5			0.6
Well	5 (100.0)	–		1 (20.0)	4 (80.0)		5 (100.0)	–	
Moderate	56 (84.8)	10 (15.2)		33 (50.0)	33 (50.0)		49 (74.2)	17 (25.8)	
Poor	19 (86.4)	3 (13.6)		11 (50.0)	11 (50.0)		16 (72.7)	6 (27.3)	
MSI			0.2			0.6			0.9
High	1 (100.0)	–		1 (100.0)	–		1 (100.0)	–	
Intact	2 (50.0)	2 (50.0)		1 (25.0)	3 (75.0)		3 (75.0)	1 (25.0)	
Stable	21 (91.3)	2 (8.7)		11 (47.8)	12 (52.2)		19 (82.6)	4 (17.4)	
Unknown	41 (82.0)	9 (18.0)		27 (54.0)	23 (46.0)		38 (76.0)	12 (24.0)	
Tumor histology			1			1			0.4416
Tubular adenocarcinoma	71 (85.5)	12 (14.5)		40 (48.2)	43 (51.8)		61 (73.5)	22 (26.5)	
Mucinous adenocarcinoma	9 (90.0)	1 (10.0)		5 (50.0)	5 (50.0)		9 (90.0)	1 (10.0)	
TNM stage at diagnosis			0.23			0.2			0.04
I	1 (100.0)	–		–	1 (100.0)		–	1 (100.0)	
II	1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)	
III	34 (82.9)	7 (17.1)		24 (58.5)	17 (41.5)		35 (85.4)	6 (14.6)	
IV	44 (89.8)	5 (10.2)		20 (40.8)	29 (59.2)		34 (69.4)	15 (30.6)	
Clinical status			0.2			0.1			0.6
Alive	28 (77.8)	8 (22.2)		13 (36.1)	23 (63.9)		26 (72.2)	10 (27.8)	
Dead	34 (89.5)	4 (10.5)		23 (60.5)	15 (39.5)		28 (73.7)	10 (26.3)	
Unknown	18 (94.7)	1 (5.3)		9 (47.4)	10 (52.6)		16 (84.2)	3 (15.8)	

FAP, familial adenomatous polyposis; MSI, microsatellite instability; CRC, colorectal cancer.

Table 5 Worldwide distribution pattern of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *APC*, and *TP53* mutations

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Middle Eastern countries			2,981	691/2,052 (33.70)	4/99 (4.0)	76/1,647 (4.60)	51/418 (12.00)	59/177 (33.0)	208/541 (38.40)	
Arabian Peninsula	2015	Next-generation sequencing	99	44 (44.00)	4 (4.0)	4 (4.00)	13 (13.00)	27 (27.3)	52 (52.00)	Current study
Egypt	2001	PCR and SequiTherm EXCEL II™ DNA sequencing; codons: 12, 13, immunohistochemistry for TP53, exons: 5–9	59	5/47 (11.00)	Not done	Not done	Not done	Not done	26/56 (46.00)	(22)
Saudi Arabia	2008	PCR amplification and direct sequencing, exons: 9, 20, exons: 5–8	448	Not done	Not done	Not done	51/418 (12.00)	Not done	130/386 (33.70)	(27)
Tunisia	2008	PCR, codons: 1240–1513	48	Not done	Not done	4/48 (8.00)	Not done	25/48 (52.0)	Not done	(28)
Iran	2011	PCR-RFLP, codon: 600	110	24/86 (28.00)	Not done	0	Not done	Not done	Not done	(29)
Jordan	2012	Hybridization-based strip assay, RT-PCR-based assay, Sanger sequencing, codons: 12, 13	100	44 (44.00)	Not done	Not done	Not done	Not done	Not done	(20)
Iraq	2012	PCR and reverse hybridization	50	24 (48.00)	Not done	Not done	Not done	Not done	Not done	(30)
Turkey	2013	AutoGenomics INFINITI® assay, codons: 12, 13, 61	53	26 (49.05)	Not done	0	Not done	Not done	Not done	(31)
Saudi Arabia	2014	LCD-array kit	83	35/83 (42.20)	Not done	Not done	Not done	Not done	Not done	(32)
Saudi Arabia	2014	Direct DNA sequencing, codons: 12, 13, codon: 600	770	216/755 (28.60)	Not done	19/757 (2.50)	Not done	Not done	Not done	(19)
Saudi Arabia	2014	PCR, codons: 12, 13	150	84/150 (56.00)	Not done	Not done	Not done	Not done	Not done	(33)
Iran	2014	PCR-RFLP, codon: 600	80	Not done	Not done	37/80 (46.25)	Not done	Not done	Not done	(34)
Iran	2014	Direct DNA sequencing, codons: 653–885, 853–1242, 1213–1482, and 1404–1613 of exon 15	30	Not done	Not done	Not done	Not done	7 (23.3)	Not done	(35)
Saudi Arabia	2015	PCR, codons: 12, 13, codon: 600	770	150/498 (30.10)	Not done	12/500 (2.40)	Not done	Not done	Not done	(24)
Turkey	2015	Pyrosequencing with PCR, codons: 12, 13, 61	31	7/31 (22.00)	Not done	Not done	Not done	Not done	Not done	(36)
Iran	2015	PCR and direct sequencing by Sanger method	100	32 (32.00)	Not done	Not done	Not done	Not done	Not done	(37)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Western countries			22,441	7,497/21,212 (35.30)	183/4,781 (3.8)	1,011/11,100 (9.10)	1,336/9,696 (13.80)	626/1,540 (40.6)	169/308 (54.90)	
Norway	2002	PCR, codons: 653–2843	218	Not done	Not done	Not done	Not done	144 (66.0)	Not done	(38)
United Kingdom	2002	Direct sequencing, codons: 12, 13, 61, denaturing HPLC "WAVE" analysis, codons: 1028–1712	106	29/106(27.40)	Not done	Not done	Not done	60/106 (56.0)	65/106 (61.30)	(39)
Portugal	2005	PCR-SSCP automated sequencing, exon: 9; PCR automated sequencing, exon: 20	150	31 (20.70)	Not done	18 (12.00)	14 (9.30)	Not done	Not done	(40)
Netherlands	2005	Nested PCR, followed by direct sequencing, exon: 1, codons: 1286–1520	656	235/656 (35.80)	Not done	Not done	Not done	245 (37.3)	Not done	(41)
USA	2007	PCR, codons: 1286–1585	90	29 (32.20)	Not done	18 (20.00)	Not done	31 (34.4)	41 (45.60)	(42)
Germany	2007	PCR, codons: 1260–1547, exons: 5–8 (TP53)	99	Not done	Not done	Not done	Not done	49 (49.0)	52 (52.00)	(43)
France	2008	PCR then direct sequencing, exon: 2, exons: 1, 2, 9, 20 (*PIK3CA*)	586	198 (33.80)	Not done	78 (13.30)	98 (16.70)	Not done	Not done	(44)
Hungary	2008	PCR and SSCP/heteroduplex analysis, codons: 1285–1465	70	Not done	Not done	Not done	Not done	15 (21.4)	Not done	(45)
USA	2008	PCR, exon: 2, exons: 11, 15, DNA sequencing using a BigDye® 3.1 Terminator kit	62	24 (38.70)	Not done	4 (5.60)	2 (3.20)	Not done	Not done	(46)
Italy	2008	HRM analysis, exon: 2, exon: 15, exons: 9, 20	116	50 (43.00)	Not done	11 (9.50)	20 (17.20)	Not done	Not done	(47)
Italy	2009	PCR, codons: 12, 13, exons: 11, 15, exons: 9, 20	32	7/29 (24.10)	Not done	3/31 (9.67)	4/31 (12.90)	Not done	Not done	(48)
USA	2009	PCR and pyrosequencing, codons: 12, 13, codon: 600, exons: 9, 20	450	160/448 (35.70)	Not done	69/438 (15.80)	82 (18.20)	Not done	Not done	(49)
United Kingdom	2009	PCR, then Sequenom mass-spectrometric genotyping, (first sample), PCR, then Sanger sequencing (second sample), exon: 2, exon: 15, exons: 9, 20	168	62 (36.90)	Not done	13 (7.70)	26 (15.47)	Not done	Not done	(50)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Belgium	2010	Mass spectrometry genotyping	1,022	299/747 (40.00)	17/644 (2.6)	36/761 (4.70)	108/743 (14.50)	Not done	Not done	(13)
Germany	2010	Two multiplex PCRs: the first for *BRAF* exon 15 and *KRAS* exons 2 and 3 and the second for *PIK3CA* exons 9 and 20 and *NRAS* exons 2 and 3	294	119/245 (48.60)	6/294 (2.0)	13/245 (5.30)	32/245 (13.10)	Not done	Not done	(51)
France	2010	*KRAS*: allelic discrimination assay; checked by direct sequencing of exon 2, *BRAF*(V600E): allelic discrimination assay; checked by direct sequencing; *PIK3CA*: direct sequencing, then DNA analyzer automated sequencer	42	19 (45.20)	Not done	1 (2.38)	6 (14.28)	Not done	Not done	(52)
USA	2011	Pyrosequencing, codons: 12, 13, 61, codons: 595–600; PCR, then Sanger sequencing (*PIK3CA*), codons: 532–554 of exon 9, 1011–1062 of exon 20	504	69/367 (18.80)	2/31 (6.0)	31/361 (8.60)	54 (11.00)	Not done	Not done	(53)
Italy	2012	HRM analysis and direct sequencing, exon: 2, exon: 15, exon: 20	209	90 (43.00)	Not done	13/117 (11.10)	7 (3.30)	Not done	Not done	(54)
Sardinia	2012	Automated DNA sequencing, exons: 2, 3, exon: 15, exons: 9, 20	478	145/478 (30.30)	Not done	1/384 (0.26)	67/384 (17.44)	Not done	Not done	(55)
USA	2013	Pyrosequencing, codons: 12, 13, codon: 600, exons: 9, 20	964	336/959 (35.00)	Not done	131/959 (13.70)	161/964 (16.70)	Not done	Not done	(56)
Portugal	2013	HRM, then DNA sequencing, exons: 3, 4 (*KRAS*), exons: 11, 15, exons: 9, 20 (*PIK3CA*)	201	26 (12.90)	Not done	11 (5.50)	22 (10.90)	Not done	Not done	(57)
Russia	2013	HRM and sequencing COLD-PCR/ sequencing, allele-specific PCR	195	70 (35.90)	8 (4.1)	8 (4.10)	24 (12.30)	Not done	Not done	(58)
Australia	2013	Direct sequencing, exons: 9, 20	757	215 (28.40)	Not done	120 (15.90)	105 (14.00)	Not done	Not done	(59)
France	2013	PCR amplification followed by direct sequencing, exons: 2, 3, exon: 15, exons: 9, 20	98	23 (23.50)	Not done	2 (2.00)	4 (4.00)	Not done	Not done	(60)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Germany	2013	Pyrosequencing, exon: 2, exon: 15, exons: 9, 20	171	70 (40.90)	Not done	19 (11.10)	20 (18.70)	Not done	Not done	(61)
Albania	2014	Direct sequencing, codons: 12, 13, 61, 146, codon: 600	159	28 (17.60)	Not done	10 (6.30)	Not done	Not done	Not done	(62)
United Kingdom	2013	Pyrosequencing (*KRAS*), codons: 12, 13, Sequenom, Sanger sequencing, codons: 12, 13, codon: 600	1,976	836 (42.30)	71 (3.6)	178 (9.00)	251 (12.70)	Not done	Not done	(18)
Greece	2014	PCR, mutation analysis methodology of increased sensitivity and conventional genomic dideoxy sequencing (*PIK3CA*)	171	92 (53.80)	Not done	4/171 (2.30)	6/171 (3.50)	Not done	Not done	(63)
Brazil	2014	Direct sequencing, codons: 12, 13	8,234	2,627 (31.90)	Not done	Not done	Not done	Not done	Not done	(64)
Chile	2014	PCR, codons: 12, 13	262	98 (37.00)	Not done	Not done	Not done	Not done	Not done	(65)
Italy	2015	Pyrosequencing, codons: 12, 13, 61, 146, codon: 600, exons: 9, 20	194	92 (47.40)	7/194 (3.6)	10 (19.40)	32 (16.50)	Not done	Not done	(66)
USA	2014	Not reported	484	240 (49.60)	32 (7.4)	10 (4.10)	Not done	Not done	Not done	(67)
Greece	2015	PCR, codons: 12, 14, 61, 146, codon: 600, bidirectional sequence analysis	322	118 (36.60)	Not done	17/188 (9.00)	Not done	Not done	Not done	(68)
Italy	2015	Mass spectrometry-based single-base extension technique, codons (*KRAS*): 12, 13, 59, 61, 117, 146, codons (*NRAS*): 12, 13, 18, 59, 61, 117, 146, codons (*BRAF*): 594, 600, 601	175	25 (14.00)	4 (3.0)	13 (7.00)	Not done	Not done	Not done	(69)
Brazil	2015	Pyrosequencing method improved by nested PCR, codons: 12, 13	422	139/421 (33.00)	Not done	Not done	Not done	Not done	Not done	(70)
Belgium	2015	RT-PCR and Sequenom, exons: 2–4	193	53/165 (32.10)	4 (2.4)	26/165 (15.80)	22/165 (13.30)	Not done	Not done	(71)
USA	2015	PCR, codons: 12, 13	331	91 (27.50)	Not done	Not done	Not done	Not done	Not done	(72)
Italy	2015	Pyrosequencing, exon: 2, codon: 600	309	143/307 (46.60)	17/307 (5.5)	12 (4.00)	Not done	Not done	Not done	(73)
France	2015	Next-generation sequencing	13	7 (53.80)	Not done	Not done	Not done	13/13 (100.0)	11/13 (84.60)	(74)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Germany	2015	PCR, codons: 12, 13, codon: 600	99	33 (33.30)	Not done	9 (9.00)	Not done	Not done	Not done	(75)
France	2015	PCR, codons: 12, 13, codon: 600	180	93 (51.70)	Not done	19 (10.60)	Not done	Not done	Not done	(76)
France	2015	Direct Sanger sequencing and PCR, codons: 12, 13, codon: 600, exons: 9, 20	826	301/817 (37.00)	Not done	85/780 (11.00)	113 (14.00)	Not done	Not done	(77)
USA	2015	Next-generation sequencing	353	175/288 (49.60)	15/288 (4.3)	18/288 (5.10)	56/288 (13.90)	69/288 (24.0)	Not done	(78)
Asian countries			8,053	2,797/7,973 (35.10)	108/3,041 (3.6)	292/5,922 (4.90)	362/4,238 (8.50)	85/262 (32.4)	244/608 (40.10)	
Japan	2002	PCR-SSCP method, codons: 12, 14, codons: 582–1580, codons: 33–367	61	22/61 (36.00)	Not done	Not done	Not done	29/61 (47.5)	35/61 (57.40)	(79)
South Korea	2008	WAVE DHPLC system, codon: 12, codons: 1202–1674, exons: 4–9	78	23 (29.50)	Not done	Not done	Not done	26 (33.3)	27 (34.60)	(80)
China	2010	Multiplex PCR for *TP53* and *PTEN* amplification; singleplex PCR using HotStarTaq (QIAGEN) to amplify *PIK3CA, KRAS*, and *BRAF* amplicons, codons: 12, 13, 61 (*KRAS*), codon: 600 (*BRAF*)	181	58 (32.00)	Not done	29 (16.00)	7 (3.00)	Not done	92 (52.00)	(81)
China	2010	Pyrosequencing using a PyroMark ID system (Biotage AB, Sweden), codons: 12, 13, codon: 600	61	12 (19.70)	Not done	3 (4.90)	3 (4.90)	Not done	Not done	(82)
Korea	2011	Direct sequencing and peptide nucleic acid-mediated PCR	92	19 (20.70)	Not done	3 (3.30)	1 (1.10)	Not done	Not done	(83)
Japan	2011	Direct sequencing	134	41 (30.60)	Not done	1 (0.75)	18 (13.40)	Not done	Not done	(84)
Taiwan (China)	2012	Direct sequencing; HRM analysis, codon: 600, exons: 9, 20 (*PIK3CA*)	182	61 (33.50)	Not done	2 (1.10)	13 (7.10)	Not done	Not done	(85)
China	2012	PCR-based direct DNA sequencing, codons: 12, 13	331	137/311 (44.10)	Not done	9/156 (5.80)	4/156 (2.60)	Not done	Not done	(86)
China	2012	Automated sequencing analysis, codons: 12–14, codon: 600, codons: 542, 545, 1047	69	25/57 (53.90)	Not done	15/59 (25.40)	5/56 (8.90)	Not done	Not done	(87)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Japan	2013	Multiplex kit (Luminex xMAP tests) and direct sequencing methods, codons: 61, 146, codon: 600, codons: 542, 545, 546, 1047	82	21 (25.60)	2 (2.4)	4 (4.90)	4 (4.90)	Not done	Not done	(88)
Malaysia	2013	Direct DNA sequencing, quantitative real-time PCR, codons: 12, 13, 61, codon: 600	44	11 (25.00)	Not done	1 (2.30)	33/43 (76.70)	Not done	Not done	(89)
Japan	2013	Direct sequencing	254	85 (33.50)	Not done	17 (6.70)	Not done	Not done	Not done	(90)
Japan	2013	Automated CEQ 2000XL DNA analysis system	43	12 (27.90)	Not done	2 (4.70)	2 (4.70)	Not done	Not done	(91)
Taiwan (China)	2013	Primer extension analysis, codons: 12, 13, HRM analysis, codon: 600, direct sequencing (for TP53), exons: 5–8	165	61/165 (36.97)	Not done	7/165 (4.24)	Not done	Not done	62/165 (37.58)	(92)
India	2013	PCR, exon: 2 (*KRAS*)	1,323	271 (20.50)	Not done	Not done	Not done	Not done	Not done	(93)
India	2013	PCR-RFLP and direct sequencing	62	41 (66.10)	Not done	Not done	Not done	Not done	Not done	(94)
India	2013	PCR-restriction digestion to detect *KRAS* mutations, PCR-SSCP followed by DNA sequencing to detect mutations in *APC* and *TP53* genes	30	8 (26.70)	Not done	Not done	Not done	14 (46.7)	6 (20.00)	(95)
India	2014	Nested PCR, codons: 12, 13; PCR and direct sequencing, codon: 600; hemi-nested and nested PCR, exons: 9, 20	204	48 (23.50)	Not done	20 (9.80)	12 (5.90)	Not done	Not done	(96)
Pakistan	2014	PCR, codons: full coding region of *KRAS*	150	20/150 (13.00)	Not done	Not done	Not done	Not done	Not done	(97)
China	2014	Torrent AmpliSeq Cancer Panel	93	47 (50.50)	3 (3.2)	1 (1.10)	10 (10.80)	16 (17.2)	22 (23.70)	(98)
Japan	2015	Denaturing gradient gel electrophoresis; PCR (for *BRAF*)	813	312/812 (38.00)	Not done	40/811 (5.00)	Not done	Not done	Not done	(99)
China	2015	Sanger sequencing; mutation system PCR (nine patients), codons: 12, 13, codon: 600	535	185/488 (37.90)	Not done	20/450 (4.40)	Not done	Not done	Not done	(100)

Table 5 (*continued*)

Table 5 (*continued*)

Region	Year	Method and codons studied	Number of patients tested	Number of patients with mutations in the indicated gene (%)						References
				KRAS	*NRAS*	*BRAF*	*PIK3CA*	*APC*	*TP53*	
Japan	2015	Luminex xMAP technology, codons: 61, 146, codon: 600, codons: 542, 545, 546, 1047; Scorpion assay, codons: 12, 13	264	100/264 (37.90)	11/264 (4.2)	14/264 (5.40)	17/264 (6.40)	Not done	Not done	(101)
Singapore	2015	Direct sequencing	45	15 (33.30)	Not done	0	1 (2.20)	Not done	Not done	(102)
China	2015	RT-PCR and Sanger sequencing, codons: 12, 13, 61, 117, 146, codon: 600, codon: 1047	1,110	504/1,110 (45.40)	43/1,110 (3.9)	34/1,110 (3.10)	39/1,110 (3.50)	Not done	Not done	(9)
South Korea	2015	Not reported	100	26 (26.00)	Not done	Not done	Not done	Not done	Not done	(103)
Japan	2015	PCR and direct sequencing, exon: 2	55	30 (54.40)	Not done	Not done	Not done	Not done	Not done	(104)
Taiwan (China)	2015	PCR and Sequenom	1,492	602 (40.30)	49 (3.3)	70 (4.70)	193 (12.90)	Not done	Not done	(105)
Pooled results for all studies from all regions			33,475	10,985/31,237 (35.20)	295/7,921 (3.7)	1,379/18,669 (7.40)	1,749/14,352 (12.20)	770/1,980 (38.9)	621/1,457 (42.60)	

PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; RFLP, restriction fragment length polymorphism; HPLC, high-performance liquid chromatography; SSCP, single-strand conformation polymorphism; HRM, high-resolution melting; COLD, co-amplification at lower denaturation temperature; DHPLC, denaturing high-performance liquid chromatography.

NRAS mutation was not previously reported in an Arab population. In Western populations the mutation frequency rates were low; in one study that used NGS, the rate was 5.1% (78). In the present study, *NRAS* mutation was detected in 4% of Arab patients, which is similar to the Western cohort and consistent with the pooled frequency rates in Asian countries and Western countries (3.6% and 3.8%, respectively).

The *BRAF* gene encodes a protein that is part of the *Ras-Raf-MEK-ERK*, or *MAPK* signaling pathway (111). Activation of this pathway results in cellular growth and proliferation. Siraj *et al.* reported the frequency of *BRAF* mutation as 2.5% in 770 Saudi patients. In our study, the *BRAF* frequency rate was 4% in Arab patients, which is lower than the Western cohort and the estimated frequency in Western countries (9.2%) but similar to the frequency reported in Asian (4.9%) and Middle Eastern countries (4.6%). This difference in *BRAF* mutational frequency may be attributable to the use of different methods/assays to assess for the mutations. Interestingly, the Western population-based studies reported that *BRAF*-mutant CRC was significantly more likely to occur in females (108); however, in our study there was no statistically significant association between the *BRAF* mutation and gender. In contrast to Zhang *et al.*'s study, which showed a significant association between *BRAF* mutation and right-sided colon cancer, we did not find any significant association between *BRAF* mutation and tumor site (9) yet the findings of our study is limited by the small number of patients with *BRAF* mutation (only four patients).

The *PIK3CA* gene encodes the catalytic subunit of PI3K, which is an intracellular central mediator of cell survival signals (112). Very few studies describe the frequency of *PIK3CA* mutations in an Arab population. Abubaker *et al.* reported a *PIK3CA* frequency rate of 12.2% in a Saudi population (27), which is consistent with the frequency found in our Arab patients population (13%) and the Western patients cohort (12.1%). However, the frequency rate of *PIK3CA* mutations in Asian countries (8.5%) appear to be lower compared with Middle Eastern and Western countries. This difference could be attributable to either environmental or genetic factors. Our study found no significant differences between Western and Arab populations with regard to *PIK3CA* gene mutations and other clinical characteristics (109).

The tumor suppressor gene *APC* plays an important role in CRC development. Absence of the *APC* protein leads to accumulation of beta-catenin in the cytoplasm, resulting in constitutive transcriptional activation of TCF-responsive genes, which may contribute to tumor progression (113). The frequency from the pooled results in Middle Eastern countries is (33%), which is consistent with the frequency rate in Asian countries (32.4%), but it is lower than the frequency rate in Western countries (44.8%). In the current study the frequency was (27.3%) in Arab patients and (24.2%) in Western patients. These differences between the frequencies in our population study and pooled frequencies in Middle Eastern countries, Asian countries and Western countries may be attributable to environmental factors.

Loss of *TP53* function is one of the major events in the development of CRC. *TP53* mutations are thought to occur late in pathogenesis of CRC (39). The *TP53* mutation rate in our study was 52% in Arab patients and 47.5% in our Western cohort, which they are significantly higher than the pooled frequency rates encountered in Middle Eastern (38.4%) and Asian countries (40.1%). This difference between our study and the Middle Eastern countries frequency rate may be attributable to different sample selection. Abubaker *et al.* reported a trend of *TP53* mutations towards old age (>50 years old) (27). In the present study, there was a significant association between *TP53* mutations and age (>50 years old). This finding is in contrast to previous studies in Western countries (110,114).

Mutations in the *FBXW7* gene are thought to impair cyclin E degradation resulting in unchecked cellular growth, and subsequently in progression of CRC (115-117). The frequency of *FBXW7* mutation in the present study was 3% in Arab patients, none were found in the matched Western cohort. This is the first report of *FBXW7* gene mutation in an Arab population and potential association.

Fleming *et al.* reported the frequency of *SMAD4* mutation in 744 patients with sporadic CRC at 8.6% (118). Mutations in *SMAD4* are thought to promote tumorigenesis by allowing CRC cells evade the inhibitory effect of TGF-beta, thus contributing to progression of CRC (119,120). In the present study, the rate of *SMAD4* mutation in the Arab patients was 2% where 11.1% in the matched Western cohort. This difference in the frequency rates may be attributable to differences in sample size, ethnicities, and geographical distribution. This is the first report of *SMAD4* gene mutation in the Arab population.

EGFR signaling plays a significant role in CRC development and progression. Gene mutations in the *EGFR* signaling proteins, such as *KRAS*, *NRAS*, *BRAF*, and *TP53*, are vital factors in evaluating *EGFR* targeted treatment resistance in patients with CRC (7,107). *KRAS*-mutant CRC

do not respond to anti-*EGFR* agents such as cetuximab (14). However, only 40–60% of type patients with wild-type *KRAS* respond to *EGFR* targeted therapies (121). Therefore, it is very important to identify other molecular alterations that may affect anti-*EGFR* treatment. De Roock *et al.* demonstrated that *BRAF*, *NRAS*, and *PIK3CA* mutations affect the anti-*EGFR* treatment outcome in chemotherapy-resistant metastatic CRC patients (13).

Many environmental factors such as lifestyle and diet are implicated as risk factors for CRC. Subjects consuming a diet rich in meat and fat and poor in fiber have a higher risk for CRC (122-124). Decreased physical activity and obesity also put the subjects in a greater risk for CRC (125,126). Westernization of the developing countries along with changes in diet and lifestyle have been associated with the increasing incidence of CRC in developing countries (127,128). The increased incidence of CRC in Arabian Peninsula is parallel to similar increase incidence of CRC in Westernized countries. The results of the present study report the frequency of *KRAS*, *NRAS*, *BRAF*, *TP53*, and *APC* mutations similar to the frequencies in Western population. Many studies have previously indicated that the differences in the incidence of CRC are probably due to environmental and not genetic factors (129). In our study, we found that there was no association between incidence of CRC and clinicopathological factors except the association of *TP53* mutation and advanced age. Two studies from Qatar and Jordan have shown associations between CRC and diet with low fiber, sedentary life and obesity in Qatari and Jordanian populations (130,131). A study by Bener *et al.* evaluated the association of family history, lifestyle and dietary factors with developing CRC in Arab patients. Multivariate stepwise logistic regression analysis showed that family history, BMI, smoking, consuming bakery and soft drinks were significant predictors of development of CRC. Age, gender, a sedentary lifestyle, and being overweight were positively linked with CRC risk (130). Also, there is a recent trend for left-sided CRC in Arabs, probably related to their changing lifestyles (132). All these results may influence CRC screening and diagnostic methodologies with cancer preventive lifestyle recommendations in Arab population.

A possible limitation of current study is the relatively small sample size which, which could potentially limit the generalizability of our findings. We have attempted to decrease this risk by including patients from at least six Arab Gulf countries, which were recruited from two large U.S. institutions.

Conclusions

This is the first study to report comprehensive hotspot somatic mutations using NGS in Arab patients with CRC. The frequency of *KRAS*, *NRAS*, *BRAF*, *TP53*, *APC* and *PIK3CA* mutations were similar to reported frequencies in Western population except *SMAD4* that had a lower frequency but higher rate of *FBXW7* mutation. Identification of molecular markers can provide insights into the pathogenic process and help optimize personalized cancer therapy in this poorly studied population.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by institutional ethics board of MD Anderson Cancer Center (NCT01772771) and Mayo Clinic (15-000563).

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Supplementary

Table S1 Codons tested on AmpliSeq 46-gene (CMS46) and 50-gene (CMS50) assays

Gene	Codons tested in CMS46	Codons tested in CMS50
ABL1	237–260, 275–283, 303–319, 350–362, 387–412	232–260, 275–279, 314–360, 380–412
AKT1	16–59	16–52, 154–183
ALK	1172–1177, 1259–1277	1172–1204, 1270–1279
APC	865–886, 1105–1122, 1289–1322, 1349–1382, 1430–1467, 1487–1509	860–891, 1089–1125, 1284–1326, 1342–1384, 1426–1471, 1483–1524, 1543–1582
ATM	343–355, 395–412, 601–614, 837–862, 1307–1324, 1674–1693, 1733–1758, 1785–1802, 1935–1957, 2436–2445, 2650–2667, 2693–2715, 2721–2739, 2888–2891, 2937–2950, 2996–3016, 3037–3052	326–355, 407–412, 601–626, 834–865, 1292–1325, 1674–1707, 1726–1757, 1790–1815, 1926–1946, 2436–2454, 2650–2667, 2682–2711, 2718–2736, 2865–2891, 2933–2950, 2996–3026, 3041–3057
BRAF	439–471, 581–605	439–473, 581–611
CDH1	69–92, 351–373, 395–415	65–96, 337–374, 380–408
CDKN2A	51–76	51–90, 98–140
CSF1R	299–318, 952–973	297–319, 953–973
CTNNB1	12–45	9–48
EGFR	89–125, 280–297, 575–601, 698–722, 729–761, 766–790, 803–823, 830–866	96–123, 279–297, 575–601, 695–726, 729–796, 807–823, 855–875
ERBB2	753–769, 772–797, 832–852, 875–883	752–797, 839–882
ERBB4	136–141, 177–186, 234–247, 272–289, 303–322, 343–363, 588–619, 923–943	136–141, 167–186, 225–247, 254–290, 295–323, 333–367, 580–623, 919–948
EZH2	–	625–649
FBXW7	264–279, 381–400, 450–472, 478–506, 566–583	264–287, 378–403, 434–473, 478–509, 567–594
FGFR1	121–139, 247–268	120–148, 247–273
FGFR2	250–268, 297–313, 367–395, 546–558	250–275, 296–313, 362–399, 546–558
FGFR3	247–268, 377–409, 634–653, 681–712, 790–807	247–277, 367–402, 631–653, 690–719, 771–807
FLT3	441–458, 569–575, 589–613, 662–682, 828–846	437–466, 570–610, 663–685, 828–847
GNA11	–	202–219
GNAQ	–	206–245
GNAS	196–218	196–240
HNF1A	198–217, 253–282	192–221, 253–282
HRAS	5–23, 48–79	5–35, 42–82
IDH1	118–134	101–135
IDH2	–	133–177
JAK2	604–622	603–622
JAK3	568–578, 709–729	128–140, 568–580, 709–733
KDR	240–258, 267–280, 472–490, 872–892, 959–985, 1138–1161, 1192–1216, 1301–1321, 1336–1356	244–291, 471–480, 872–894, 961–988, 1135–1156, 1192–1221, 1283–1310, 1324–1357
KIT	47–69, 501–514, 536–549, 550–585, 641–684, 714–728, 807–828, 836–854	23–58, 494–514, 525–587, 627–661, 664–684, 714–724, 802–828, 832–858
KRAS	5–28, 40–67, 136–150	5–66, 114–150
MET	160–187, 362–379, 992–1017, 1105–1126, 1247–1268	159–188, 339–378, 816–856, 981–1012, 1105–1132, 1246–1274
MLH1	373–393	373–415
MPL	499–522	501–522
NOTCH1	1566–1605, 1673–1697	1566–1602, 1673–1680, 2536–2476
NPM1	283–295	283–295
NRAS	6–22, 53–69	3–31, 43–69, 124–150
PDGFRA	552–570, 647–688, 819–847	552–583, 644–668, 671–709, 819–854
PIK3CA	77–98, 328–351, 418–422, 533–551, 688–716, 1019–1049, 1065–1069	54–90, 106–118, 316–351, 390–422, 449–468, 522–549, 677–720, 898–924, 1017–1051, 1065–1069
PTEN	5–24, 55–70, 167–184, 212–222, 240–266, 282–300, 316–342	1–25, 55–70, 99–135, 165–184, 212–215, 231–267, 282–300, 312–342
PTPN11	53–82, 486–506	46–82, 485–527
RB1	132–154, 195–203, 350–371, 549–565, 566–585, 655–680, 703–724, 743–765	130–159, 196–203, 314–345, 350–366, 452–463, 547–582, 655–691, 703–724, 743–770
RET	609–627, 630–654, 762–774, 880–901, 914–931	608–654, 762–786, 875–924
SMAD4	109–128, 167–184, 228–247, 304–319, 330–363, 385–404, 444–472, 497–526	98–136, 142–146, 165–202, 242–263, 307–319, 326–365, 384–424, 443–474, 494–532
SMARCB1	39–55, 154–167, 182–203, 376–386	35–72, 144–206, 373–386
SMO	186–218, 310–340, 399–418, 516–542, 626–646	186–228, 307–331, 391–419, 511–542, 608–646
SRC	514–534	499–533
STK11	30–62, 174–199, 253–281, 325–360	22–64, 155–181, 191–207, 253–285, 317–361
TP53	1–18, 81–114, 126–135, 149–181, 187–223, 230–253, 269–306, 332–344	1–20, 68–113, 126–138, 149–223, 225–258, 263–307, 332–367
VHL	88–110, 120–149, 147–175	78–108, 114–150, 155–174