Original Article

Integrated molecular profiling of *RAS*, *BRAF* mutations, and mismatch repair status in advanced colorectal carcinoma: insights from gender and tumor laterality

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Background: Colorectal carcinoma (CRC) is one of the most frequently diagnosed forms of cancer worldwide. The *RAS* (*KRAS*, *NRAS*) and *BRAF* genes encode proteins that are important therapeutic targets for the treatment of CRC and, together with the mismatch repair (MMR) system, are closely related to patient prognosis and survival in advanced CRC. Here we evaluate the mutational profile and the frequency of mutations in the *KRAS*, *NRAS* and *BRAF* genes, along with the expression of MMR in advanced CRC, at a tertiary hospital in southern Brazil.

Methods: A cross-sectional retrospective study was carried out, where molecular analysis of mutations in the *KRAS*, *NRAS* and *BRAF* genes was carried out, as well as immunohistochemistry for MMR proteins.

Results: Next-generation sequencing (NGS) analysis of 310 tumors revealed that 202 patients (65.2%) had mutations. The *KRAS* gene (53.2%) was the most frequently mutated in our sample, with G12D being the most frequent, representing 30.5% of the mutations in this gene. The most frequent mutation found in *BRAF* was V600E (n=25; 89.3%) and differed significantly in women and in the right colon in patients with MMR deficiency. Among the 283 patients tested for MMR, the rate of loss of expression was 8.8% (25/283).

Conclusions: Deficiency in the MMR system is associated with the presence of the *BRAF* V600E mutation, tumors located in the right colon, and the female sex. In our case series, more than 60% of patients had at least one mutation in *KRAS*, *NRAS*, or *BRAF*. The presence of mutations in these genes is closely related to CRC prognosis and helps define the best therapeutic approach in patients with metastatic CRC.

Keywords: Colorectal carcinoma (CRC); *KRAS*; *NRAS*; *BRAF*; mismatch repair (MMR)

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Introduction

Colorectal carcinoma (CRC) is one of the most frequently diagnosed forms of cancer worldwide (1). Excluding non-melanoma skin tumors, colon and rectal cancer ranks third among the most common cancer types (1). According to the National Cancer Institute (INCA), in Brazil, the CRC is the third most prevalent type. In the southern region, it is the second most common type of cancer in women and the third most common in men (2). CRC is observed more frequently in the left colon than in the right colon. Based on gene expression data, CRC has been divided into four consensus molecular subtypes (CMS): CMS1 (microsatellite instability), CMS2 (canonical epithelial), CMS3 (metabolic), and CMS4 (mesenchymal)—each subtype reflects significant biological differences (3). The most frequent clinically actionable types of CRC currently belong to CMS type 1 [microsatellite instability (MSI), *BRAF* mutations] and type 3 (*KRAS* mutations, mixed MSI status) (3,4).

Tumors arising in the left colon and right colon differ not only in incidence, but also in their biology and histology, which consequently influences patient

prognosis (5). Another major challenge is the clinical management of metastatic CRC (mCRC); more recently, combined therapies have shown benefits for specific subgroups (6), and several drugs have been approved for the treatment of this disease. However, to effectively benefit patients' lives, the optimal combination and sequence of these drugs likely depend on many factors, including the mutational status of tumor cells (7).

The analysis of the mutational status of *RAS* and *BRAF* genes is becoming increasingly relevant in CRC treatment, especially for determining the course of treatment in patients with metastatic CRC. Patients with *KRAS* mutations also show a low response to the epidermal growth factor receptor (EGFR) inhibitors (8), and the presence of a *BRAF* gene mutation is an indicator of a worse prognosis (9). The *RAS* (*KRAS*, *NRAS*) and *BRAF* genes encode proteins that play a crucial role in the treatment of CRC and are closely linked to the outcome and longevity of patients (10-13). The constitutive activation of the RAS-RAF-MEK-ERK (MAPK) pathway plays a critical role in the development and progression of CRC (14). Monoclonal antibodies against EGFR, such as cetuximab and panitumumab, have been shown to bind to the extracellular domain and block the signaling of this pathway (15).

The mutational status of *RAS* genes (*KRAS* and *NRAS*) is a predictive marker for therapeutic decisions in therapies targeting EGFR in metastatic CRC (15). The *KRAS* G12C mutation (c.34G>T in exon 2), which represents the substitution of a glycine for a cysteine in codon 12, occurs in around 3–4% of CRC (16). The KRAS G12C mutant has been identified as a potential target for novel therapies (17). First selective KRAS G12C inhibitors to succeed in clinical trials were sotorasib and adagrasib, which are potent and irreversible inhibitors of the mutant KRAS G12C isoform, available orally, for the treatment of solid tumors with the oncogenic *KRAS* G12C mutation, including non-small cell lung cancer and colorectal cancer (18,19).

Mutations in the key protein BRAF in the MAPK pathway result in the constitutive activation of this pathway, which suggests that BRAF mutation plays a crucial role in CRC (14). The V600E mutation, which is predominant in the *BRAF* gene, results from an activating mutation, with the substitution of valine for glutamic acid at amino acid 600 (20). *BRAF* mutations occur in about 8% of patients with advanced CRC and in 14% of patients with localized CRC, stages II or III (21). Previous studies substantiate the fact that the combined MSI/*BRAF* test plays a prognostic role in colorectal cancer (21,22).

Highlight box

Key findings

- G12D was the most frequent mutation found in the *KRAS* gene and the deficient mismatch repair (MMR) system system was associated with the presence of the *BRAF* V600E mutation and absence of the *KRAS* mutation. Lung metastasis did not present the V600E mutation.

What is known and what is new?

- The *RAS* and *BRAF* genes encode proteins that are important therapeutic targets for the treatment of colorectal carcinoma (CRC) and, together with the MMR system are closely related to patient prognosis and survival in advanced CRC.
- The deficiency in the MMR system was associated with the presence of the *BRAF* V600E mutation, absence of the *KRAS* mutation, tumors located in the right colon, and the female sex. The lung metastasis did not have the V600E mutation and only had a mutation in exon 2 of the *KRAS* gene.

What is the implication, and what should change now?

- This study provided results that can contribute to the clinical diagnosis, establish the prognosis, and improve the treatment of patients with advanced CRC. We emphasize the relevance of the investigation into the KRAS G12C mutation, the result of which opens up another alternative for the treatment of patients with a mutation in the *KRAS* gene, in this pioneering study in the far south of Brazil.

Another well-known biomarker is MSI, which is present in tumors with deficient mismatch repair (dMMR) systems. Mismatched bases that arise during DNA replication, recombination, or chemical/physical damage are identified and repaired by proteins of the MMR system, which is a highly conserved cellular process (21). However, a deficient MMR system produces a MSI phenotype. The MSI pathway is widely recognized as an important carcinogenic pathway in CRC, representing the molecular signature of Lynch Syndrome, which is often linked to a germline mutation in the MMR genes and 15% of sporadic CRC, most often due to the epigenetic inactivation of *MLH1* (23). The V600E mutational analysis should be performed in dMMR tumors with loss of MLH1 to assess Lynch syndrome risk. The presence of a *BRAF* mutation is strongly associated with sporadic pathogenesis. Risk of Lynch syndrome is not excluded by the absence of the *BRAF* mutation (24).

The increasing number of molecular markers in CRC, the development of immunotherapy, and the approval of agnostic treatments by regulatory agencies, along with the identification of markers with prognostic and predictive value, currently play an important role in CRC treatment (25). Therefore, in the present context, it is important to understand the epidemiology of CRC in each population in order to better plan access to new therapeutic possibilities (26,27). The purpose of this study is to assess the mutational profile and the frequency of mutations in *KRAS*, *NRAS*, and *BRAF*, along with the expression of MMR in advanced CRC, at a tertiary hospital in southern Brazil. We present this article in accordance with the STROBE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-1017/rc).

Methods

Study population and sample

This retrospective study used data from a series of cases of stage III or IV CRC in patients treated at the Hospital de Clínicas de Porto Alegre (HCPA). Patients included in the study consented to the use of their samples, which were obtained from the Surgical Pathology Service and subjected to molecular analyses by the Personalized Medicine Program of HCPA from 2018 to 2022. Tumor samples from 310 patients were included in this study. Clinical data were obtained from a review of patient medical records. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by

the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, under CAAE (Certificate of Presentation for Ethical Consideration) number 56230122200005327.

Tumor selection and DNA extraction

The molecular analysis of mutations in the *KRAS*, *NRAS*, and *BRAF* genes was performed on samples from 310 patients. The paraffin block with the best representation of the tumor was selected from the corresponding H&E (hematoxylin and eosin) slide and cut on a microtome regulated to a thickness of 10 µm. Following the manufacturer's recommendations, DNA was extracted from the samples using the ReliaPrep FFPE gDNA Miniprep System kit (Promega, Madison, WI, USA). The fluorescence method was used to quantify DNA samples after extraction (Qubit 2.0 Fluorometer, Invitrogen, Carlsbad, CA, USA).

Molecular analysis by next-generation sequencing (NGS)

NGS was used for the molecular analysis of the *KRAS*, *NRAS*, and *BRAF* genes, with the Ion Torrent™ Ion GeneStudio™ S5 System, server version 5.0 (Thermo Fisher Scientific, Waltham, MA, USA), using a customized panel for the identification of mutations in *KRAS* (exons 2, 3, and 4), *NRAS* (exons 2, 3, and 4), and *BRAF* (exon 15) (13,24,28). Data were analyzed using the Ion Torrent Suite and Ion Reporter bioinformatics platform, version 5.0, considering a minimum coverage of 800×. The NM_0033360.3 (*KRAS*), MM_002524.3 (*NRAS*), and NM_004333.4 (*BRAF*) sequences were used as references. The tests were conducted using research use reagents with internal validation. The limit of detection (LOD) for variant allele frequency (VAF) was 2% VAF.

For NGS analysis, primary and secondary analyses were performed with the Ion Torrent™ Ion GeneStudio™ S5 System, server version 5.12.3. The Torrent Mapping Alignment Program was used to map the human reference genome hg19. Initial quality control and evaluation of the coverage of the amplification product for the regions of interest were carried out using the Torrent CoverageAnalysis plugin implemented in version 5.12.3 of the Torrent Suite software (Thermo Fisher Scientific, Waltham, MA, USA). After filtering the uniformity (>85%), the readings on the target (>60%) and the minimum mapped readings of 25,000, the regions of interest were obtained. Ion Reporter version 5.12 (Thermo Fisher

Table 1 Clinical characteristic of patients

Clinical data	n	%
Sex		
Male	153	49.4
Female	157	50.6
Site		
Right colon	68	21.9
Left colon	145	46.8
Rectum	89	28.7
Not specified	8	2.6
Age at diagnosis		
<60 years	147	47.4
≥60 years	163	52.6
Metastasis		
Liver	86	27.7
Nodes	51	16.5
Liver + lung	34	11.0
Lung	30	9.7
Peritoneum	22	7.1
Other sites	87	28.0

Scientific, Waltham, MA, USA) was used to identify variants, with the following somatic parameters: minimum variant quality of 10, minimum coverage of 100, maximum chain polarization of 0.95 and minimum variant score of 6.

Mismatch repair (MMR) protein analysis

The preparation of slides for the immunohistochemical analysis of the MMR system proteins MLH1, PMS2, MSH2, and MSH6 was performed on tumor samples from 283 patients using an automated platform (Benchmark ULTRA Ventana Medical Systems Inc., Tucson, Arizona, USA). The paraffin-embedded block, which contained tumor tissue and, when available, tissue devoid of morphological alterations serving as an internal control, was chosen based on the corresponding H&E stained slide. It was then sectioned using a microtome adjusted to a thickness of 3 μm. This selection was not necessarily restricted to the same block designated for NGS. The following antibodies and detection kit were used: MLH1 clone M1 Roche™ USA, PMS2 clone A16-4 Roche™ USA,

MSH2 clone G219-1129 Roche™ USA, MSH6 clone SP93 Roche™ USA, all in ready-to-use format, and the Optiview Roche™ USA reagent kit. All slides were examined under an optical microscope and contained a positive control for each antibody. Internal sample control was also evaluated. Markers were assessed for positivity in the tumor area, and samples with brown-stained nuclei were considered positive. When all four proteins were positive, the tumor was considered pMMR (proficient MMR), and when the expression was negative in at least one of the proteins, the tumor was considered dMMR (deficient MMR).

Statistical analysis

The prevalence of mutations was assessed using absolute and relative frequencies, with a confidence interval of 95%. Statistical analyses were conducted using the Statistical Package for Social Science for Windows (SPSS) version 29. To investigate the association of the molecular profile with sex, age, and tumor location, researchers performed the χ^2 test or Fisher's exact test. Results were considered statistically significant when $P<0.05$.

Results

Clinical characteristics of patients

This study included 310 patients (157 women and 153 men). The mean age at diagnosis of these patients was 60 years (range, 18–84 years). The tumor was located in the right colon in 68 cases, in the left colon in 145 cases, and in the rectum in 89 cases. In eight cases, the tumor location was not specified. Eighty-six patients (27.7%) had liver metastasis, 51 (16.5%) had lymph node metastasis, and 34 (11%) had concomitant liver and lung metastasis, while other patients had metastasis at different sites (*Table 1*).

Relationship between MMR protein expression and clinical characteristics

From 310 patients included in this study, 283 were tested for MMR. The remaining patients did not have a sufficient sample. The rate of loss of expression was 8.8% (25/283), and the frequency of loss of expression for each of the four MMR proteins (MLH1, PMS2, MSH2, MSH6) was 6.36% (18/283), 6.36% (18/283), 2.12% (6/283), and 2.47% (7/283), respectively. The rate of loss of expression of MLH1 and PMS2 was significantly higher than that of MSH2 and

Table 2 Relationship between MMR expression and clinical characteristics

| Clinical data | Total | MMR | | | | χ^2 | P |
| | | dMMR | | pMMR | | | |
		n	%	n	%		
Sex						3.864	0.049
Male	138	7	5.1	131	94.9		
Female	145	18	12.4	127	87.6		
Site						36.411	<0.001
Right colon	67	19	28.4	48	71.6		
Left colon	128	6	4.7	122	95.3		
Rectum	83	0	0.0	83	100.0		
Not specified	5	0	0.0	5	100.0		
Age at diagnosis						0.000	>0.99
<60 years	137	12	8.8	125	91.2		
≥60 years	146	13	8.9	133	91.1		

MMR, mismatch repair; dMMR, deficient MMR; pMMR, proficient MMR.
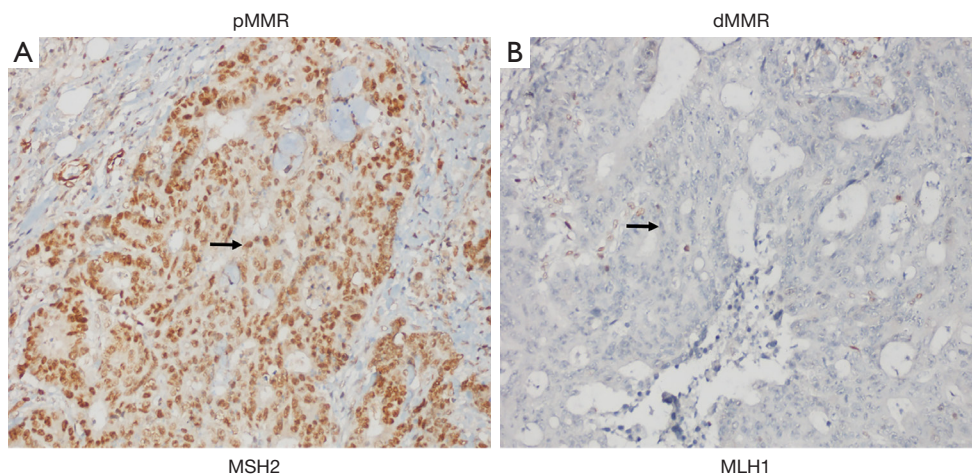
Figure 1 Mismatch repair immunohistochemistry showing typical patterns of (A) intact MSH2 and (B) loss of MLH1. Original magnification 200×. The arrows indicate positively stained cells (left) and negatively stained cells (right). pMMR, proficient mismatch repair; dMMR, deficient mismatch repair.

MSH6 (P<0.001). Patients with different ages at diagnosis did not significantly differ in terms of MMR expression loss (P>0.05) using the cut-off point of 60 years old. However, a significant difference in expression loss was observed in women (P=0.049) and in tumors located in the right colon (P<0.001) (*Table 2*). *Figure 1* represents the results of immunohistochemistry for pMMR and dMMR, for the Mismatch Repair System proteins MSH2 and MLH1.

Relationship between mutations in KRAS, NRAS, BRAF, and clinical characteristics

NGS analyses conducted on 310 tumors revealed the presence of mutations in 202 patients (65.2%). The
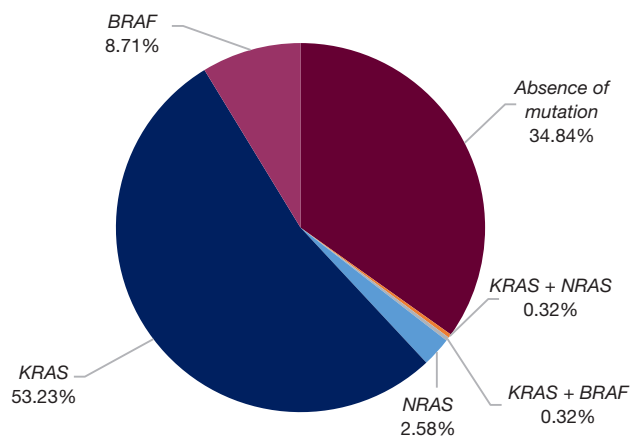
Figure 2 Frequency of somatic mutations in *KRAS*, *BRAF*, and *NRAS* in advanced colorectal tumors at the Hospital de Clínicas de Porto Alegre.

mutational profile in this sample showed that 167 patients had mutations in *KRAS* (53.23%), 27 had mutations in *BRAF* (8.71%), eight had mutations in *NRAS* (2.58%), one had concomitant mutations in *KRAS* and *NRAS* (0.32%), and one had mutations both in *KRAS* and *BRAF* (0.32%). In 108 patients (34.84%), no mutations were detected with the panel used (*Figure 2*).

The frequency of mutations in *KRAS* was 7% higher in women than in men; however, this difference was not statistically significant (P=0.26). Most patients who had no mutations detected by the panel were men, but this difference between sex was also not statistically significant (P=0.09) (*Table 3*). Among the mutations in *KRAS*, G12D was the most common, accounting for 30.5% of the mutations found in this gene, followed by G12V (n=36; 21.6%), G13D (n=25; 15%), and G12C (n=11; 6.6%), all in

Table 3 Relationship between mutational profile in *KRAS*, *NRAS*, *BRAF* genes, and clinical characteristics

Clinical data	Total	KRAS					NRAS					BRAF V600E				
		Wild		Mutated		P	Wild		Mutated		P	Not mutated		Mutated		P
		n	%	n	%		n	%	n	%		n	%	n	%	
Sex						0.26					0.10					0.01
Male	153	76	49.7	77	50.3		146	95.4	7	4.6		147	96.1	6	3.9	
Female	157	67	42.7	90	57.3		155	98.7	2	1.3		138	87.9	19	12.1	
Site						0.97					0.70					<0.001
Right colon	68	31	45.6	37	54.4		67	98.5	1	1.5		47	69.1	21	30.9	
Left colon	145	67	46.2	78	53.8		141	97.2	4	2.8		141	97.2	4	2.8	
Rectum	89	42	47.2	47	52.8		85	95.5	4	4.5		89	100.0	0	0.0	
Not specified	8	3	37.5	5	62.5		8	100.0	0	0.0		8	100.0	0	0.0	
Age at diagnosis						0.40					>0.99					0.07
<60 years	147	72	49.0	75	51.0		143	97.3	4	2.7		140	95.2	7	4.8	
≥60 years	163	71	43.6	92	56.4		158	96.9	5	3.1		145	89.0	18	11.0	
Metastasis						0.045					0.77					0.005
Liver	86	42	48.8	44	51.2		84	97.7	2	2.3		83	96.5	3	3.5	
Nodes	51	32	62.7	19	37.3		50	98.0	1	2.0		42	82.4	9	17.6	
Liver + lung	34	15	44.1	19	55.9		32	94.1	2	5.9		32	94.1	2	5.9	
Lung	30	8	26.7	22	73.3		29	96.7	1	3.3		30	100.0	0	0.0	
Peritoneum	22	10	45.4	12	54.6		21	95.5	1	4.5		17	77.3	5	22.7	
Other sites	87	36	41.4	51	58.6		85	97.7	2	2.3		78	89.7	9	10.3	

Table 4 Frequency of different mutations in *KRAS*, *NRAS*, and *BRAF* genes

Gene name	Mutation	No. of patients	Frequency (%)
KRAS			
Exon 2		144	86.3
	G12D	51	30.5
	G12V	36	21.6
	G13D	25	15.0
	G12C	11	6.6
	G12A	9	5.4
	G12S	5	3.0
	G13C	2	1.2
	G12E	1	0.6
	G12R	1	0.6
	G13V	1	0.6
	G12V + G12S	1	0.6
	dupG13	1	0.6
Exon 3		13	7.8
	Q61H	9	5.4
	Q61R	2	1.2
	Q61L	1	0.6
	S65N	1	0.6
Exon 4		10	6.0
	A146T	5	3.0
	K117N	3	1.8
	A146V	2	1.2
Total		167	100
NRAS			
Exon 2		3	33.3
	G12D	2	22.2
	G12S	1	11.1
Exon 3		6	66.6
	Q61K	2	22.2
	Q61L	3	33.3
	Q61R	1	11.1
Total		9	100
BRAF			
Exon 15			
	V600E	25	89.3
	D594G	1	3.6
	G596V	1	3.6
	N581S	1	3.6
Total		28	100

exon 2 of the *KRAS* gene (*Table 4*). Eight other mutations were found in exon 2. In exon 3, four different mutations were detected, and in exon 4, three mutations were found. The lung metastasis only had mutations in exon 2 of the *KRAS* gene, while the liver metastasis had mutations in exons 2, 3 and 4 (*Table 5*).

Only nine patients (seven men and two women) had mutations in the *NRAS* gene. A total of 28 patients had mutations in the *BRAF* gene. The most frequently found mutation in *BRAF* was V600E (n=25; 89.3%), but three patients had mutations D594G, G596V, and N581S (*Table 4*). Of the 30 CRC that metastasized to the lung, none had the *BRAF* V600E mutation (P=0.005). The *BRAF* V600E mutation also showed a significant difference by sex: it was more common in women (P=0.01) and also more prevalent in the right colon (P<0.001). *BRAF* V600E was more frequent in patients aged 60 or over; however, this difference was not statistically significant (P=0.07) (*Table 3*).

Association between MMR protein expression and mutations in the KRAS, NRAS, and BRAF genes

In this study, we found statistically significant differences when investigating the association between MMR expression loss and mutations in the *KRAS* and *BRAF* genes. When there was MMR expression loss (dMMR), the frequency of *KRAS* mutations was significantly lower than when there was no MMR expression loss (pMMR) (P<0.001). In contrast, the frequency of the *BRAF* V600E mutation was significantly higher in dMMR MLH1/PMS2 than in pMMR. There was no significant difference between dMMR and the *NRAS* gene (*Table 6*).

Discussion

In this study, we evaluated the mutational profile and the frequency of mutations in the *KRAS*, *NRAS*, and *BRAF* genes, along with the expression of MMR system proteins in advanced CRC, in patients from a tertiary hospital in southern Brazil, correlating these findings with each other.

Data from the literature demonstrate the importance of performing a molecular analysis of tumors in patients with metastatic CRC before initiating treatment, as this leads to improved overall survival and progression-free survival in patients with wild-type *KRAS* treated with anti-EGFR therapy (13-15). Other studies have extended the analysis to include testing for mutations in other genes, such as *NRAS* and *BRAF*, which are predictors of treatment failure with

Table 5 Frequency of *KRAS* mutations by metastasis site

Metastasis	Most frequent *KRAS* mutations							
	G12D	G13D	G12V	G12C	Q61H	A146T	G12A	G12S
Liver	13	7	13	2	1	2	0	3
Lung	4	4	4	3	0	0	2	1
Peritoneum	3	1	5	0	2	0	0	0
Nodes	6	5	3	1	1	1	1	0
Liver + lung	8	3	2	0	2	1	1	1
Other sites	17	5	9	5	3	1	5	0

Table 6 Relationship between MMR proteins and *KRAS*, *NRAS*, and *BRAF* genes

Gene name	Total	MMR					P
		dMMR			pMMR		
		MLH1/PMS2 (n)	MSH2/MSH6 (n)	%	n	%	
KRAS							<0.001
Wild	135	17	2	14.1	116	85.9	
Mutant	148	1	5	4.1	142	95.9	
NRAS							0.19
Wild	275	18	6	8.7	251	91.3	
Mutant	8	0	1	12.5	7	87.5	
BRAF V600E							<0.001
Not mutant	259	7	7	5.4	245	94.6	
Mutant	24	11	0	45.8	13	54.2	

MMR, mismatch repair; dMMR, deficient MMR; pMMR, proficient MMR.

EGFR inhibitors (12,15,29,30). In our case series, 34.8% of patients did not have mutations in the studied genes, which indicates that these patients would be eligible for treatment with EGFR inhibitors. A portion of the studied population could benefit from this targeted therapy, which suggests that this type of testing is justified for potential use in treatment decisions.

In CRC, the prevalence of mutation rates in the *KRAS*, *NRAS*, and *BRAF* genes has been reported to range between 15–60%, 2–15%, and 3–10%, respectively (12,31-34). In Brazil, the study by Gil Ferreira *et al.*, which analyzed the frequency of mutations in exon 2 of the *KRAS* gene in metastatic CRC in the Brazilian population, found that the mutation rate in *KRAS* was 31.9% (35). In the southern region of Brazil, the same study showed that the KRAS mutation rate was 32% in metastatic CRC (35).

In southeastern Brazil, a study published by Dos Santos *et al.* showed that the rates of mutation in *KRAS*, *NRAS*, and *BRAF* were 52.7%, 4.4%, and 8.8%, respectively (36). Also in the southeast, Ribeiro *et al.* found a KRAS mutation rate of 49.2% (37). In the present study, higher frequencies, compared to Gil Ferreira *et al.* study of mutations in *KRAS* (52.3%) were found, and the reason for this finding could be the fact that we also analyzed exons 3 and 4 (35). We are not aware of any other study in the southern Brazilian population that has evaluated *KRAS*, *NRAS* and *BRAF* mutations by NGS and associated them with MMR expression and clinical data. In contrast to *KRAS* findings, lower frequencies of *NRAS* mutations (2.6%) were observed. Among *KRAS* mutations, G12D (Gly12Asp) was the most frequent, which is also in line with previous studies (33,34,37-39). The frequency of the G12C (Gly12Cys)

mutation was 6.6% (11/167), which is especially interesting given the use of the drugs adagrasib and sotorasib which specifically target this mutation, opening up another alternative for treating patients with a mutation in the *KRAS* gene (40-43).

The frequency of *BRAF* mutations (8.7%) we found is in line with the data from Dos Santos *et al.*, as well as the fact that the *BRAF* V600E mutation was the most common (89.3%) (36). In our study, the *BRAF* V600E mutation showed a significant difference by sex, being predominant in women, and by tumor location, occurring more commonly in the right colon, a fact already reported in the literature (44,45). Among metastasis, *BRAF* V600E was more frequent in CRCs that metastasized to the lymph nodes, while this mutation was not observed in exclusive lung metastasis. A previous study indicated that colorectal tumors located in different sites have completely different therapeutic results and specific biomolecular characteristics (44). The knowledge about the different rates of *BRAF* V600E mutation in distinct tumor sites can be useful in the development of treatment therapies for CRC located in different tumor sites (14). In CRC, the presence of the *BRAF* mutation is associated with lower survival time and resistance to standard therapeutic approaches (46). CRC with a *BRAF* mutation is an aggressive subpopulation of metastatic CRC (47). The therapeutic approach to CRC when there are mutations in the *BRAF* gene is challenging due to resistance, and this treatment does not achieve the same success as that of *BRAF* inhibitors that revolutionized the treatment of *BRAF* V600E mutated metastatic melanomas. In part, this can be explained by the fact that metastatic CRC is as a more complex disease compared to melanoma. The use of regimens combining targeted therapy and chemotherapy is the most suitable strategy to overcome resistance (48). Some guidelines recommend targeted therapy for patients with metastatic CRC and *BRAF* mutations. This subgroup seems to benefit from anti-VEGF therapies, although the available data are still limited and inconclusive (49,50).

Regarding the expression of MMR proteins, we observed that the loss of MLH1 and PMS2 was significantly higher than that of MSH2 and MSH6, which is in line with the literature (4). The dMMR status was more common in women (12.4%) than in men (5.1%) (P=0.049). Patients with tumors located in the right colon were found to be more likely to have dMMR and the *BRAF* V600E mutation than patients with tumors in the left colon and rectum. These results are consistent with those of previous studies

(31,36). A meta-analysis (14) demonstrated an association between the *BRAF* V600E mutation and high microsatellite instability, corroborating the findings of this study. When evaluating the group of patients with dMMR, it was observed that most of these patients did not have mutations in the *KRAS* gene. During the *BRAF* analysis, we found that the *BRAF* V600E mutation was significantly more common in patients with dMMR. This mutation is quite common in these tumors and has prognostic value, being associated with worse survival (51). The site of origin of the tumor is considered an independent prognostic factor that affects treatment response. In this sense, tumors differ in various aspects, including histology and mutational profile (52). Studies have shown that CRC located in the right colon is more common in women, whereas tumors located in the left side are more common in men (45,53-56). Other studies have shown that overall survival is higher in patients with stage I, III, and IV CRC located in the left side than in those affected by this disease in the right side (57-60). Right-sided tumors carry many adverse characteristics, including MSI and a higher rate of *BRAF* V600E mutations (52,53,56), and are associated with worse clinical outcomes in patients with metastatic CRC (60,61).

Despite our findings, this study has some limitations; the main limitation of this study is that we do not have data on the clinical treatment, prognosis, and survival of these patients, and therefore we cannot explain the association of the study findings with the performance of treatment in patients. A methodological limitation is that only the exons recommended for defining the prognosis and treatment of the disease according to the National Comprehensive Cancer Network and other guidelines were sequenced. This approach did not allow us to observe other rare or as yet unreported alterations.

Conclusions

This study analyzed the frequency of mutations in the *KRAS*, *NRAS*, and *BRAF* genes, as well as the loss of expression in the MMR system. We found that deficiency in the MMR system is associated with the presence of the *BRAF* V600E mutation, tumors located in the right colon, and the female sex. In our case series, more than 60% of patients had at least one mutation in *KRAS*, *NRAS*, or *BRAF*. The presence of mutations in these genes is closely related to CRC prognosis and helps define the best therapeutic approach in patients with metastatic CRC.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-1017/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-1017/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-1017/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, under CAAE (Certificate of Presentation for Ethical Consideration) number 56230122200005327. Patients included in the study consented to the use of their samples.

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