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Prevalence of the *BRAF* p.v600e variant in patients with colorectal cancer from Mexico and its estimated frequency in Latin American and Caribbean populations

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ABSTRACT

This study aimed to investigate the frequency of the somatic *BRAF* p.V600E in patients with colorectal cancer (CRC) in Mexico and compare it with those estimated for Latin American and Caribbean populations. One hundred and one patients with CRC with AJCC stages ranging I–IV from Western Mexico were included, out of which 55% were male and 61% had AJCC stage III–IV, with a mean age of 60 years. PCR-Sanger sequencing was used to identify the BRAF p.V600E variant. In addition, a systematic literature search in PubMed/Medline database and Google of the 42 countries in Latin America and the Caribbean led to the collection of information on the *BRAF* p.V600E variant frequency of 17 population reports. To compare the *BRAF* variant prevalence among populations, a statistical analysis was performed using GraphPad Prism V.6.0. We found that 4% of patients with CRC were heterozygous for the p.V600E variant. The χ^2 test showed no significant difference ($p>0.05$) in p.V600E detection when comparing with other Latin American and Caribbean CRC populations, except for Chilean patients ($p=0.02$). Our observational study provides the first evidence on the frequency of *BRAF* p.V600E in patients with CRC from Western Mexico, which is 4%, but increases to 7.8% for all of Latin America and the Caribbean. The patient mean age and genetic descent on the observed frequencies of the variant in populations could influence the frequency differences.

Significance of this study

What is already known about this subject?
- The *BRAF* p.V600E is an activating variant associated with several cancer types.
- The reported prevalence of this pathogenic variant is 2.5%–20% in colorectal cancer.

What are the new findings?
- The *BRAF* p.V600E variant frequency in patients with colorectal cancer is 4% for Western Mexican, and 7.8% for Latin American and Caribbean populations.
- Variations in patient mean age and genetic characteristics in Latin American and Caribbean populations could underlie the significant differences in *BRAF* p.V600E variant frequency.

How might these results change the focus of research or clinical practice?
- Determination of the *BRAF* p.V600E variant frequency in patients with colorectal cancer from Latin America and the Caribbean improves our understanding of the molecular characteristics of colorectal cancer, within this region, and provides an estimate of the need to increase molecular studies to support the treatment of the patient.

INTRODUCTION

Colorectal cancer (CRC) is the third most common neoplasia worldwide. In Latin America and the Caribbean, accounting for Brazil, Argentina and Mexico, CRC is also the third most prevalent cancer among patients aged more than 50 years. Bray *et al*[1] estimated that the CRC incidence in Latin America and the Caribbean will increase by 44.6% by 2030, with almost 177,000 new cases and more than 94,000 deaths.

To provide precision medicine for CRC treatment, the molecular variation present in tumors must be defined. Among the genetic changes encountered in CRC is the p.V600E pathogenic variant of the *BRAF* gene, which encodes a serine/threonine kinase involved in the EGFR–MAPK signaling pathway. This variant is a vital

feature of this cancer as it causes constitutive activation of the protein, resulting in inhibition of apoptosis and uncontrolled cell proliferation.[2]

The *BRAF* p.V600E variant (c.1799T>A, rs113488022, chr7:140 753 336 position in GRCh38.p12) causes a substitution of valine with glutamic acid at codon 600 (GTG→GAG).[3] Besides the common allele with an A substitution, the occurrence of lower-frequency alleles with C and G substitutions make this pathogenic variant multiallelic.[4 5] This variant represents approximately 90% of all *BRAF* variants detected in CRC, with a prevalence of 2.5%–20% in this disease associated with a reduced survival of patients with metastases.[6] It is also often observed in other cancer types, such as 40%–60% of melanoma cases.[7]

With the advent of the monoclonal antibodies cetuximab and panitumumab, there has been improvement in the treatment of metastatic CRC, as these antibodies target the epidermal growth factor receptor (EGFR) overexpressed in CRC. However, it has been shown that pathogenic variants of the *BRAF* gene interfere with the treatment response; therefore, analyzing the occurrence of this variant in primary CRC tumors will benefit clinical treatment.[8] Our goal in this study was to determine the prevalence of *BRAF* p.V600E variant in patients with primary CRC from Western Mexico and compare the rate of occurrence with that in Latin American and Caribbean populations estimated based on a systematic review of a collection of studies. It might aid in improving treatment outcomes and encourage further research on the molecular traits of CRC within the area.

MATERIALS AND METHODS

Patients and tissue samples

Primary tumor specimens from 101 Western Mexican patients with sporadic CRC were collected following surgical resection between September 2010 and July 2017 at the Civil Hospital of Guadalajara "Dr. Juan I. Menchaca", Jalisco, Mexico. At the time of resection, none of the patients had undergone radiation or chemotherapy. Fresh tissue of approximately 25–50 mg was removed from each tumor; diagnoses of colonic or rectal adenocarcinomas were confirmed by histopathology.

BRAF p.V600E variant screening

Subsequent to tissue acquisition, genomic DNA extraction was carried out with the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany) followed by quantification (260/280 nm) using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The fragment of interest was amplified by PCR; the reaction volume was 25 µL, including 100 ng of DNA, 1.5 mM $MgCl_2$, 1× Taq buffer, 0.8 mM dNTPs, 0.08 U/µL Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 0.1 mM of each primer (forward 5′-TCATAATGCTTG CTCTGATAGGA-3" and reverse 5′-TCCACTGATTAA ATTTTTGGCC-3′). The amplified fragment length was 224 bp and the PCR conditions were initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C, annealing at 62°C, extension at 72°C, for 30 s per step, and finally elongation at 72°C for 10 min. To identify the p.V600E variant by Sanger sequencing, the BigDye Terminator V.3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the Applied Biosystems ABI prism 310 Genetic Analyzer were used. The sequencing reaction conditions were initial denaturation at 96°C for 4 min followed by 25 cycles of denaturation at 96°C for 10 s, annealing at 55°C for 5 s, extension at 60°C for 4 min, and finally elongation at 60°C for 7 min. To verify variant sequences, sequencing was duplicated. The reference *BRAF* gene sequence available on GenBank M95712.2 and Chromas software V.2.6.4 (Technelysium Pty Ltd, Australia) was used for interpreting sequencing results.

Determination of *BRAF* p.V600E variant prevalence in Latin American and Caribbean populations

A systematic search in the PubMed/Medline database using the keywords "frequency", "BRAF", "V600E", "colorectal" and "cancer" led to 197 publications; however, only six were related to any of the 42 countries in Latin America and the Caribbean listed in the Latin American Network Information Center at the University of Texas.[9] In addition, an exhaustive search on Google was performed, searching for each of the 42 countries by name, typed in Spanish and English, generating 769 entries that were reviewed individually. Only 17 publications related to sporadic CRC were identified. Among the selected publications, six reports had been previously identified on PubMed, 12 were articles, four were published abstracts with no complete population data but reported the *BRAF* p.V600E frequency, and one study was a postgraduate dissertation. From each study, we collected information on the *BRAF* p.V600E variant frequency, sex, mean and range of age of the study population, as well as pathological data, disease stage and tumor location.

Statistical analysis

The *BRAF* p.V600E variant frequency was estimated by quantification of the instances of its detection in Western Mexicans. A statistical analysis was performed using GraphPad Prism V.6.0 to compare the *BRAF* variant prevalence between West Mexican to Latin American and the Caribbean populations. Statistical significance was defined as p value <0.05.

RESULTS

Frequency of *BRAF* p.V600E variant in Western Mexican CRC population

Table 1 displays the characteristics of the 101 CRC Western Mexican patients recruited in this study. Four subjects were found to be heterozygous for the *BRAF* p.V600E variant (figure 1): the neoplasms were always located in the right colon of patients aged 62–76 years, three of which were female. One tumor was poorly differentiated (stage III) and the rest were graded as moderately differentiated (two staged IV and one staged II). No statistical comparison between clinicopathological features of patients with or without the variant could be performed due to the low variant frequency.

Frequency of *BRAF* p.V600E variant in Latin American and Caribbean CRC populations

Of the 42 populations selected, reports on *BRAF* p.V600E prevalence were only found for Argentina, Brazil, Chile, Mexico, Paraguay, Peru and Puerto Rico for a total of 17

Table 1 Clinical and pathological characteristics of patients with colorectal cancer (CRC)

CRC patient characteristics	n=101, %*
Age (years)	
Mean	60 (range 19–96)
≤50	24
>50	76
Gender	
Female	45
Male	55
Tumor localization	
Right colon	26
Left colon	24
Ubiquitous colon	12
Rectum	38
Pathological grade	
Well	3
Moderate	74
Poor	19
U	4
AJCC stage	
I and II	37
III and IV	61
U	2

*Except for mean age.
U, undetermined.

publications. This pathogenic variant occurred in 0%–15% of the populations and, taken together, these data indicate that the *BRAF* p.V600E frequency for Latin America and the Caribbean equals 7.8% (117 positive findings out of 1492 analyzed patients). Table 2 illustrates the characteristics of the analyzed patients with CRC and the comparison of variant frequency between populations. Based on the criteria of Yamane *et al*[10] and to facilitate analysis, tumor localization described in the studies is specified as right colon for tumors reported in the cecum, the ascending and the transverse colon, and as left colon for tumors located in the descending colon, sigmoid colon and rectum.

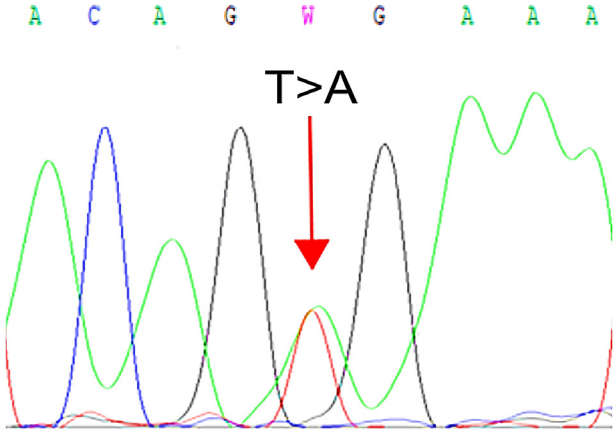

Figure 1 Partial electropherogram of DNA from a patient with colorectal cancer from Western Mexico showing heterozygosity for the *BRAF* p.V600E variant.

DISCUSSION

The presence of the most common pathogenic variant of the *BRAF* gene, p.V600E, is associated with reduced effectiveness of CRC monoclonal antibody treatment that targets the EGFR–MAPK pathway.[11] This invokes a clinical imperative to measure the prevalence of this variant in patients with CRC.

Via a screening of 101 patients with CRC, we determined the prevalence of the variant to be 4% in Western Mexico. The four patients bearing the p.V600E variant were three women and a man, all older than 60 years, with tumors located in the proximal colon, three of which were at stages III–IV. Although no statistical approach was possible due to the low number of patients positive for the pathogenic variant, the characteristics of these subjects are in agreement with the clinicopathological features associated with *BRAF* p.V600E described in three meta-analyses.[12–14] Chen *et al*[12] reported an analysis of 25 studies, published before 2013, and estimated a frequency of this pathogenic variant of 10.8% among 11,955 patients with CRC. Li and Li[13] studied 13,208 patients with CRC from 25 selected study populations published after 2005 and estimated an average variant allele frequency of 11.1%. A recent meta-analysis of 61 studies published from 2006 to 2018 analyzing a total of 32,407 patients with CRC was reported by Wang *et al*,[14] with a *BRAF* pathogenic variant frequency of 11.38%. Given the overlap period in the selected studies, several reports used in these meta-analyses are shared, leading to similar results regarding the increased frequency of *BRAF* p.V600E in female patients, patients older than 60 years, the proximal colon tumor location, the TNM III–IV stage, poor differentiation of the tumor and poor outcome of CRC. A comparison between the reported pathogenic variant frequencies of 10.8%, 11.1% and 11.38% showed no statistical differences, assuming a p=0.18, between the three meta-analyses, and the average estimate of the general frequency of *BRAF* p.V600E in patients with CRC is 11%.

Since the three meta-analyses did not include studies on Latin American patients with CRC with the *BRAF* p.V600E, we conducted a search for publications with data from Latin American and Caribbean populations to compare the variant frequency. From the 17 selected reports, the estimated frequency ranged from 0% to 15% with sample sizes ranging from 36 to 120 patients. By comparing the selected populations, we only identified a significant difference in the frequency of *BRAF* p.V600E with one of the four Chilean populations included, the study of Wielandt *et al*,[15] with statistical significance (p=0.02). It must be noted that these authors described 53 patients who showed the highest variant frequency (15%) among the selected studies, with a median age of 70 years. The latter perhaps increases the possibility of detecting this pathogenic variant because, as previously described in the meta-analyses by Chen *et al*[12] and Wang *et al*,[14] patient age above 60 years is associated with its presence.

Differences in allele frequency could also be explained through the methodological approaches chosen in the studies. Colomba *et al*[16] and Roma *et al*[17] used Sanger sequencing for detection of the variant, which is characterized by 5%–30% lower sensitivity than RT-PCR, but 12% higher specificity. Colomba *et al*,[16] Lopez-Rios *et al*[18]

Table 2 Comparison of the prevalence of the *BRAF* p.V600E variant among Latin American and Caribbean populations and description of the clinicopathological data of positive individuals

Country/authors	Number of patients	Male/female %	Mean or median age (range)	Tumor location %	Tumor stage (%)	*BRAF* p.V600E frequency*	P value
Western Mexico/ This study	101	55/45	60 (19–96)	Right colon 26 Left colon 62 Ubiquitous 12	I–II=37 III–IV=61 ND=2	4/101 (4%)	–
Northeast Mexico/ Luévano-González *et al*[33]	106	55/45	<50=58 ≥50=48	Right colon 45 Left colon 55	T2=23† T3=19 T4=58	0/97 (0%)	0.12
Central Mexico/ González-Colunga *et al*[34]	135‡	58/42	55 (45–65)§	Right colon 59 Left colon 41	I–II=28 III–IV=72	13/135 (9.6%)	0.13
Peru/ Montenegro *et al*[35]	90	ND	ND	ND	ND	9/90 (10%)	0.14
Peru/ Egoavil *et al*[36]	90 patients (91 samples)	49/51	59.3 (22–89)	Right colon 36 Left colon 63 ND 1	I–II=51 III–IV=49	9/91 (9.9%)	0.14
Brazil/ Rasuck *et al*[37]	77	40/60	63 (ND)	Right colon 35 Left colon 61 ND 4	All stages	5/77 (6.5%)	0.50
Brazil/ Yamane *et al*[10]	155	53/47	66 (50–89)	Right colon 47 Left colon 53	ND	9/103 (8.7%)¶	0.25
Brazil/ Pereira-Zambalde[38]	84	45/55	65 (36–89)	Colon 54 Rectum 34 ND 12	0–II=36 III–IV=31 ND=33	0/84 (0%)	0.12
Brazil/ dos Santos *et al*[39]	91	54/46	61.1 (29–88)	Right colon 22 Left colon 78	0–II=78 III–V=22	6/91 (6.6%)	0.52
Chile/ Roa *et al*[28]	100	44/56	61 (ND)	Colon 88 Rectum 6 Hepatic metastasis 5 ND 1	"Advanced" stages	11/94 (12%)	0.06
Chile/ Hurtado *et al*[29]	58‡	48/52	63.5 (35–90)	Right colon 33 Left colon 67	I–II=59 III–IV=41	5/58 (9%)	0.28
Chile/ Alvarez *et al*[30]	56	59/41	64 (45–97)	Right colon 37 Left colon 63	0–II=61 III–IV=36 ND=3	At least 4/56 (7.1%)	0.45
Chile/ Wielandt *et al*[15]	53	55/45	70 (41–97)	Right colon 38 Left colon 62	I–II=45 III–IV=55	8/53 (15%)	**0.02**
Argentina/ Perazzo *et al*[40]	146	58/42	58.1 (17–88)	Right colon 28 Left colon 69	I–II=27 III–IV=73	6/49 (12.2%)	0.08
Argentina/ Lopez-Ruitti *et al*[41]	85	54/46	64 (28–85)	ND	ND	4/45 (8.9%)	0.25
Argentina/ González *et al*[42]	155	56/44	65.6 (ND)	Right colon 35 Left colon 65	I–II=54 III–IV=46	11/112 (9.8%)	0.11

Continued

Table 2 Continued

Country/authors	Number of patients	Male/female %	Mean or median age (range)	Tumor location %	Tumor stage (%)	*BRAF* p.V600E frequency*	P value
Puerto Rico/ Perez-Mayoral *et al*[43]	120	ND	ND	ND	ND	11/120 (9.17%)	0.17
Paraguay/ Fleitas-Kanonnikoff *et al*[44]	36	64/36	52 (20–74)	Right colon 39 Left colon 61	I–III=17 IV=83	2/36 (5.5%)	0.65
Total	1738					117/1492 (7.8)	

**BRAF* p.V600E frequency established based only on the number of patients analyzed for the variant.
†Primary tumor invasion.
‡Patient with colon cancer.
§Median (IQR) only for wild-type *BRAF* patients.
¶Described as precursor lesions, not as patients.
ND, no data.

and Jurkowska *et al*[19] suggested that differences in methodologies could alter p.V600E frequencies, principally for patients with melanoma, but it was also for patients with CRC, as described by Løes *et al*[20] and Roma *et al*.[17] However, the discrepancy between variant frequencies reported by Wielandt *et al*[15] and the present study is still unclear since detection of the variant allele was performed by Sanger sequencing in both studies. In fact, this approach was used in 56% of the selected studies in Latin America and the Caribbean.

Differences in the variant prevalence among populations have also been attributed to race/ethnicity. Yoon *et al*[21] studied the variant frequency in patients with stage III colon cancer, showing that *BRAF* p.V600E was detected half as often in black (6.4%) and Asian (5.6%) patients as compared with white patients (13.9%). Heath *et al*[22] reported similar results for patients with CRC with 1.7% *BRAF* p.V600E occurrence in African-American patients compared with 8.5% positive cases of Caucasians. As described by Ruiz-Linares *et al*[23] and Belbin *et al*,[24] the genetics of Latin American and Caribbean populations are an admixture of Native American, European and African characteristics. With respect to ancestries, Salazar-Flores *et al*[25] showed that the European ancestry is more prevalent in South America by contrast to Central America and Mexico, where the predominant ancestry is Native American. More specifically, this mixture of genetic traits produces differences based on geographical location and social structure of each country. Rubí-Castellanos *et al*[26] outlined the ancestry of Western Mexicans, showing that 53.2% are of Native American, 30.8% of European and 15.9% of African descent. Moreover, Santiago City in Chile, where the Wielandt *et al*[15] study population originated from, is within the central zone of Chile characterized by Eyheramendy *et al*,[27] with 40.43% Native American ancestry, 2.46% African and 57.11% European ancestry, which is the highest percentage among Chilean populations. The genetic descent of patients with CRC from Western Mexico and Central Chile might be a crucial factor in producing the differences in frequency of *BRAF* p.V600E, which is further supported by the statistical discrepancy in the comparison of the whole variant frequency of 10.7%, considering the conclusive results for 28 of the 261 Central Chilean patients with CRC analyzed,[15 28–30] in contrast to the 4% frequency estimated for Western Mexican patients with CRC (p=0.04). Whether advanced patient age or ethnicity contributed more to p.V600E frequency differences remains to be elucidated.

Altogether, a total of 1738 patients with CRC were included in the Latin American and Caribbean studies, but only 1492 of them were analyzed for the *BRAF* p.V600E, with 117 (7.8%) positive results. This frequency is lower than the estimate of 11% deduced from meta-analytical reports.

With respect to the CRC burden in Latin America and the Caribbean, GLOBOCAN estimates 6.7% of the worldwide CRC cases of patients older than 50 years to be registered in these areas.[1] Although Carioli *et al*[31] observed a slight decrease in CRC cases in Latin America and the Caribbean in recent years, with the highest CRC rates accounted for in Argentina and the lowest in Mexico, Araghi *et al*[32] expressed that the CRC mortality rate in this region is

expected to increase by 2035. Nevertheless, investigations on the molecular profile of CRC tumors, exploring the prevalence of *BRAF* p.V600E and other variants, on Latin American and Caribbean patients are limited due to several factors, including economical and health infrastructure, that discourage research efforts. The reduced number of patients analyzed for *BRAF* p.V600E presence was also identified as a limitation of the estimate produced in this study, although most of the selected Latin American and Caribbean populations studies showed statistical similarity in variant frequency, regardless of the number of cases per study.

In conclusion, we found a 4% *BRAF* p.V600E prevalence among 101 CRC Western Mexican patients. This result was statistically similar to frequency estimates from studies in Latin America and the Caribbean, except of the variant prevalence in Chilean patients. As a whole, the *BRAF* p.V600E frequency in Latin America and the Caribbean was estimated to be 7.8%. Further research on the frequency of this pathogenic variant and on the molecular profile of CRC within the region will benefit treatment success and patient survival rates.

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