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ORIGINAL ARTICLE

Case Control Study

Association of insertion-deletions polymorphisms with colorectal cancer risk and clinical features

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Abstract

AIM

To investigate the association between 16 insertion-deletions (INDEL) polymorphisms, colorectal cancer (CRC) risk and clinical features in an admixed population.

METHODS

One hundred and forty patients with CRC and 140 cancer-free subjects were examined. Genomic DNA was extracted from peripheral blood samples. Polymorphisms and genomic ancestry distribution were assayed by Multiplex-PCR reaction, separated by capillary electrophoresis on the ABI 3130 Genetic Analyzer instrument and analyzed on GeneMapper ID v3.2. Clinicopathological data were obtained by consulting the patients' clinical charts, intra-operative documentation, and pathology scoring.

RESULTS

Logistic regression analysis showed that polymorphism variations in *IL4* gene was associated with increased CRC risk, while *TYMS* and *UCP2* genes were associated with decreased risk. Reference to anatomical localization of tumor Del allele of *NFKB1* and *CASP8* were associated with more colon related incidents than rectosigmoid. In relation to the INDEL association with tumor node metastasis (TNM) stage risk, the Ins alleles of *ACE*, *HLAG* and *TP53* (6 bp INDEL) were associated with higher TNM stage. Furthermore, regarding INDEL association with relapse risk, the Ins alleles of *ACE*, *HLAG*, and *UGT1A1* were associated with early relapse risk, as well as the Del allele of *TYMS*. Regarding INDEL association with death risk before 10 years, the Ins allele of *SGSM3* and *UGT1A1* were associated with death risk.

CONCLUSION

The INDEL variations in *ACE*, *UCP2*, *TYMS*, *IL4*, *NFKB1*, *CASP8*, *TP53*, *HLAG*, *UGT1A1*, and *SGSM3* were associated with CRC risk and clinical features in an admixed population. These data suggest that this cancer panel might be useful as a complementary tool for better clinical management, and more studies need to be conducted to confirm these findings.

Key words: Colorectal cancer; Ins-del polymorphism; Admixed population; Potential biomarker; Diagnostic; Risk stratification; Prognostic; Clinical features

Core tip: The insertion-deletions (INDEL) variations in *IL4* gene was associated with increased colorectal cancer (CRC) risk, while *TYMS* and *UP2* genes were associated with decreased risk. The Del-alleles of *NFKB1* and *CASP8* were associated with more colon related incidents than rectosigmoid. The Ins-alleles of *ACE*, *HLAG* and *TP53* were associated with higher TNM stage. The Ins-allele of *ACE*, *HLAG*, and *UGT1A1* were associated with early relapse risk, as well as the Del-allele of *TYMS*. The Ins-alleles of *SGSM3* and *UGT1A1* were associated with death risk. These data suggest that these INDEL might be useful as a complementary tool for better CRC clinical management.

Marques D, Ferreira-Costa LR, Ferreira-Costa LL, Correa RS, Borges AMP, Ito FR, Ramos CCO, Bortolin RH, Luchessi AD, Ribeiro-dos-Santos A, Santos S, Silbiger VN. Association of insertion-deletions polymorphisms with colorectal cancer risk and clinical features. *World J Gastroenterol* 2017; 23(37): 6854-6867 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i37/6854.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i37.6854

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer type in men and the second in women, considering 1477402 new cases in both sexes in 2015[1]. In that same year, Brazil was the tenth country with the highest CRC incidence, with 37167 new cases in both sexes[1], and making matters worse, its incidence and mortality continue to increase in the country.

Both genetic and environmental factors cause CRC[2], especially when combined[3,4]. Interestingly, these factors are ample and they vary pursuant to the cancer geographical regions[5]. However, inherited susceptibility is a major component of CRC predisposition, with an estimated 12%-35% risk attributed to genetic factors[6-8].

In relation to genetic factors, there are several mutations that might occur in human DNA, such as substitution, insertion, and deletion[9]. The second most abundant form of genetic variation in humans, after single nucleotide polymorphisms (SNPs), are the insertion-deletions (INDEL)[10]. INDELs are important because they are common genetic variations within genomes and among different ethnic groups[11,12], that may alter human traits and cause diseases[10,13], including CRC[14], by modifying the coding region[10,13] or mRNA stability[15]. The polymorphisms investigated in this study exhibit common features, given they are

all functional polymorphisms that alter the expression of genes participating in metabolic pathways associated with carcinogenesis. Also, these genes are associated with different types of cancer with high incidence in the Brazilian population, such as stomach and CRC.

Furthermore, allele frequency varies among different populations[16], and genomic ancestry distribution may influence cancer development[17,18] by affecting polymorphisms distribution[19,20]. Few studies have been evaluated INDEL association in CRC in admixed population, mainly in Brazil. Thus, the aim of this study was to determine the association between CRC risk and prognostic follow-up with 16 INDELs in genes involved in apoptosis signaling (*CASP8*), GTPase-activating (SGSM3), steroids metabolism (*CYP2E1*, *CYP19A1*, and *UGT1A1*), immune system (*HLAG*, *IL1A*, *IL4*, and *NFKB1*), MDM2-P53 pathway (*MDM2* and *TP53*), DNA replication and repair (*TYMS* and *XRCC1*) and angiogenesis (*UCP2* and *ACE*) in an admixed population from Rio Grande do Norte state (in the Northeast Region of Brazil).

MATERIALS AND METHODS

A statement of ethics

The protocol used in this study was approved by the Research Ethics Committee of Liga Norte Riograndense Contra o Câncer (Rio Grande do Norte, Brazil) by number 211/211/2011. Moreover, all participants signed a consent form prior to providing a blood sample.

Casuistic distinctions

The patients in the case group (n = 140) were diagnosed with CRC as primary cancer and treated in the Proctologist Clinic and Colorectal Surgery Department of Liga Norte Riograndense Contra o Câncer (Rio Grande do Norte, Brazil). The control subjects were cancer-free blood donors (n = 140) from the hemotherapy service (Hemovida, Rio Grande do Norte, Brazil) and were recruited in 2014.

Both Peripheral blood samples and questionnaire answers were collected from all subjects. The clinicopathological data were obtained by consulting the patients' clinical charts, intra-operative documentation, and pathology scoring. Furthermore, the CRC patients were followed up to 20 years by medical records.

Definitions

Alcohol consumption was classified as having the habit of alcohol consumption (Yes) or not having the habit of alcohol consumption (No). The subjects who have the habit of consuming alcohol were subcategorized according to consumption frequency (Eventually: $\leqslant$ 3 d per month; Frequently: > 3 d per month). The tobacco consumption was classified as have already smoked (Already) or not (Never). The subjects who have already smoked were subcategorized in Former (Stopped smoking for at least 1 year) and Current.

Tumor location was classified as rectosigmoid (sigmoid colon and rectum) and colon (ascending, transverse and descending colon) based on colonoscopy or on radiographic exam. The relapse records were obtained from histological or radiographic exams with subsequent clinical/radiological progression.

Overall survival was defined as the time from the date of surgery to the date of death or the date of the last follow-up of patients who were still alive. The relapse time was defined as the time from the date of surgery to the date of the first local. Patients with no local or distant relapse evidence at the date of death or the date of the last follow-up were censored.

DNA extraction and quantification

Genomic DNA was extracted by using a DNA extraction commercial kit, QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), and quantified with Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, United States).

Polymorphism selection

Recently, INDELs have been the focus of multiple investigations[21-25]. This type of polymorphism presents interesting features as genetic markers: (1) INDELs are spread throughout the human genome; (2) INDELs derive from a single event (they do not present homoplasy); (3) since the allele frequencies of many INDELs are significantly different in separated populations; (4) small INDELs can be analyzed using short amplicons, which improves the amplification of degraded DNA and facilitates multiplexing; and (5) INDELs can be easily genotyped with a simple dye-labeling electrophoretic approach. Furthermore, all these genes evaluated in the present study show potential activity in pathway and may contribute to the carcinogenesis process (Table 1). Their genetic variations could contribute to: (1) risk of developing CRC; (2) impact on treatment response; or (3) in prognosis.

Genotyping of polymorphism

Multiplex PCR was used to simultaneously amplify the 16 investigated markers, as shown in Supplementary Table 1. The amplification was performed on ABI Verity thermocycler (Life Technologies, Foster City, CA, United States). A single multiplex reaction used Master Mix QIAGEN Multiplex PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The samples were incubated at 95 ℃ for 15 min, followed by 35 cycles at 94 ℃ for 45 s, 60 ℃ for 90 s, and 72 ℃ for 1 min, with a final extension at 70 ℃ for 30 min.

For fragment analysis, we used capillary electrophoresis on the ABI 3130 Genetic Analyzer instrument (Life Technologies). 1.0 μL of PCR product was added to 8.5 μL of HI-DI deionized formamide (Life Technologies) and 0.5 μL of GeneScan 500 LIZ pattern size standard (Life Technologies). After data collection,

Table 1 Potential biological effects of insertion-deletions polymorphism selected in this study

Gene	dbSNP	Localization[1]	INDEL lenght	Region	Potential biological effect[84]				Potential impact on carcinogenesis
					mRNA splicing	mRNA stability	Gene expression	Protein function	
ACE	rs4646994	17:63488539	289	Intron	X	X		X	Angiogenesis, proliferation, progression and metastases[85]
CASP8	rs3834129	2:201232809	6	Promoter			X		Apoptosis[86-88]
SGSM3	rs56228771	22:40410092	4	3'-UTR		X	X		Proliferation and apoptosis [83,89-91]
CYP2E1	-	-	96	5'-Flanking			X		Metabolism of endo- and exogenous[92-97]
CYP19A1	rs11575899	15:51227749	3	Intron	X	X		X	Metabolism of endo- and exogenous[92-97]
HLAG	rs371194629	6:29830804	14	3'-UTR		X	X		Immune surveillance[98-102]
IL1A	rs3783553	2:112774138	4	3'-UTR		X	X		Induce chronic inflammation and proliferation[103,104]
IL4	rs79071878	5:132680584	70	Intron	X	X		X	Immune surveillance and proliferation[40,39,43,105,106]
MDM2	rs3730485	12:68807065	40	Promoter			X		Proliferation and apoptosis[107,108]
NFKB1	rs28362491	4:102500998	4	Promoter			X		Differentiation, proliferation and apoptosis [109,110]
TP53	rs17878362	17:7676372	16	Intron	X	X		X	Proliferation, apoptosis, repair, differentiation[111-114]
TP53	rs17880560	17:7668169	6	3'-Flanking		X	X		Proliferation, apoptosis, repair, differentiation[111-114]
TYMS	rs151264360	18:673444	6	3'-UTR		X	X		Differentiation, replication and repair[50,105]
UCP2	-	-	45	3'-UTR		X	X		Tumor aggressiveness and metastasis[56]
UGT1A1	rs8175347	2:233760235	2	3'-UTR		X	X		Metabolism of endo- and exogenous[92-97]
XRCC1	rs3213239	19:43576907	4	5'- Flanking			X		Repair[115-117]

[1]According to the single nucleotide polymorphism database (dbSNP); UTR: Untranslated region; INDEL: Insertion-deletions.

samples were analyzed on the GeneMapper ID v.3.7 software (Life Technologies).

Analysis of genetic ancestry

Genomic ancestry analysis was performed based on the method described by Santos *et al*[25] using 62 autosomal ancestry informative markers (AIMs). Two multiplex PCR reactions of 20 and 22 markers were performed and amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and GeneMapper ID v.3.2 software. The individual proportions of European, African, and Amerindian genetic ancestries were estimated using STRUCTURE v.2.3.3 software, assuming three parental populations (European, African, and Amerindian).

Statistical analysis

The categorical variables case and control participants were tested by the Chi-squared test. For ancestry index and age at diagnosis variables we used the Mann-Whitney test. Logistic regression analyses between the genotype model and CRC risk were performed by the SNPassoc package v.1.9-2, along with clinical features variables. The association between genotype and free-relapse survival time was evaluated by Kaplan-Meier plots, performed by the survival package v.2.41-3. Log-rank and Wilcoxon tests were used to examine the genetic effect on survival outcomes. The statistical power was estimated by 10000 simulations. All statistical analyses and plotting were performed with R package v.3.1.2[26]. Differences between groups were considered significant at $P < 0.05$.

RESULTS

Demographic characteristics

We analyzed 140 subjects with CRC and 140 cancer-free individuals. The demographic characteristics of participants were summarized in Table 2, which shows demographic features of the groups. Regarding genomic ancestry, significance was observed with the distribution of African ancestry ($P = 0.049$), Table 3. However, there was no difference between groups when an analysis of multinomial logistic regression was performed.

Distribution of genotypes associated with susceptibility to CRC

All INDEL polymorphisms are in Hardy-Weinberg equilibrium ($P > 0.05$). The genotypic and allelic frequencies of the subjects are presented in the Table 4. Genotypic frequency ($P = 0.01$) of *IL4* gene polymorphism was significantly different between case-controls, and higher frequency of Del allele were observed in cases than in controls.

The significant logistic regression analyses between case-controls are summarized in Table 5. Del allele polymorphism in *IL4* gene ($P = 0.0110$) was associated with an increased risk of CRC development, while Ins allele in *UCP2* ($P = 0.0210$) was decreased

CRC risk. Furthermore, the Del allele in the *TYMS* (P = 0.0120) gene was associated with decreased CRC risk.

Distribution of genotypes associated with prognostic follow-up in CRC

The baseline characteristics of CRC patients are summarized in Table 6. The follow-up time median was 5.28 years among 78 patients who had complete genotype and follow-up information. The 5-year free-relapse rate was 70% and the 10-year free-relapse rate was 66.4%. The 5-year survival rate was 91.4% and the 10-year survival rate was 87.9%.

We also evaluated the genetic impact in the clinical features. The Del allele in *NFKB1* and *CASP8* were associated with more incidents to colon than rectosigmoid (Table 7). In relation to the INDEL association with TNM stage risk, the Ins alleles of *ACE*, *HLAG* and *TP53* (6 bp INDEL) were associated with a higher TNM stage (Table 8). Regarding the INDEL association with relapse risk, the Ins alleles of *ACE*, *HLAG*, and *UGT1A1* were associated with relapse risk, as well as the Del allele of *TYMS* (Table 9). Moreover, these findings corroborate those observed in the free-relapse survival curve (Figure 1). Regarding INDEL association with death risk, the Ins alleles of *SGSM3* and *UGT1A1* were associated with death risk (Table 10).

DISCUSSION

Despite the effective strategies for prevention, early detection, and treatment[27-32], there are ethnic differences in the CRC incidence and survival[33,34], specifically in individuals with African American ancestry, who have higher CRC incidence and lower 5-years survival rates than other ethnic groups[33-38].

In this work, we evaluated the association between 16 INDEL [*ACE, CASP8, SGSM3, CYP19A1, CYP2E1, HLAG, IL1A, MDM2, NFKB1, TP53* (16 and 6 bp), *TYMS, UCP2, XRCC1*, *IL4* and *UGT1A1*] and the risk of developing CRC in a Brazilian population, as well as their clinical features. We found significant association between three investigated INDEL polymorphisms and CRC risk, two associated with anatomical localization, three associated with TNM stage, four associated with early relapse risk, and two associated with death risk before 10 years.

Variations in the IL-4 activity or in the IL-4 receptor due to mutations have been associated with cell proliferation and might affect signal transduction pathways in cancer[39]. We evaluated INDEL of 70 bp in intron 3 of the *IL4* gene (rs79071878), a variation which may influence the production of this cytokine. The higher IL-4 production may result in diminished cell-mediated immune response, and escape from immune surveillance in the tumor cells. The cell-mediated immune response may be inhibited by downregulating the expression of Th1 cytokines, decreasing the CD8+ T-cell response in the tumor microenvironment[39-41]. Furthermore, this INDEL has been associated with gastric cancer[39] and other immune diseases[42,43]. However, this is the first study indicating an association between this *IL4* polymorphism and the risk of developing CRC. Our results indicate that the Del allele in *IL4* was associated with the risk of developing CRC.

The *TYMS* gene plays an essential role in the biosynthesis of the DNA-component thymidylate (dTTP) and is required for DNA replication and repair[44]. The insertion of 6 bp in the 3'-UTR of *TYMS* primary transcript (rs151264360) may significantly influence gene expression as shown by using a luciferase assay[15]. Mandola *et al*[15] observed that a Del allele might decrease the TYMS mRNA stability, and the TYMS protein expression. Moreover, Rahman *et al*[45] showed in vitro that TYMS overexpression might induce the transformation of mammalian cells into a malignant phenotype. Studies indicate that this INDEL is associated with many cancers[46-49], especially colorectal[48]. These results suggested that this INDEL variation might decrease CRC risk, as showed in the present work. However, this finding diverges from data from Mexico[50], in which association was not observed. On the other hand, our results showed that this Del allele was associated with an increase relapse risk.

Uncoupling proteins (UCPs) are a family of mitochondrial proteins, which were originally reported to play essential roles in reducing the reactive oxygen species[51,52]. *UCP2* plays a role in carcinogenesis in various tissues, including colon cancer, and regulates the responsiveness of carcinomas to chemotherapy[53-56]. Adaptive mechanisms of cancer cells include resistance to tumor growth inhibition and evasion of apoptosis, and cellular events that are appreciably affected by oxidative stress[57,58]. The UCP2 expression level is significantly higher in colon cancer tissue than in its adjacent tissue and *UCP2* may play a role in intestinal epithelial cells from benign to malignant transformation[59]. However, the role of *UCP2* in development of colon cancer is unclear. INDEL polymorphism may regulate *UCP2* mRNA stability via post-transcriptional modification of UCP2 protein expression[60,61]. Indeed, in the present study was observed that INDEL polymorphism might be associated with colorectal cancer. However, this is the first study indicating an association between this *UCP2* polymorphism and the risk of developing CRC.

The renin-angiotensin system (RAS), which regulates systemic blood pressure, also exerts local effects on cell proliferation, apoptosis, inflammation and angiogenesis in different tissues[62]. In addition, there is evidence linking the RAS with tumorigenesis and tumor angiogenesis[63]. The polymorphisms in the various components of the RAS that may possess clinical relevance[62], and the most common polymorphism in the gene encoding angiotensin converting enzyme (*ACE*) is INDEL of a 287-bp fragment in intron 16 and is responsible for the inter-individual variation in the

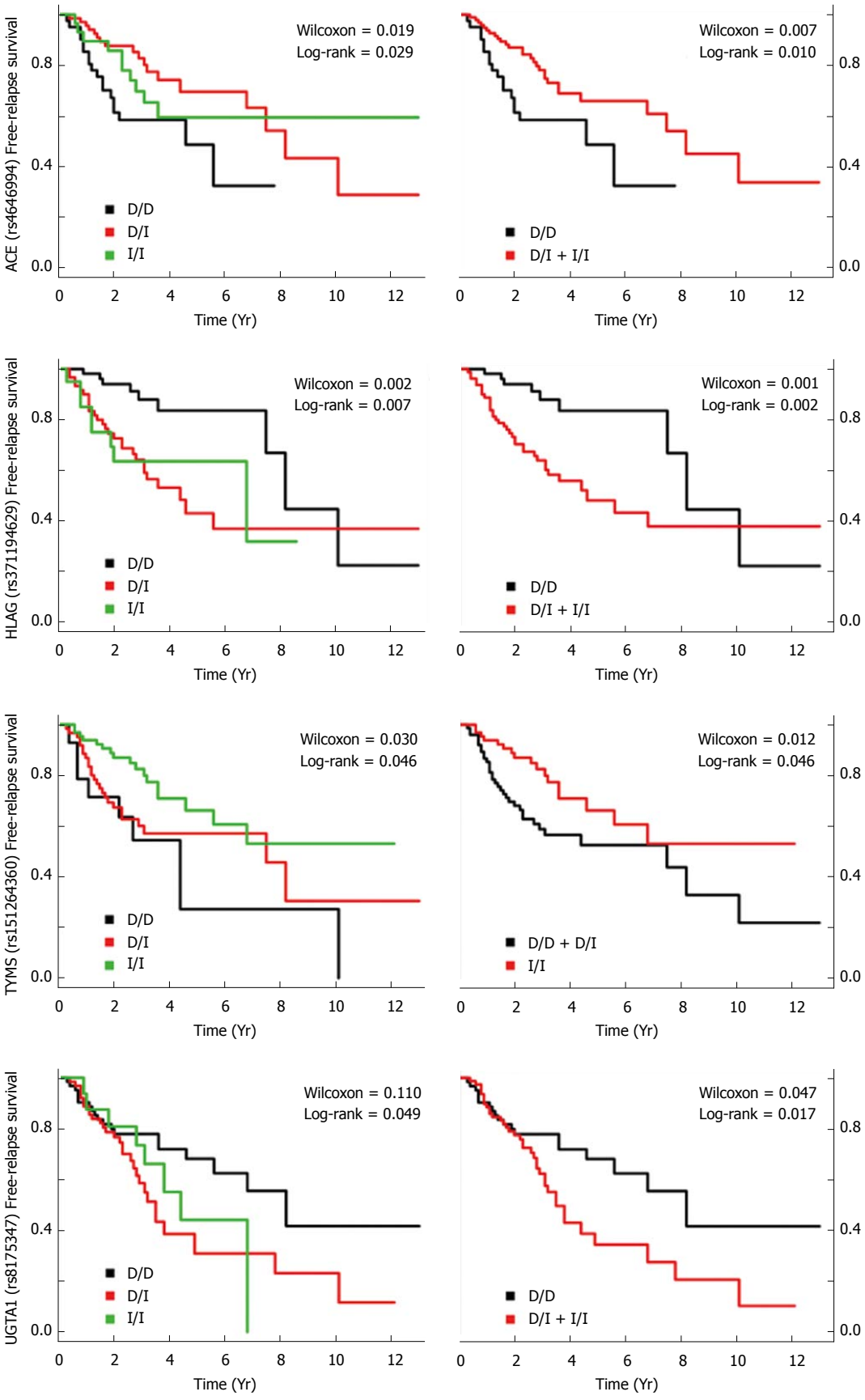

Figure 1 Free-relapse survival of patients with colorectal cancer related with significant insertion-deletions. Logistic regression adjusted for confounders. The analyses and graphic were performed by survival packages, in *R* statistical software.

Table 2 Participant demographic and clinical characteristics and their stratification by case and control groups

Characteristic	Total, *n* = 280	Cases, *n* = 140	Controls, *n* = 140	*P* value
Age (yr)	48 (21-93)	59 (23-93)	37 (21-81)	< 0.001
< 45	136 (48.7)	23 (16.5)	113 (80.7)	
⩾ 45	144 (51.3)	116 (83.5)	27 (19.3)	
Gender				< 0.001
Male	172 (61.6)	62 (44.6)	110 (78.6)	
Female	108 (38.4)	78 (55.4)	30 (21.4)	
Alcohol consumption				< 0.001
No	180 (65.1)	118 (85.5)	62 (44.9)	
Yes	96 (34.9)	20 (14.5)	76 (55.1)	
Eventually	56 (20.4)	11 (8.0)	45 (32.6)	
Frequently	40 (14.5)	9 (6.5)	31 (22.5)	
Tobacco consumption				< 0.001
Never	182 (65.4)	68 (48.9)	114 (82.0)	
Already	96 (24.6)	71 (48.1)	25 (18.0)	
Former	66 (23.8)	55 (39.6)	11 (7.9)	
Current	30 (10.8)	16 (11.5)	14 (10.1)	

Categorized data are presented by absolute numbers of individuals (percentage) and analyzed by Chi-square test. Continuous data are presented by mean (min-max) and analyzed by Mann-Whitney test.

Table 3 Genetic ancestry distribution between case and control groups

Genetic ancestry (%)	Total, *n* = 280	Cases, *n* = 140	Controls, *n* = 140	OR (95%CI)	*P* value
European	65.3 ± 15.5	64.2 ± 15.6	66.4 ± 15.3		0.243
95-80	50 (17.9)	21 (15.1)	29 (20.7)	1.0 (Reference)	
80-70	70 (25.1)	33 (23.7)	37 (26.4)	1.23 (0.59-2.56)	0.577
70-60	67 (24.0)	38 (27.3)	29 (20.7)	1.81 (0.86-3.80)	0.117
60-50	47 (16.8)	22 (15.8)	25 (17.9)	1.21 (0.54-2.71)	0.634
50-40	26 (9.3)	14 (10.1)	12 (8.6)	1.61 (0.62-4.18)	0.327
40-30	11 (3.9)	6 (4.3)	5 (3.6)	1.66 (0.45-6.16)	0.451
30-20	7 (2.5)	5 (3.6)	2 (1.4)	3.45 (0.61-19.54)	0.161
20-10	1 (0.4)	-	1 (0.7)	-	
Amerindian	16.2 ± 10.1	16.0 ± 10.3	16.3 ± 9.9		0.645
02-10	90 (32.3)	45 (32.4)	45 (32.1)	1.0 (Reference)	
10-20	113 (40.5)	60 (43.2)	53 (37.9)	1.13 (0.65-1.97)	0.661
20-30	47 (16.8)	18 (12.9)	29 (20.7)	0.62 (0.30-1.27)	0.193
30-40	21 (7.5)	11 (7.9)	10 (7.1)	1.10 (0.42-2.85)	0.844
40-50	7 (2.5)	4 (2.9)	2 (2.1)	2.00 (0.35-11.47)	0.437
50-60	1 (0.4)	1 (0.7)	-	-	
African	18.6 ± 12.0	19.8 ± 12.3	17.3 ± 11.7		0.049
02-10	81 (29.0)	36 (25.9)	45 (32.1)	1.0 (Reference)	
10-20	89 (31.9)	41 (29.5)	48 (34.3)	1.07 (0.58-1.95)	0.832
20-30	66 (23.7)	38 (27.3)	28 (20.0)	1.70 (0.88-3.27)	0.114
30-40	28 (10.0)	15 (10.8)	13 (9.3)	1.44 (0.61-3.42)	0.405
40-50	8 (2.9)	5 (3.6)	3 (2.1)	2.08 (0.47-9.31)	0.337
50-60	5 (1.8)	3 (2.2)	2 (1.4)	1.87 (0.30-11.83)	0.504
60-70	2 (0.7)	1 (0.7)	1 (0.7)	1.25 (0.07-20.68)	0.876

Categorized data are presented by absolute numbers of individuals (percentage) and analyzed by Chi-square test. Continuous data are presented by mean ± standard variation and analyzed by Mann-Whitney test.

ACE levels in blood and tissues[64]. The insertion allele in this gene was associated with ACE levels, the rate of disease progression, shorter TTF, and lower circulating levels of ACE[62,65]. This INDEL has been associated with cancer risk susceptibility[66-68], including CRC[65,68], and with response to bevacizumab[62]. Our results indicate that Ins allele was not associated with CRC risk development, as showed by Yang *et al*'s meta-analysis[69] and Liu *et al*[70] case-control study (241 cases and 299 control, China). On the other hand, our results also showed that this INDEL was also associated with TNM stage risk and relapse risk.

The *HLAG* is an important immunomodulatory molecule related to several mechanisms of tolerance[71]. Since the discovery of the HLA-G protein expression in cancer[72], several pieces of evidence have supported a considerable role for HLA-G in tumor cell escape from immuno-surveillance and antitumor immune responses[73]. The 14 bp INDEL (rs371194629) has been suggested to have functional significance. The Ins allele has been shown to be associated with alternative splicing, resulting in deletion of 92 bp in

Table 4 Genotype and allele frequency in percentage of patients with colorectal cancer and controls

Gene	dbSNP	Case/control (*n* = 140/140)						
		Genotype frequency				Allele frequency		HWE
		II	ID	DD	*P* value	I	D	*P* value
ACE	rs4646994	21.0/14.3	49.3/54.3	29.7/31.4	0.335	45.7/41.4	54.3/58.6	0.161
CASP8	rs3834129	34.5/30.0	46.0/46.4	19.4/23.6	0.605	57.6/53.2	42.4/46.8	0.424
SGSM3	rs56228771	7.2/3.6	30.2/36.4	62.6/60.0	0.274	22.3/21.8	77.7/78.2	0.415
CYP19A1	rs11575899	35.8/37.9	49.6/50.0	14.6/12.1	0.82	60.3/62.9	39.7/37.1	0.402
CYP2E1	-	0.7/0.7	17.3/12.9	82.0/86.4	0.588	9.4/7.1	90.6/92.6	0.716
HLAG	rs371194629	14.7/13.6	43.4/50.0	41.9/36.4	0.538	36.4/38.6	63.6/61.4	0.514
IL1A	rs3783553	46.0/52.1	38.8/37.9	15.1/10.0	0.368	65.5/71.1	34.5/28.9	0.348
IL4	rs79071878	48.9/60.0	40.3/37.1	10.8/2.9	0.017	69.1/78.6	30.9/21.4	0.223
MDM2	rs3730485	50.4/50.7	43.2/37.1	6.5/12.1	0.219	71.9/69.3	28.1/30.7	0.132
NFKB1	rs28362491	38.1/40.7	46.0/42.1	15.8/17.1	0.806	61.2/61.8	38.8/38.2	0.203
TP53	rs17878362	3.6/1.4	27.3/32.1	69.1/66.4	0.383	17.3/17.5	82.7/82.5	0.181
TP53	rs17880560	7.2/7.1	39.6/32.9	53.2/60.0	0.489	27.0/23.6	73.0/76.4	0.297
TYMS	rs151264360	46.0/42.9	43.9/42.1	10.1/15.0	0.459	68.0/63.9	32.0/36.1	0.308
UCP2	-	6.6/9.3	35.3/44.3	58.1/46.4	0.149	24.3/31.4	75.7/68.6	0.745
UGT1A1	rs8175347	11.5/10.8	44.6/46.0	43.9/43.2	0.785	33.8/33.8	66.2/66.2	0.735
XRCC1	rs3213239	41.7/42.9	47.5/45.0	10.8/12.1	0.894	65.5/65.4	34.5/34.6	0.941

Genotype frequencies are presented as the percentage of patients with colorectal cancer/percentage of controls, and analysis by Chi-square test. dbSNP: Register of genetic variation on NCBI database; bp: Base pairs of DNA sequence; HWE: Hardy-Weinberg Equilibrium.

Table 5 The logistic regression analyses between case-control and insertion-deletions polymorphism

Gene	Model	OR (95%CI)	*P*-value
IL4	Ins/Ins *vs* Del/Ins + Del/Del	2.26 (1.20-4.31)	0.0110
TYMS	Ins/Ins + Del/Ins *vs* Del/Del	0.26 (0.08-0.75)	0.0120
UCP2	Del/Del *vs* Del/Ins + Ins/Ins	0.48 (0.25-0.90)	0.0210

Adjusted by age at diagnosis, gender, alcohol consumption, tobacco consumption and ancestry distribution. The Supplementary Table 2 shows the genotype frequency and all logistic regression.

Table 7 The significant insertion-deletions associations with anatomic localization

Gene	Model	OR (95%CI)	*P* value
CASP8	Ins/Ins *vs* Del/Ins + Del/Del	0.28 (0.08-0.97)	0.0303
NFKB1	Ins/Ins *vs* Del/Ins+Del/Del	0.31 (0.10-0.93)	0.0276

Logistic regression adjusted for confounders. The Supplementary Table 3 shows the genotype frequency and all logistic regression.

Table 8 The significant insertion-deletions associations with tumor node metastasis stage risks

Gene	Model	OR (95%CI)	*P* value
ACE	Del/Del *vs* Del/Ins + Ins/Ins	2.82 (1.26-6.31)	0.0092
HLAG	Del/Del + Del/Ins *vs* Ins/Ins	2.74 (1.01-7.42)	0.0416
TP53 06 bp	Del/Del *vs* Del/Ins + Ins/Ins	2.50 (1.23-5.06)	0.0099

Logistic regression adjusted for confounders. The Supplementary Table 3 shows the genotype frequency and all logistic regression.

Table 6 Clinical characteristics of patients with colorectal cancer at diagnosis and follow-up

Characteristics	Cases (*n* = 140)
Tumor localization	
Colon	25 (17.9)
Rectosigmoid	115 (82.1)
Tumor grade	
G1, G2	130 (92.9)
G3, G4	10 (7.1)
Depth of invasion	
T1, T2	37 (26.6)
T3, T4	89 (64.0)
Tx	13 (9.4)
Lymph node involvement	
N0	77 (55.4)
N1, N2	47 (33.8)
Nx	15 (10.8)
Distant metastasis	
M0	109 (78.4)
M1	13 (9.4)
Mx	17 (12.2)
AJCC stage	
Stage Ⅰ	31 (22.3)
Stage Ⅱ	43 (30.9)
Stage Ⅲ	43 (30.9)
Stage Ⅳ	15 (10.8)
Unknown	7 (5.1)
Relapse, Yes	48 (34.5)
Death, Yes	18 (12.9)

Categorized data are presented by absolute numbers (percentage) and continuous data are presented as median (min-max). Tumors were classified according to the guidelines of the American Joint Committee on Cancer (AJCC) staging system.

exon 5 from mature mRNA, which then leads to low levels of soluble HLA-G (sHLA-G)[74]. Furthermore, our results indicated that the Ins allele was associated with a higher TNM stage and relapse up to 5 years. These findings suggest that low levels of sHLA-G might influence in poor prognostics.

UGT1 is a family of membrane-bound enzymes involved in the inactivation and elimination of lipophilic molecules through glucorination. Moreover, variants

Table 9 The significant insertion-deletions associations with relapse risks

Gene	Model	Time of follow-up	OR (95%CI)	*P* value
ACE	Del/Del *vs* Del/Ins + Ins/Ins	2 yr	0.32 (0.13-0.77)	0.0113
ACE	Del/Del *vs* Del/Ins + Ins/Ins	3 yr	0.37 (0.15-0.91)	0.0298
HLAG	Del/Del *vs* Del/Ins + Ins/Ins	2 yr	2.75 (1.07-7.08)	0.0281
HLAG	Del/Del *vs* Del/Ins + Ins/Ins	4 yr	2.83 (1.07-7.52)	0.0332
HLAG	Del/Del *vs* Del/Ins + Ins/Ins	5 yr	3.47 (1.20-9.99)	0.0194
TYMS	Ins/Ins *vs* Del/Ins + Del/Del	2 yr	3.35 (1.36-8.28)	0.0058
TYMS	Ins/Ins *vs* Del/Ins + Del/Del	3 yr	3.42 (1.41-8.28)	0.0046
UGT1A1	Del/Del *vs* Del/Ins + Ins/Ins	4 yr	3.23 (1.27-8.22)	0.0116
UGT1A1	Del/Del *vs* Del/Ins + Ins/Ins	5 yr	3.50 (1.24-9.84)	0.0145

Logistic regression adjusted for confounders. The Supplementary Table 4 shows the genotype frequency of all insertion-deletions polymorphism.

Table 10 The significant insertion-deletions associations with death risks

Gene	Model	Time of follow-up	OR (95%CI)	*P* value
SGSM3	Del/Del *vs* Del/Ins + Ins/Ins	6 yr	3.61 (1.01-12.92)	0.0487
SGSM3	Del/Del *vs* Del/Ins + Ins/Ins	7 yr	4.60 (1.16-18.23)	0.0260
UGT1A1	Del/Del *vs* Del/Ins + Ins/Ins	6 yr	5.30 (1.43-19.73)	0.0084
UGT1A1	Del/Del *vs* Del/Ins + Ins/Ins	7 yr	4.64 (1.19-18.10)	0.0202
UGT1A1	Del/Del *vs* Del/Ins + Ins/Ins	8 yr	6.50 (1.47-28.80)	0.0091

Logistic regression adjusted for confounders. The Supplementary Table 5 shows the genotype frequency of all insertion-deletions polymorphism.

in this gene have been shown to be useful tools to identifying patients more likely to experience severe toxicity related to irinotecan-containg regimens[75]. In particular, INDEL variants in *UGT1A1* (rs8175347) were associated with significantly decreased glucuronidation activity, which results in reduced SN-38 clearance[76] and an increased risk of these toxicities in patients homozygous for the Ins allele[75,77-80]. Our results showed that the Ins allele in UGT1A1 was associated with early relapse risk, as well as with death risk prior to 8 years. This genetic variation may identify patients who might benefit from increased irinotecan dosing, as observed by Chen *et al*[75].

The *SGSM3* belongs to a novel protein family consisting of three members and appears to be associated with small G-protein coupled receptor signal transduction pathways, and could control cellular functions by a Ras-mediated signaling pathway[81]. Studies have linked Rab dysfunction to various human diseases including cancer[82,83], and our results have shown that the Ins allele might also be associated with death risk prior to 8 years.

The aims of this study were to determine the association between CRC risk and the clinical features with 16 INDEL in genes involved with carcinogenesis pathways in an admixed population from Brazil. Although we have achieved our goal, there are limitations regarding sample number. We suggest, therefore, that an extensive study should be conducted in the Brazilian population to confirm the findings, as well as in other admixed populations.

In summary, the present work indicates that polymorphisms in *ACE* (rs4646994), *TYMS* (rs151264360), *UCP2* (45 bp), *IL4* (rs79071878), *NFKB1* (rs28362491), *CASP8* (rs3834129), *TP53* (rs17880560), *HLAG* (rs371194629), *UGT1A1* (rs3213239), and *SGSM3* (rs56228771) genes were associated with CRC risk and clinical features in an admixed population. These data suggest that this cancer panel might be useful as a complementary tool for better clinical management, and more studies need to be conducted to confirm these findings.

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COMMENTS

Background

Colorectal cancer (CRC) is the third most common cancer type in men and the second in women. Despite the effective strategies for prevention, early detection, and treatment, there are ethnic differences in CRC incidence and survival. These variances occur specifically in African Americans, who have higher CRC incidence and lower survival rates than other ethnic groups. Thus, the present study evaluated the association between 16 insertion-deletions (INDEL) polymorphisms with colorectal cancer risk in an admixture population, as well with clinical features.

Research frontiers

The second most abundant form of genetic variation in humans, after single nucleotide polymorphisms (SNPs), are the INDEL. The INDEL understanding is important because they are common genetic variations within genomes, and they may alter human traits and cause diseases, including colorectal cancer, by modifying the coding region or mRNA stability. One of the challenges for genetic polymorphism association studies is the lack of knowledge regarding the frequency of the polymorphism in the targeted population, mainly in admixed populations (*e.g.* Brazil).

Innovations and breakthroughs

This is the first case-control study to evaluate the association between these 16 INDEL polymorphisms with colorectal risk, clinical features and prognostic follow-up in an admixture population, adopting the methodology that can be easily used to perform multiplexing assays.

Applications

This pilot study is design and findings could be used to determine sample size for a larger randomized controlled study aiming to test the impact of these INDEL polymorphism panel in colorectal risk, clinical features and prognostic follow-up.

Terminology

Ancestry informative marker - In population genetics, an ancestry informative marker (AIM) is a polymorphism that exhibits substantially different frequencies between populations from different geographical regions. A set of many AIMs can be used to estimate the proportion of ancestry of an individual derived from each geographical region.

Peer-review

This is an interesting study aiming to determine the association between CRC risk, and the clinical features with 16 INDEL in genes involved with carcinogenesis pathways in an admixed population from Brazil. The overall structure of the manuscript is complete and conforms to the academic rules.

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