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ORIGINAL ARTICLE

Retrospective Study

Analysis of 12 variants in the development of gastric and colorectal cancers

Giovanna C Cavalcante, Marcos AT Amador, André M Ribeiro dos Santos, Darlen C Carvalho, Roberta B Andrade, Esdras EB Pereira, Marianne R Fernandes, Danielle F Costa, Ney PC Santos, Paulo P Assumpção, Ândrea Ribeiro dos Santos, Sidney Santos

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Abstract

AIM

To evaluate the relation between 12 polymorphisms and the development of gastric cancer (GC) and colorectal cancer (CRC).

METHODS

In this study, we included 125 individuals with GC diagnosis, 66 individuals with CRC diagnosis and 475 cancer-free individuals. All participants resided in the North region of Brazil and authorized the use of their samples. The 12 polymorphisms (in *CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1* genes) were genotyped in a single PCR for each individual, followed by fragment analysis. To avoid misinterpretation due to population substructure, we applied a previously developed set of 61 ancestry-informative markers that can also be genotyped by multiplex PCR. The statistical analyses were performed in Structure v.2.3.4, R environment and SPSS v.20.

RESULTS

After statistical analyses with the control of confounding factors, such as genetic ancestry, three markers (rs79071878 in *IL4*, *rs3730485* in *MDM2* and *rs28362491* in *NFKB1*) were positively associated with the development of GC. One of these markers (rs28362491) and the marker in the *UGT1A1* gene (rs8175347) were positively associated with the development of CRC. Therefore, we investigated whether the joint presence of the deleterious alleles of each marker could affect the development of cancer and we obtained positive results in all analyses. Carriers of the combination of alleles RP1 + DEL (rs79071878 and rs28361491, respectively) are at 10-times greater risk of developing GC than carriers of other combinations. Similarly, carriers of the combination of DEL + RARE (rs283628 and rs8175347) are at about 12-times greater risk of developing CRC than carriers of other combinations.

CONCLUSION

These findings are important for the comprehension of gastric and CRC development, particularly in highly admixed populations, such as the Brazilian population.

Key words: Inflammatory processes; Immune response; Genomic and cellular stability; Gastric cancer; Colorectal cancer; Amazon

Core tip: Gastric cancer and colorectal cancer (CRC) are among the most incident and aggressive types of cancer in Brazil, especially in the Amazon region. Alterations in genes involved in pathways of immune responses, inflammatory processes or genomic and cellular stability may generate cellular imbalances and lead to tumorigenesis. Therefore, it is vital to understand the effect of different alleles in the development of gastric and CRC, which could contribute to the early detection of these types of cancer, increasing the survival chances of the patient.

Cavalcante GC, Amador MAT, Ribeiro dos Santos AM, Carvalho DC, Andrade RB, Pereira EEB, Fernandes MR, Costa DF, Santos NPC, Assumpção PP, Ribeiro dos Santos Â, Santos S. Analysis of 12 variants in the development of gastric and colorectal cancers. *World J Gastroenterol* 2017; 23(48): 8533-8543 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i48/8533.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i48.8533

INTRODUCTION

Cancer is one of the main causes of death worldwide[1]. In Brazil, it is considered a severe problem of public health, and in the North region of this country gastric cancer (GC) and colorectal cancer (CRC) are among the three most incident and aggressive types of cancer[2].

Carcinogenesis is a multifactorial process. Gastritis and colitis have been related to the development of GC[3,4] and CRC[5,6], respectively, but they are not determinant. Infection by *Helicobacter pylori*, one of the most common human infectious agents, is also very important for the development of gastritis and GC[7]. However, it should not be considered the only cause for development of this type of cancer[8]. Genetics also play a major role in the carcinogenesis, and there is much to be discovered regarding this subject.

Genes involved in important pathways, such as inflammatory processes, metabolism of carcinogens, cell stability and hormonal pathways, are possible susceptibility factors to cancer[9-14]. Alterations in these genes may generate imbalances in such pathways and trigger tumor development. In this study, we investigated the following 12 polymorphisms of important genes of these pathways: *CASP8* (rs3834129), *CYP2E1* (96 bp-deletion), *CYP19A1* (rs11575899), *IL1A* (rs3783553), *IL4* (rs79071878), *MDM2* (rs3730485), *NFKB1* (rs28362491), *PAR1* (rs11267092), *TP53* (rs17878362), *TYMS* (rs16430), *UGT1A1* (rs8175347) and *XRCC1* (rs3213239).

These genes and polymorphisms have been studied in association with various types of cancer in different populations, *e.g*, breast cancer[15-19], bladder cancer[20], endometrial cancer[21], acute lymphoblastic leukemia[22], chronic lymphoblastic leukemia[23], oral carcinoma[24,25], lung cancer[26], nasopharyngeal cancer[27], thyroid cancer[28], hepatocellular carcinoma[29], GC[30-39] and CRC[40-50]. Therefore, these markers were chosen based on the importance of each gene as a potential influencing factor in the susceptibility of tumor development. All are functional polymorphisms that correspond to insertion/deletion (INDEL) of small DNA fragments and can be analyzed in a single multiplex PCR, which makes it a cheap and accessible methodology that could be used in different laboratories worldwide.

Thus, the aim of this work was to investigate the association between 12 polymorphisms in genes related to pathways of immune/inflammatory response (*CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *NFKB1* and *PAR1*) and cellular or genomic stability (*CASP8*, *MDM2*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) and the development of GC

Table 1 Technical characteristics of the studied markers

Gene	ID	Type	Length, bp	Primers	Amplicon, bp
CASP8	rs3834129	INDEL	6	F-5′CTCTTCAATGCTTCCTTGAGGT3′ R-5′CTGCATGCCAGGAGCTAAGTAT3′	249-255
CYP2E1	-	INDEL	96	F-5′TGTCCCAATACAGTCACCTCTTT3′ R-5′GGCTTTTATTTGTTTTGCATCTG3′	303-399
CYP19A1	rs11575899	INDEL	3	F-5′TGCATGAGAAAGGCATCATATT3′ R-5′AAAAGGCACATTCATAGACAAAAA3′	122-125
IL1A	rs3783553	INDEL	4	F-5′TGGTCCAAGTTGTGCTTATCC3′ R-5′ACAGTGGTCTCATGGTTGTCA3′	230-234
IL4	rs79071878	VNTR	70	F-5′AGGGTCAGTCTGGCTACTGTGT3′ R-5′CAAATCTGTTCACCTCAACTGC3′	147/217/287
MDM2	rs3730485	INDEL	40	F-5′GGAAGTTTCCTTTCTGGTAGGC3′ R-5′TTTGATGCGGTCTCATAAATTG3′	192-232
NFKB1	rs28362491	INDEL	4	F-5′TATGGACCGCATGACTCTATCA3′ R-5′GGCTCTGGCATCCTAGCAG3′	366-370
PAR1	rs11267092	INDEL	13	F-5′AAAACTGAACTTTGCCGGTGT3′ R-5′GGGCCTAGAAGTCCAAATGAG3′	265-277
TP53	rs17878362	INDEL	16	F-5′GGGACTGACTTTCTGCTCTTGT3′ R-5′GGGACTGTAGATGGGTGAAAAG3′	148-164
TYMS	rs16430	INDEL	6	F-5′ATCCAAACCAGAATACAGCACA3′ R-5′CTCAAATCTGAGGGAGCTGAGT3′	213-219
UGT1A1	rs8175347	VNTR	2	F-5′CTCTGAAAGTGAACTCCCTGCT3′ R-5′AGAGGTTCGCCCTCTCCTAT3′	133/135/137/139
XRCC1	rs3213239	INDEL	4	F-5′GAACCAGAATCCAAAAGTGACC3′ R-5′AGGGGAAGAGAGAGAAGGAGAG3′	243-247

F: Forward; INDEL: Insertion/deletion; R: Reverse; VNTR: Variable number tandem repeat.

and CRC in a population in Northern Brazil. In addition, we investigated the influence of genetic ancestry in the development of these types of cancer in the studied population.

MATERIALS AND METHODS

Samples

In this study, we included three groups: (1) 125 individuals with GC diagnosis; (2) 66 individuals with CRC diagnosis; and (3) 475 cancer-free individuals that were considered the control group. The cancer-free individuals did not have personal or familial histories of any kind of cancer and they did not show any symptoms or signs of cancer. All participants resided in Belém, which is a city located in the Northern region of Brazil, and signed an informed consent, with approval by the Committee for Research Ethics of Hospital João de Barros Barreto under Protocol No. CAAE 25865714.6.0000.0017.

DNA Extraction and Quantification

Samples of peripheral blood were collected from all individuals of the study and the DNA extraction was performed accordingly[51]. DNA quantification was performed with NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States).

Genotyping

Samples were then submitted to multiplex PCR and fragment analysis in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, United States) according to the protocol described[22]. Technical characteristics of the studied markers are presented in Table 1. Due to the high level of genetic admixture in the studied population, we applied a panel of 61 ancestry-informative markers to avoid misinterpretations caused by population substructure, as described[52,53].

Statistical Analyses

Statistical analyses were conducted with different programs. Ancestry analyses were performed in Structure v.2.3.4[54], and tests concerning the genotyping analyses (Student's *t*-test, Pearson's χ^2 test, Mann-Whitney test and logistic regression) were performed in R[55] and in SPSS v.20.0 (IBM Corp., Armonk, NY, United States).

The genotype distribution was assessed as established by Hardy-Weinberg equilibrium (HWE), with post-test correction by the Bonferroni method for multiple tests. *P*-value $\leqslant$ 0.05 was considered statistically significant.

RESULTS

All population distributions were according to HWE ($P > 0.004$) for the analyzed polymorphisms, with the exception of the *IL4* marker in the control group. The observed deviation seems to be due to a significant increase of heterozygotes in this population ($P = 0.0003$).

We also investigated the possible confounding factors of age, sex and genetic ancestry. Table 2 shows these results. When considered statistically significant in the comparison between groups (GC patients *vs*

Table 2 Demographic data for patient and control groups

Variable	GC	CRC	Control	*P*-value	
				GC *vs* Control	CRC *vs* Control
n	120	64	475	-	-
Age, yr[1]	57.02 ± 1.29	52.84 ± 1.90	55.59 ± 0.91	0.522	0.294
Sex, % of male/female	55.0/45.0	45.3/54.70	34.7/65.3	0.000[3]	0.098
European ancestry[2]	0.42 ± 0.01	0.53 ± 0.02	0.47 ± 0.01	0.002[3]	0.003[3]
African ancestry[2]	0.26 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.071	0.016[3]
Amerindian ancestry[2]	0.32 ± 0.01	0.27 ± 0.02	0.30 ± 0.01	0.114	0.100

[1]Values are expressed as mean ± SD. Significance was obtained by Student's *t*-test; [2]Values are expressed as mean ± SD. Significance was obtained by Mann-Whitney test; [3]Statistically significant. CRC: Colorectal cancer; GC: Gastric cancer.

cancer-free individuals, and CRC patients *vs* cancer-free individuals; $P \leq 0.05$), such characteristics were controlled in the logistic regression that assessed whether there are significant differences in the following tests: (1) carriers of INS/INS genotype *vs* carriers of other genotypes (INS/DEL + DEL/DEL); (2) carriers of DEL/DEL genotype *vs* carriers of other genotypes (INS/DEL + INS/INS); and (3) additive effect of the alleles (joint presence of the significant alleles from tests Ⅰ and Ⅱ).

In the analyses with GC patients, positive associations were observed for the markers rs79071878 (*IL4* gene), rs3730485 (*MDM2* gene) and rs28362491 (*NFKB1* gene) after correction of confounding factors for this group (sex and European ancestry) (Table 3). For rs79071878, carriers of the RP1/RP1 genotype have approximately 3-fold increased chances of developing GC than carriers of other genotypes (RP1/RP1 + RP1/RP2) [$P = 0.002$; odds ratio (OR) = 2.857; 95% confidence interval (CI) = 1.490-5.479]. For rs3730485, INS/INS genotype shows a protection effect for the development of GC in comparison with different genotypes ($P = 0.021$; OR = 0.409; 95%CI: 0.192-0.872). For rs28362491, carriers of the DEL/DEL genotype have more chances of developing GC than carriers of the other genotypes ($P = 0.006$; OR = 2.918; 95%CI: 1.352-6.298).

In the analyses with CRC patients, markers rs28362491 (*NFKB1* gene) and rs8175347 (*UGT1A1* gene) showed positive association after the correction of confounding factors (European and African ancestries) (Table 4). Similar to the result for GC, carriers of the DEL/DEL genotype for rs28362491 should present more chances of developing CRC in comparison to carriers of other genotypes ($P = 0.006$; OR = 3.732; 95%CI: 1.451-9.599). For rs8175347, which has multiple alleles (*1, *28, *36 and *37), our results show that 8% of the CRC patients and 0.6% of the cancer-free individuals carry at least one of the rare alleles (*36 and *37). Comparing both groups, we observed that such allele presence could lead to almost 13-fold increased chances of developing CRC ($P = 0.001$; OR = 12.849; 95%CI: 2.906-56.817).

In addition, we analyzed whether the joint presence of the alleles that were statistically significant when in homozygosis (RP1 allele of rs79071878, INS allele of rs3730485, DEL allele of rs28362491 and *36 and *37 alleles in rs8175347) may affect the development of GC and CRC. After controlling for the confounding factors, we obtained statistically significant results for both GC ($P = 0.004311$) and CRC ($P = 3.52 \times 10^{-6}$) analyses. These findings are shown in Figure 1 for GC and in Figure 2 for CRC.

We highlight some positive associations of these alleles due to the absence of neutral effect (logOR = 0 or OR = 1) in the 95%CI for GC [IL4(RP1): OR = 3.068, 95%CI: 1.036-9.088; NFKB1(DEL): OR = 3.414, 95%CI: 1.347-8.654; IL4(RP1) + NFKB1(DEL): OR = 10.475, 95%CI: 4.845-22.624); IL4(RP1) + NFKB1(DEL) + MDM2(INS): OR = 4.437, 95%CI: 2.948-6.686] and CRC [NFKB1(DEL): OR = 2.552, 95%CI: 2.014-3.238; NFKB1(DEL) + UGT1A1(RARE): OR = 11.929, 95%CI: 1.732-82.187)].

DISCUSSION

In the HWE analysis for the *IL4* marker in the control group, the large amount of heterozygotes could be explained either by selective advantage of the heterozygote or by an intense or continuous process of admixture between populations with different genetic backgrounds. Allele frequencies for this marker vary greatly between the three main populations that contributed to the formation of the Brazilian population; the frequency of the RP2 allele has been described as 0.74 among Europeans, 0.23 among Amerindians and 0.42 among Africans[56]. Due to the recent formation of the Brazilian population, we believe that the admixture process is more fitted to explain the observed disequilibrium.

In the analysis for GC, we observed a positive association between the *IL4* marker (rs79071878) and the development of this type of cancer. This polymorphism is a 70-bp variable number tandem repeat located in an intron of *IL4*, which is an interleukin involved in inflammatory pathways. We did not find other studies relating to this polymorphism and GC, but the increased risk of the development of bladder cancer among the carriers of RP1 allele has been previously described[14,57]. Recently, we reported

Table 3 Genotypic and allelic distributions of the investigated polymorphisms for patients with gastric cancer in comparison to control group

Genotype	GC	Control	*P* value[1]	OR (95%CI)[1]	Genotype	GC	Control	*P* value[1]	OR (95%CI)[1]
CASP8	120	475			RP2/RP2	18 (15.1)	154 (32.5)	0.189	0.673 (0.372-1.216)
DEL/DEL	11 (9.2)	90 (19.0)	0.650	0.892 (0.545-1.461)	Allele RP1	0.54	0.41		
INS/DEL	70 (58.3)	230 (48.4)			Allele RP2	0.46	0.59		
INS/INS	39 (32.5)	155 (32.6)	0.080	1.936 (0.924-4.058)	*NFKB1*	120	473		
Allele DEL	0.38	0.43			DEL/DEL	34 (28.3)	117 (24.7)	0.006[2]	2.918 (1.352-6.298)
Allele INS	0.62	0.57			INS/DEL	71 (59.2)	246 (52.0)		
MDM2	120	475			INS/INS	15 (12.5)	110 (23.3)	0.88	0.959 (0.5662-1.610)
DEL/DEL	13 (10.8)	33 (6.9)	0.199	1.365 (0.849-2.192)	Allele DEL	0.58	0.51		
INS/DEL	46 (38.3)	168 (35.4)			Allele INS	0.42	0.49		
INS/INS	61 (50.9)	274 (57.7)	0.021[2]	0.409 (0.192-0.872)	*PAR1*	113	473		
Allele DEL	0.30	0.25			DEL/DEL	66 (58.4)	273 (57.7)	0.068	0.482 (0.221-1.054)
Allele INS	0.70	0.75			INS/DEL	36 (31.9)	169 (35.7)		
TP53	120	475			INS/INS	11 (9.7)	31 (6.6)	0.949	0.984 (0.601-1.610)
DEL/DEL	91 (75.8)	350 (73.7)	0.999	138214253.0 (0.000)	Allele DEL	0.74	0.76		
INS/DEL	27 (22.5)	116 (24.4)			Allele INS	0.26	0.24		
INS/INS	2 (1.7)	9 (1.9)	0.247	0.708 (0.395-1.270)	*CYP2E1*	116	475		
Allele DEL	0.87	0.86			DEL/DEL	94 (81.0)	398 (83.8)	0.999	276187721.0 (0.000)
Allele INS	0.13	0.14			INS/DEL	21 (18.1)	73 (15.4)		
TYMS	120	475			INS/INS	1 (0.9)	4 (0.8)	0.574	1.193 (0.644-2.212)
DEL/DEL	16 (13.3)	65 (13.7)	0.409	1.231 (0.752-2.015)	Allele DEL	0.90	0.91		
INS/DEL	53 (44.2)	224 (47.2)			Allele INS	0.10	0.09		
INS/INS	51 (42.5)	186 (39.2)	0.867	1.060 (0.536-2.096)	*CYP19A1*	120	475		
Allele DEL	0.35	0.37			DEL/DEL	18 (15.0)	76 (16.0)	0.654	1.127 (0.669-1.897)
Allele INS	0.65	0.63			INS/DEL	67 (55.8)	248 (52.2)		
XRCC1	119	474			INS/INS	35 (29.2)	151 (31.8)	0.415	1.334 (0.667-2.671)
DEL/DEL	10 (8.4)	35 (7.4)	0.346	1.257 (0.781-2.021)	Allele DEL	0.43	0.42		
INS/DEL	48 (40.3)	179 (37.8)			Allele INS	0.57	0.58		
INS/INS	61 (51.3)	260 (54.8)	0.396	0.697 (0.303-1.604)	*UGT1A1*	120	464		
Allele DEL	0.29	0.26			*1/*1	49 (40.8)	206 (44.5)	0.792	1.109 (0.515-2.386)
Allele INS	0.71	0.74			*1/*28	57 (47.5)	209 (45.0)		
IL1A	120	475			*28/*28	12 (10.0)	46 (9.9)	0.445	1.205 (0.746-1.946)
DEL/DEL	17 (14.2)	86 (18.1)	0.626	0.882 (0.522-1.460)	*36/*1	2 (1.7)	3 (0.6)		
INS/DEL	63 (52.5)	246 (51.8)			*36/*37	0 (0.0)	0 (0.0)	0.585	1.941 (0.180-20.973)
INS/INS	40 (33.3)	143 (30.1)	0.143	1.705 (0.835-3.482)	*1/*37	0 (0.0)	0 (0.0)		
Allele DEL	0.40	0.44			Allele *36	0.01	0.01		
Allele INS	0.60	0.56			Allele *1	0.65	0.67		
IL4	119	474			Allele *28	0.34	0.32		
RP1/RP1	28 (23.6)	69 (14.5)	0.002[2]	2.857 (1.490-5.479)	Allele *37	0.00	0.00		
RP1/RP2	73 (61.3)	251 (53.0)							

Data for GC and Control columns are presented as *n* or *n* (%). [1]Analysis of combined genotypes (INS/INS *vs* others, or DEL/DEL *vs* others) with adjusted values for confounding factors (sex and European ancestry) in logistic regression; [2]Statistically significant. GC: Gastric cancer.

that the frequency of the RP1 allele of rs79071878 is higher in the North of Brazil (0.414) than in the other regions of the country (mean = 0.233), probably due to the elevated frequency of this marker in Amerindian populations[56]. Data have revealed that the highest incidence of GC in Brazil occurs in the North region. The apparent overlap between the greater incidence of GC and the elevated frequency of RP1 (rs78071878) in the North region of Brazil seems to corroborate the results that indicate that the carriers of homozygous RP1 allele have greater chances of developing GC than the carriers of other genotypes, possibly due to the close relation of this type of cancer with increased inflammation. More studies involving this polymorphism in different admixed populations in this country are recommended.

As for the polymorphism in the *MDM2* gene (rs3730485), we observed that the carriers of INS/INS genotype have less chances of developing GC than carriers of the other genotypes of this marker. To the best of our knowledge, there are no other studies reporting the positive association of this polymorphism and GC development, but the DEL allele has been shown to be associated with increased risk of developing various types of cancer, *e.g*, hepatocellular carcinoma[29], breast cancer[58], prostate cancer[59] and colon cancer[60] in different populations. *MDM2* is an oncogene responsible for the regulation of *TP53* expression[61]. The INS allele of rs3730485 may reduce the activity of *MDM2*, possibly increasing the activity of the tumor suppressor *TP53* and then reducing the chances of developing cancer.

In the current study, we observed an association of the DEL/DEL genotype of the polymorphism in *NFKB1* (rs28362491) with increased chances of developing both GC and CRC. This is an INDEL polymorphism that is

Table 4 Genotypic and allelic distributions of the investigated polymorphisms for patients with colorectal cancer in comparison to control group

Genotype	CRC	Control	*P* value[1]	OR (95%CI)[1]	Genotype	CRC	Control	*P*-value[1]	OR (95%CI)[1]
CASP8	63	475			RP2/RP2	16 (25.4)	154 (32.5)	0.871	1.068 (0.482-2.368)
DEL/DEL	13 (20.6)	90 (19.0)	0.676	0.888 (0.508-1.552)	Allele RP1	0.44	0.41		
INS/DEL	28 (44.4)	230 (48.4)			Allele RP2	0.56	0.59		
INS/INS	22 (35.0)	155 (32.6)	0.939	0.974 (0.503-1.887)	*NFKB1*	63	473		
Allele DEL	0.43	0.43			DEL/DEL	16 (25.4)	117 (24.7)	0.006[2]	3.732 (1.451-9.599)
Allele INS	0.57	0.57			INS/DEL	42 (66.7)	246 (52.0)		
MDM2	64	475			INS/INS	5 (7.9)	110 (23.3)	0.829	0.935 (0.508-1.723)
DEL/DEL	7 (10.9)	33 (6.9)	0.412	1.166 (0.143-9.487)	Allele DEL	0.60	0.51		
INS/DEL	25 (39.1)	168 (35.4)			Allele INS	0.40	0.49		
INS/INS	32 (50.0)	274 (57.7)	0.986	0.995 (0.546-1.811)	*PAR1*	63	473		
Allele DEL	0.30	0.25			DEL/DEL	37 (58.7)	273 (57.7)	0.464	0.704 (0.275-1.801)
Allele INS	0.70	0.75			INS/DEL	20 (31.8)	169 (35.7)		
TP53	64	475			INS/INS	6 (9.5)	31 (6.6)	0.813	0.937 (0.546-1.608)
DEL/DEL	47 (73.4)	350 (73.7)	0.886	1.166 (0.143-9.487)	Allele DEL	0.75	0.76		
INS/DEL	16 (25.0)	116 (24.4)			Allele INS	0.25	0.24		
INS/INS	1 (1.6)	9 (1.9)	0.986	0.995 (0.546-1.811)	*CYP2E1*	62	475		
Allele DEL	0.86	0.86			DEL/DEL	56 (90.3)	398 (83.8)	0.999	189364591.0 (0.000)
Allele INS	0.14	0.14			INS/DEL	6 (9.7)	73 (15.4)		
TYMS	63	475			INS/INS	0 (0.0)	4 (0.8)	0.351	0.655 (0.269-1.593)
DEL/DEL	11 (17.5)	65 (13.7)	0.304	1.342 (0.765-2.354)	Allele DEL	0.95	0.91		
INS/DEL	31 (49.2)	224 (47.2)			Allele INS	0.05	0.09		
INS/INS	21 (33.3)	186 (39.2)	0.429	0.751 (0.369-1.526)	*CYP19A1*	64	475		
Allele DEL	0.42	0.37			DEL/DEL	7 (10.9)	76 (16.0)	0.297	0.747 (0.431-1.293)
Allele INS	0.58	0.63			INS/DEL	33 (51.6)	248 (52.2)		
XRCC1	64	474			INS/INS	24 (37.5)	151 (31.8)	0.313	1.532 (0.669-3.508)
DEL/DEL	4 (6.2)	35 (7.4)	0.771	1.082 (0.637-1.838)	Allele DEL	0.37	0.42		
INS/DEL	27 (42.2)	179 (37.8)			Allele INS	0.63	0.58		
INS/INS	33 (51.6)	260 (54.8)	0.445	1.528 (0.515-4.535)	*UGT1A1*	63	464		
Allele DEL	0.27	0.26			*1/*1	20 (31.7)	206 (44.5)	0.098	0.541 (0.262-1.120)
Allele INS	0.73	0.74			*1/*28	32 (50.8)	209 (45.0)		
IL1A	64	475			*28/*28	6 (9.5)	46 (9.9)	0.370	1.282 (0.745-2.205)
DEL/DEL	10 (15.6)	86 (18.1)	0.657	0.880 (0.500-1.548)	*36/*1	3 (4.8)	3 (0.6)		
INS/DEL	33 (51.6)	246 (51.8)			*36/*37	1 (1.6)	0 (0.0)	0.001[2]	12.849 (2.906-56.817)
INS/INS	21 (32.8)	143 (30.1)	0.610	1.208 (0.584-2.368)	*1/*37	1 (1.6)	0 (0.0)		
Allele DEL	0.41	0.44			Allele *36	0.03	0.01		
Allele INS	0.59	0.56			Allele *1	0.60	0.67		
IL4	63	474			Allele *28	0.35	0.32		
RP1/RP1	8 (12.7)	69 (14.5)	0.195	1.493 (0.814-2.740)	Allele *37	0.02	0.00		
RP1/RP2	39 (61.9)	251 (53.0)							

Data for CRC and Control columns are presented as *n* or *n* (%). [1]Analysis of combined genotypes (INS/INS *vs* others, or DEL/DEL *vs* others) with adjusted values for confounding factors (European and African ancestries) in logistic regression; [2]Statistically significant. CRC: Colorectal cancer; INDEL: Insertion/deletion.

located in the promoter region of the gene, which is highly involved in inflammatory pathways. The DEL/DEL genotype has been previously associated with an increased risk of developing GC in a Japanese population[37] and bladder cancer in a Chinese population[62]. In addition, the DEL allele of this polymorphism has been related to the development of ulcerative colitis and *H. pylori* infection[63,64], which can increase the risk of CRC and GC. Regarding the INS/INS genotype, it has been associated with decreased development risk of ovarian cancer[65] and with increased risk of developing melanoma[66], while the DEL/DEL genotype has also been associated with reduced risk of developing other types of cancer[67]. Previous studies have suggested that the effects of rs28362491 on the risk of carcinogenesis may be ethnic- and cancer type-specific, as described by two meta-analyses involving Asian and Caucasian populations[68,69].

The *UGT1A1* gene is involved in hepatic detoxification and metabolization of different substances. The studied marker in this gene (rs8175347) has four possible alleles [*36 (5 repeats), *1 (6 repeats), *28 (7 repeats) and *37 (8 repeats)]. Allele *1 is considered the wild-type and the most common allele, *28 is the second most common allele and *36 and *37 are considered rare alleles. In this study, we observed that the presence of at least one of the rare alleles of this polymorphism appears to increase the chances of developing CRC by 13-times. In the literature, some studies show that alleles *36 and *37 are absent or extremely rare in different populations[70,71], but there are no studies relating the association of these alleles with the development of CRC. Although little is known about *36 and *37 alleles, it

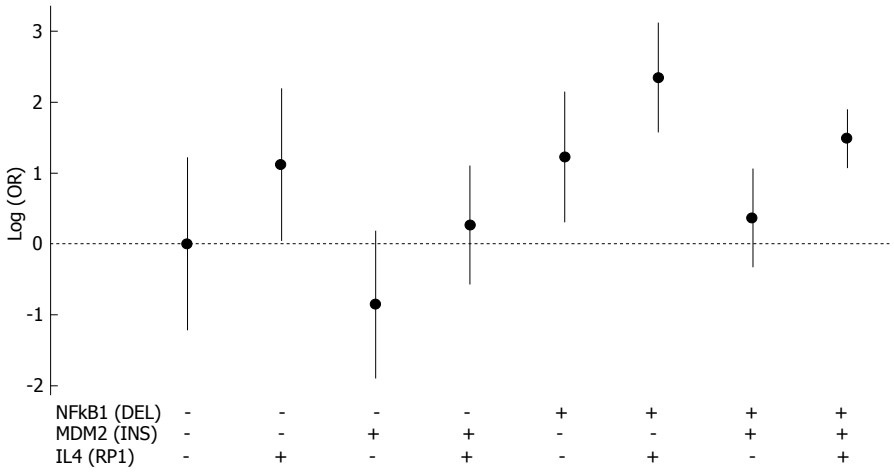

Figure 1 Analysis of the joint presence of three alleles regarding gastric cancer development. DEL allele of rs28362491 is represented by NFKB1 (DEL), INS allele of rs3730485 is represented by MDM2 (INS) and RP1 allele of rs79071878 is represented by IL4(RP1). All possible combinations were considered. Allele presence is represented by (+) and allele absence is represented by (-). DEL: Deletion; GC: Gastric cancer; INS: Insertion.

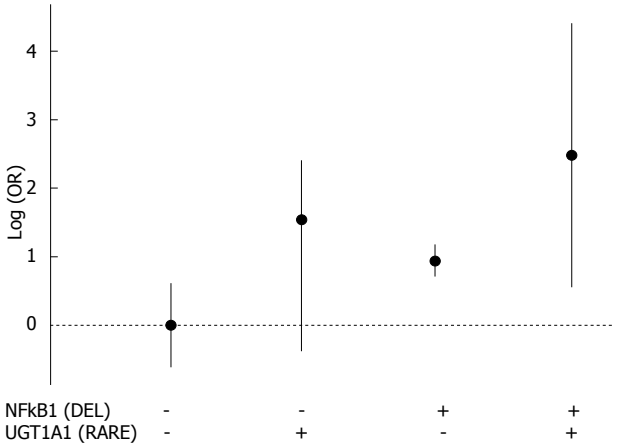

Figure 2 Analysis of the joint presence of two alleles regarding colorectal cancer development. DEL allele of rs28362491 is represented by NFKB1 (DEL) and *36 and *37 alleles in rs8175347 are represented by UGT1A1 (RARE). All possible combinations were considered. Allele presence is represented by (+) and allele absence is represented by (-). CRC: Colorectal cancer; DEL: Deletion.

is possible that the presence of such alleles could lead to a decreased activity of the *UGT1A1* gene, inducing the carcinogenesis process. We understand that the sample size of CRC patients may have influenced the observed result in this study, but we believe that our findings indicate the need to expand the investigation to a great number of patients from other Brazilian admixed populations, considering the important increase rate we observed.

In addition, we investigated the joint presence of the alleles that were statistically significant in homozygosis in the analyses discussed above. This is important because the interaction of alleles in different loci could lead to an increased effect in the carcinogenesis. Recently, this kind of additive effect has been reported for multiple types of cancer in different populations[72,74], but there is a lack of this type of study involving GC and CRC in the Brazilian population. To the best of our knowledge, this is the first study using this approach for these types of cancer in a Brazilian population.

The analyses of combined effect showed statistical significance for both types of cancer, presenting some interesting results. Among these, it is notable that: (1) individuals carrying both RP1 (*IL4* marker) and DEL (*NFKB1* marker) alleles have more than 10-fold increased chances of developing GC than carriers of the other alleles; and (2) individuals carrying the DEL allele (*NFKB1* marker) and at least one of the rare alleles *36 and *37 (*UGT1A1* marker) have almost 12-fold increased chances of developing CRC than carriers of other alleles of these markers. These results reinforce the importance of knowing which markers may play a role in cancer development.

In conclusion, we investigated 12 polymorphisms in genes with functions in inflammatory pathways, immune response or cellular and genomic stability (*i.e. CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) regarding the development of GC and CRC. Our findings indicate that some of these markers may be related to the development of GC and CRC. Moreover, the interaction between such polymorphisms may increase the risk of developing these types of cancer. These results contribute to a greater knowledge of possible risk factors in the development of GC and CRC.

ARTICLE HIGHLIGHTS

Research background

Our research group, located in the North region of Brazil, has been working with population genetics for many years. More recently, we have designed a set of 12 markers that are able to be genotyped in a single multiplex PCR and capillary electrophoresis, which is faster than Sanger sequencing and cheaper than real-time PCR. All markers in this set are in genes related to different pathways (*e.g.* inflammatory and immune response, and cellular and genomic stability). We have previously investigated not only the association of this set with the development of different diseases (*i.e.* acute lymphoblastic leukemia and leprosy), but also the distribution of these markers in individuals from the five regions of Brazil (North, Northeast, Midwest, Southeast and South) and in individuals representative of the main parental populations of this country

(Europeans, Africans and Native Americans). However, we believe it also is important to investigate the association of this set with the development of other types of cancer, such as gastric cancer (GC) and colorectal cancer (CRC).

Research motivation

GC and CRC are two of the most incident and aggressive types of malignant neoplasms in Brazil. A notable aspect of the Brazilian population is that it is highly admixed and, then, it is important not to extrapolate results from one region to another. For instance, these types of cancer are particularly frequent in the North region of Brazil. In general, most cases of GC and CRC are diagnosed in advanced stages and the death rate related to these types of cancer is high. To help early diagnosis, many research groups worldwide have been working to identify biomarkers able to detect increased risk of developing such types of cancer. Considering the high incidence of GC and CRC in the North region, we believe that it is important to study such neoplasms in this region.

Research objectives

In this study, we analyzed the association of 12 polymorphisms in genes involved in inflammatory pathways, immune response or cellular and genomic stability (namely, *CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) regarding GC and CRC development in a population from the North region of Brazil. Understanding the distribution of these markers in the studied population helps to improve the knowledge of the different factors that lead to cancer development.

Research methods

We collected blood samples from the participants (125 GC patients, 66 CRC patients and 475 cancer-free individuals), from which we extracted the DNA using a phenol-chloroform-based method. The studied 12-polymorphism set can be genotyped through amplification in a single multiplex PCR, followed by capillary electrophoresis. The different statistical analyses were performed in Structure v.2.3.4 and SPSS v.20 programs, and the R language. We analyzed the allelic and genotypic distribution of these markers, as well as the combined effect of the statistically significant alleles. The latter approach is not a common approach for studying GC and CRC. In fact, to the best of our knowledge, this is the first study using this kind of approach for these types of cancer in the Brazilian population. It gave us interesting results.

Research results

After performing the statistical analyses with correction of confounding factors, we observed positive associations between the markers rs79071878 (*IL4* gene), rs3730485 (*MDM2* gene) and rs28362491 (*NFKB1* gene) and GC development, as well as between the markers rs28362491 (*NFKB1* gene) and rs8175347 (*UGT1A1* gene) and CRC development. When we analyzed the combined effect of the alleles of the statistically significant genotypes of each marker (RP1 allele of rs79071878, INS allele of rs3730485, DEL allele of rs28362491 and *36 and *37 alleles in rs8175347), we obtained statistically significant results for both types of cancer. From these results, we highlight that: (1) individuals carrying both RP1 (IL4 marker) and DEL (NFKB1 marker) alleles have more than 10-fold increased chances of developing GC than carriers of the other alleles; and (2) individuals carrying the DEL allele (NFKB1 marker) and at least one of the rare alleles *36 and *37 (UGT1A1 marker) have almost 12-fold increased chances of developing CRC than carriers of other alleles of these markers. Our results reinforce the importance of knowing the role that different markers play in the development of cancer, which may contribute to the early detection of GC and CRC.

Research conclusions

In this study, we observed that the individual or joint presence of some alleles of the 12 polymorphisms of the set may affect the development of GC (RP1 allele of rs79071878, INS allele of rs3730485 and DEL allele of rs28362491) and/or CRC (DEL allele of rs28362491 and *36 and *37 alleles in rs8175347) in a population from the North region of Brazil. To the best of our knowledge, this is the first time it has been reported, and it supports the notion that more attention should be given to these polymorphisms in relation to the development of GC and CRC. Considering the results we obtained, we recommend that the individual and the joint presence of these markers should be further investigated in the other regions of Brazil, due to the high levels of admixture in this country, and in other types of cancer.

Research perspectives

Although there have been many advances in the complex field of oncogenetics, there is still a lot remaining to be discovered. The present study investigated 12 polymorphisms, some of them not frequently studied, and showed statistically significant association between four of these markers and the development of GC and CRC in a population from the North region of Brazil. It shows the importance of studying different polymorphisms in important genes, some of which may be involved not only in the development of GC and CRC but also of other types of malignant neoplasms. In addition, our study reinforces the notion of investigating different types of cancer in genetically admixed populations, such as the Brazilian population.

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