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Original Article

Polymorphisms of xenobiotic-metabolizing and transporter genes, and the risk of gastric and colorectal cancer in an admixed population from the Brazilian Amazon

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Abstract: Colorectal (CRC) and gastric (GC) cancers are associated with increased morbidity and mortality. Single nucleotide polymorphisms (SNPs) of xenobiotic metabolism and transporter genes may play a role in the individual responses to exposure to substances implicated in susceptibility to cancer. The investigation of the genetic variation related to the activation and detoxification of xenobiotics may thus help to clarify the prevalence of neoplasms. We analyzed the role of 30 SNPs in xenobiotic-metabolizing and transporter genes in susceptibility to CRC and GC. The study included individuals diagnosed with CRC (n = 121) and GC (n = 95), and 141 controls (non-cancer patients) from the population of Belém, in the Brazilian Amazon. The results indicated an association between the polymorphisms rs2231142 (P = 0.013; OR = 3.01; 95% CI = 1.26-7.13), in the ABCG2 gene, and rs1801159 (P = 0.03; OR = 2.35; 95% CI = 1.14-5.05), in DPYD gene, with the risk of developing GC. The polymorphism rs17116806 of the DPYD gene was found to be associated with a lower risk of developing gastric (P≤0.0001; OR = 0.043; 95% CI = 0.015-0.12) or colorectal (P≤0.0001; OR = 0.076; 95% CI = 0.33-0.18) cancers, indicating that the same variant may play a similar role in different types of cancer tissue. Additionally, the carriers of the TT genotype of the polymorphism in the ABCB1 gene (rs1128503) presented a reduced probability of developing CRC (P = 0.0001; OR = 0.16; 95% CI = 0.06-0.41) as well as GC (P = 0.007; OR = 0.27; 95% CI = 0.1-0.7). Our findings indicate that polymorphisms in xenobiotic-metabolizing and transporter genes may modulate susceptibility to colorectal and gastric cancers.

Keywords: Xenobiotics, polymorphism, admixed, Brazil, gastric cancer, colorectal cancer

Introduction

Gastric (GC) and colorectal (CRC) cancers have very high rates of mortality and are among the main causes of death, worldwide [1, 2]. In the northern (Amazon) region of Brazil, the incidence of these types of neoplasms is relatively high in comparison with the mean rates observed in other regions of the country. In the state of Pará, part of the Brazilian Amazon region, CRC and GC are the fifth and sixth most prevalent cancers, respectively [2]. Despite the high number of cases in the region, the genetic susceptibility of the population of the Brazilian Amazon region to GC and CRC is poorly known.

While the carcinogenesis of gastric and colorectal cancers is still unclear, the available research has identified a number of environmental and lifestyle risk factors, such as obesity, sedentary behavior, a diet rich in fat and calories, the consumption of alcohol and tobacco [3-6]. However, genetic factors almost certainly also play an important role in the development of these neoplasms.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome, and from a clinical perspective, they may be used as potential diagnostic and therapeutic biomarkers for many

types of cancer. The SNPs found in xenobiotic-metabolizing or transporter genes are known to modify the activity of their encoded enzymes, resulting in either an increase or a decrease in the predisposition of the individual for the development of GC or CRC [6-9]. However, few studies have demonstrated the role of these polymorphisms in the susceptibility to CRC or GC of genetically admixed populations. The investigation of populations with high levels of miscegenation is important, given that the frequency of an allele or trait may vary considerably among different ethnic groups, which may confound the interpretation of associations with specific polymorphisms [10].

Given this, we investigated the role of 30 polymorphisms in 15 xenobiotic-metabolizing and transporter genes, and the influence of genetic ancestry in the susceptibility of individuals to CRC and GC in a population from the Brazilian Amazon region with a high degree of interethnic admixture.

Materials and methods

Study participants

Three groups were included in this study: (1) 95 individuals with diagnosed GC; (2) 121 individuals with diagnosed CRC, and (3) 141 cancer-free individuals. The cancer-free individuals did not have personal or familial histories of any type of cancer, and they did not present any symptoms or signs of cancer. All individuals resided in Belém, a city located in the northern Brazilian Amazon region. All the patients were in treatment at two local hospitals, the Ophir Loyola Hospital and the João de Barros Barreto University Hospital. All the participants selected for the study were from the same geographical area and had similar socioeconomic status.

Ethics, consent and permissions

All 357 individuals provided written informed consent for their participation and the publication of this study. The protocol used in the study was approved by the Ethics Committee of João de Barros Barreto University Hospital (protocol number 231.244/2013) and Ophir Loyola Hospital (protocol number 298.994/2013).

DNA extraction and quantification

Peripheral blood samples were collected from all the participants of the study, and the DNA was extracted with the commercial Biopur Kit-Plus Mini Spin Extract-250 kit (Biopur, Brazil), according to the manufacturer's instructions. The DNA was quantified in a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States).

Genotyping

The polymorphisms were genotyped using the QuantStudio™12K Flex Real-Time PCR System by TaqMan Open Array Genotyping (Applied Biosystems, Life Technologies, Carlsbad, USA), following the protocol published by Applied Biosystems. The QuantStudio tool is based on real-time Polymerase Chain Reaction (RT-PCR). The results were imported into the TaqMan® Genotyper software, where they were interpreted based on the amplification and emission of fluorescence at wavelengths specific to each genotype.

Analysis of genetic ancestry

To avoid misinterpretations related to the high level of genetic admixture of the study population, we applied a set of 61 ancestry informative genetic markers (AIMs), as described previously [10, 11]. These analyses were conducted by capillary electrophoresis in an ABI PRISM 3130 sequencer with the GeneMapper ID v3.2 software. The ancestral populations included representatives of three major ethnic groups: Amerindian tribes from the Brazilian Amazon (Tiriyó, Waiãpi, Zoé, Urubu-Kaapor, Awa-Guajá, Parakanã, Wai Wai, Gavião, and Zoró), and African (Angola, Mozambique, Republic of the Congo, Cameroon, and Ivory Coast) and European (Portugal and Spain) populations. Details on these populations are presented in [12]. The genomic ancestry was determined by the Structure v.2.3.4 software.

Statistical analysis

The genotype frequencies of each SNP in the control subjects were evaluated using the Hardy-Weinberg equilibrium (HWE), considering a $P = 0.0017$ significance level following the Bonferroni adjustment. Minor allele frequency (MAF) was also used to remove SNPs with insufficient genotyping quality. The genotype frequencies of the case and control groups were compared using Pearson's χ^2 test, while the Student's t-test and the Mann-Whitney test were used to analyze continuous variables (age

Table 1. Quality control of the candidate SNPs analyzed in the present study

SNP ID	Allele	Gene	HWE (p)[a]	MAF[b]	Status
rs717620	C>T	*ABCC2*	0.3724	0.16	Included
rs9524885	C>T	*ABCC4*	0.0077	0.30	Included
rs4148551	C>T	*ABCC4*	0.1884	0.47	Included
rs3742106	A>C	*ABCC4*	0.4614	0.44	Included
rs1045642	G>A	*ABCB1*	0.5593	0.49	Included
rs1128503	C>T	*ABCB1*	0.0042	0.46	Included
rs2231142	G>T	*ABCG2*	0.0797	0.19	Included
rs8192726	C>A	*CYP2A6*	0.3155	0.22	Included
rs28399433	A>C	*CYP2A6*	0.0883	0.18	Included
rs55886062	A>C	*DPYD*	0.0000	0.04	Not in HWE/MAF below 15%
rs17376848	A>G	*DPYD*	0.6889	0.10	MAF below 15%
rs67376798	T>A	*DPYD*	0.0000	0.20	Not in HWE
rs4970722	T>A	*DPYD*	0.0110	0.26	Included
rs3918290	C>T	*DPYD*	0.3705	0.05	MAF below 15%
rs1760217	A>G	*DPYD*	0.8057	0.21	Included
rs1801159	T>C	*DPYD*	0.0075	0.31	Included
rs17116806	C>A	*DPYD*	0.3566	0.38	Included
rs1801265	A>G	*DPYD*	0.3595	0.28	Included
rs4451422	A>C	*FPGS*	0.0000	0.46	Not in HWE
rs3758149	G>A	*GGH*	0.8558	0.43	Included
rs10049380	T>C	*ITGB5*	0.6953	0.38	Included
rs1801133	G>A	*MTHFR*	0.0022	0.38	Not in HWE
rs1801131	G>A	*MTHFR*	0.00002	0.40	Not in HWE
rs4149178	A>G	*SLC22A7*	0.8202	0.23	Included
rs2270860	C>T	*SLC22A7*	0.3086	0.39	Included
rs760370	A>G	*SLC29A1*	0.8483	0.35	Included
rs747199	G>C	*SLC29A1*	0.00001	0.19	Not in HWE
rs12806698	C>A	*RRM1*	0.0000	0.23	Not in HWE
rs1042927	A>C	*RRM1*	0.0257	0.13	Not in HWE/MAF below 15%
rs1801019	G>C	*UMPS*	0.8456	0.32	Included

[a]*P*-value adjusted by Bonferroni correction. [b]Minor allele frequency (MAF).

and ancestry). The Odds Ratios (ORs) and 95% confidence intervals (CIs) were determined using a multivariate logistic regression analysis with adjustments for age, sex, and genetic ancestry, to control for possible confounding effects. All the data analyses were run in SPSS version 20.0 (SPSS, Chicago, IL, USA). The statistical tests were all two-tailed and a $P \leq 0.05$ significance level was considered in all cases.

Results

Quality control and genotyping

We investigated a total of 30 polymorphisms in the present study. The polymorphisms that had a minor allele frequency (MAF) of less than 15% or were not in HWE were excluded from the analysis. The identification of the SNPs, the gene, HWE and MAF of the case and control groups and the status of each SNP are shown in **Table 1**. Six polymorphisms (rs67376798, rs4451422, rs1801133, rs1801131, rs747199 and rs12806698) were out of HWE and two of the markers were above the threshold of 15% of missing data in the MAF analysis (rs17376848 and rs3918290). Two of the polymorphisms (rs55886062 and rs1042927) were both out of HWE and above the MAF threshold. These ten polymorphisms were excluded from our association analysis, while all the others were included.

Table 2. Demographic parameters of the patients with gastric or colorectal cancer in comparison with the control group

Cancer type	*Variable*	Case	Control	*P-value*
Gastric	*No.*	95	140	
	Age, in years[a]	55.59±9.94	69.44±6.89	<0.001
	Sex (Female/Male)	36/59	101/39	<0.001
	Genetic Ancestry[b]			
	European	0.45±0.16	0.45±0.17	0.87
	African	0.22±0.12	0.23±0.14	0.68
	Amerindian	0.33±0,13	0.32±0.15	0.21
Colorectal	*No.*	121	140	
	Age, in years[a]	54.05±12.06	69.44±6.89	<0.001
	Sex (Female/Male)	67/54	101/39	0.006
	Genetic Ancestry[b]			
	European	0.50±0.14	0.45±0.17	0.015
	African	0.20±0.10	0.23±0.14	0.11
	Amerindian	0.30±0,12	0.32±0.15	0.77

The genetic ancestry analysis was based on the three principal ancestral groups that form the study population. [a]Student's *t*; [b]Mann-Whitney's *U*.

Demographic characteristics

A total of 357 individuals were analyzed in the present study. Demographic data on the case (GC and CRC) and control groups are presented in **Table 2**. Whereas GC was more common in men, women predominated in the CRC case group. The GC case group was composed predominantly of men while the CRC had a predominance of women, although both case groups were significantly younger, on average, than the control group, and were significantly less male-biased. These potentially confounding variables were controlled for in the logistic regression.

European ancestry predominated in all three groups, followed by Amerindian and African ancestry. However, while there was some variation among the groups in their genomic ancestry, there was a significant difference between case and control groups only for European ancestry, which was significantly higher in the CRC group in comparison with the control (**Figure 1**).

Associations between the polymorphisms and susceptibility to GC and CRC

The investigation of the different polymorphisms identified specific associations that imply an increased risk of carcinogenesis (**Table 3**). In particular, the rs2231142 variant of the *ABCG2* transporter gene was associated with a three-fold risk of developing GC (P = 0.013; OR = 3.01, 95% CI = 1.26-7.13). In the case of the *DPYD* gene, the rs1801159 variant was also associated with an increase in the risk of developing GC (P = 0.03, OR = 2.35, 95% CI = 1.14-5.05).

The rs17116806 variant had the opposite effect, providing protection against the development of GC (P≤0.0001; OR = 0.043; 95% CI = 0.015-0.12). Interestingly, this variant had a similar effect on the carcinogenesis of CRC (P≤0.0001; OR = 0.076; 95% CI = 0.33-0.18). This shows that the same variant can confer a reduction in the risk of both types of neoplasia investigated in this population.

We also found that the rs1128503 polymorphism of the ABCB1 gene correlates with a lower risk of developing of both CRC (P = 0.0001; OR = 0.16; 95% CI = 0.06-0.41) and GC (P = 0.007; OR = 0.27; 95% CI = 0.1-0.7). The carriers of the TT genotype presented an approximately 73% decrease in the risk of developing these neoplasia. No evidence was found of any association with the other polymorphisms analyzed (Tables S1 and S2).

Discussion

Colorectal (CRC) and gastric cancers (GC) are among the leading causes of death worldwide [1]. In the Brazilian Amazon region, GC is the second most prevalent type of neoplasia in men and the fourth most common in women, whereas CRC is the fourth most prevalent in men and the third in women [2]. In Brazil, estimates for the 2020-2022 period predict 13,360 new cases of stomach cancer per annum in men and 7,870 in women, as well as 16,590 new cases of CRC [2].

Although the causes of cancer have not yet been completely elucidated, a number of studies have shown that a large group of mutagen-carcinogenic agents require metabolic activation to allow them to bind to DNA, RNA, and

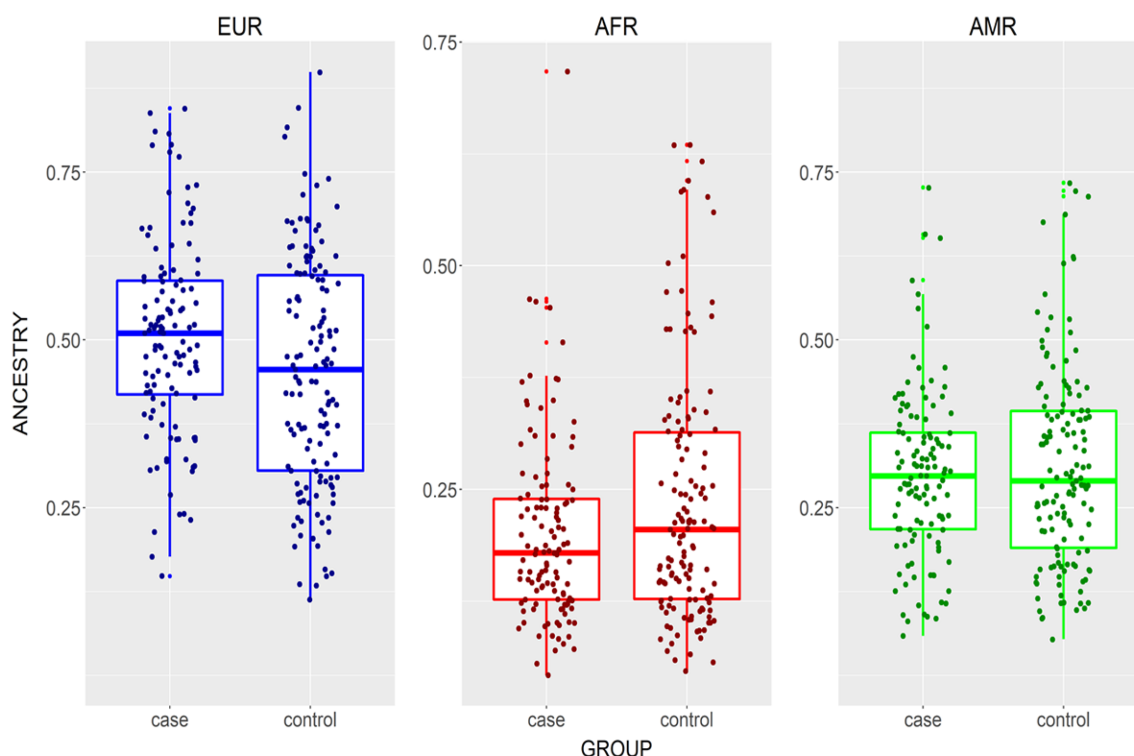

Figure 1. Box plot graph elucidates the difference between with colorectal (CRC) cancer and control groups for Amerindian, African and European ancestries. The genetic ancestry of the CRC group was 50% European, 20% African, and 30% Amerindian, whereas the control group was 45% European 24% African, and 32% Amerindian. There was a statistically different difference for European ancestry between groups (P = 0.015).

proteins. Given this, a range of environmental factors are known to be strong risk factors for the development of GC and CRC [13, 14]. Combined, it is demonstrated that environmental factors have a 55% influence on susceptibility to GC and a 26% influence in the case of CRC [15]. Association studies of the genetic pathways related to the metabolism and transport of environmental risk factors have provided a better understanding of carcinogenesis in several organs [16-18]. Most genetic association studies of cancers have investigated tumor suppressors genes or oncogenes. However, we have proposed focusing on xenobiotic-metabolizing and transporter genes, which can also modulate the susceptibility of an individual to different types of cancer.

Few of the available studies of these genes have been focused on admixed populations, such as that of Brazil [11, 12]. Case-control studies in admixed populations may be influenced by the variation in the allelic frequencies of polymorphisms found in different ethnic groups, which may create biases in the outcome, especially in investigations of susceptibility to complex diseases, such as cancer [19]. Clearly, then, estimates of ethnic admixture must be taken into consideration in any study of genetic association. In the present study, the genomic ancestry analysis was based on the set of 61 AIMs used in a previous genetic study of complex diseases, which was designed and, posteriorly, expanded by our research group [10, 11]. Specifically, our research group demonstrated the influence of ethnic admixture in childhood B-cell Leukemia [20, 21] and breast, gastric cancer [22] in northern Brazil. In the present study, European ancestry was correlated with the risk of CRC. Previous studies have shown that there is genetic heterogeneity in the patterns of CRC in Americans of African or European descent [23]. Some of the studies of in Latin American populations have demonstrated a role of genetic ancestry in the development of CRC. In these populations, however, African ancestry was associated with an increased risk of developing this type of tumor [24, 25]. Using a validated set of 105 ancestry informative markers (AIMs) to estimate genetic

Table 3. Distribution of the genotypes of the polymorphisms investigated in the present study that presented a statistical association with cancer (GC or CRC) in comparison with the control group

Cancer	Genotype	N (%) case	N (%) control	*p-value*[a]	OR (95% IC)
GC	ABCB1 (rs1128503)	90	101	0.007	TT *vs.* others: 0.27 (0.1-0.7)
	CC	32 (35.6%)	40 (39.6%)		
	CT	44 (48.9%)	35 (34.7%)		
	TT	14 (15.6%)	26 (25.7%)		
	Allele C	0.6	0.6		
	Allele T	0.4	0.4		
GC	ABCG2 (rs2231142)	93	122	0.013	GG *vs.* others: 3 (1.26-7.13)
	GG	65 (69.9%)	68 (55.7%)		
	GT	25 (26.9%)	51 (41.8%)		
	TT	3 (3.2%)	3 (2.5%)		
	Allele G	0.8	0.77		
	Allele T	0.2	0.23		
GC	DPYD (rs1801159)	94	134	0.03	TT *vs.* others: 2.35 (1.14-5.05)
	TT	55 (58.5%)	49 (36.6%)		
	TC	35 (37.2%)	76 (56.7%)		
	CC	4 (4.3%)	9 (6.7%)		
	Allele T	0.8	0.6		
	Allele C	0.2	0.4		
GC	DPYD (rs17116806)	92	121	<0.0001	AA *vs.* others: 0.043 (0.015-0.012)
	CC	35 (38%)	95 (78.5%)		
	CA	41 (44.6%)	26 (21.5%)		
	AA	16 (17.4%)	0 (0.0%)		
	Allele C	0.6	0.9		
	Allele A	0.4	0.1		
CRC	ABCB1 (rs1128503)	116	101	0.0001	TT *vs.* others: 0.16 (0.06-0.41)
	CC	31 (26.7%)	40 (39.6%)		
	CT	72 (62.1%)	35 (34.7%)		
	TT	13 (11.2%)	26 (25.7%)		
	Allele C	0.6	0.6		
	Allele T	0.4	0.4		
CRC	DPYD (rs17116806)	112	121	<0.0001	AA *vs.* others: 0.076 (0.33-0.18)
	CC	29 (25.9%)	95 (78.5%)		
	CA	57 (50.9%)	26 (21.5%)		
	AA	26 (23.2%)	0 (0.0%)		
	Allele C	0.5	0.9		
	Allele A	0.5	0.1		

[a]Logistic regression adjusted for the confounding variables (age and sex).

ancestry in a Puerto Rican population, a study showed that African ancestry is associated with an increased risk of developing rectal tumors (OR = 1.55, 95% CI 1.04-2.31) [25]. Although genetic ancestry appears to be an important factor in the susceptibility of the individual to CRC, then, there have been no analyses of Brazilian populations, prior to the present study. Our results thus reinforce the need for further research in order to determine whether the high number of CRC cases recorded in this population reflects, in addition to other factors, its genetic heterogeneity in relation to the key molecular markers for this type of tumor.

The polymorphisms found to have an association with colorectal or gastric cancer in the

present study are related to the metabolism of xenobiotics. As mutagenic-carcinogenic agents require metabolic activation, polymorphisms in these xenobiotic-metabolizing and transporter genes may account for much of the individual variation observed in response to exposure to environmental risk factors [18, 19].

The rs2231142 variant of the *ABCG2* gene was associated with an approximately three-fold increase in the risk of development of gastric cancer (**Table 3**). The *ABCG2* gene encodes the breast cancer resistance protein (BCRP)/ATP-binding cassette subfamily G member 2, which is an ATP-binding cassette (ABC) transporter responsible for the active transport of a number of compounds through the extra and intracellular membranes [26]. This protein is expressed primarily in the liver and the apical membrane of the intestinal epithelium, where it plays an important role in the intestinal absorption and mediation of hepatobiliary excretions (such as potentially carcinogenic xenobiotics and anticancer drugs) [27]. The BRCP is known as a molecular cause of multidrug resistance (MDR) in several types of cancer cell, but recently, some research has focused on understanding its role as a biomarker of susceptibility in human carcinoma cells [28, 29]. Studies have shown down-regulation of BCRP/ABCG2 in colorectal [30]. To promote carcinogenesis, the expression of BCRP would decrease to allow the accumulation of genotoxins and nitric oxide, but in the more advanced stages, BRCP may be expressed positively to permit the efficient transport of chemotherapeutic drugs out of the cancerous cells, thus enabling drug resistance [30]. Supporting this finding, another investigation demonstrated differential expression of the BCRP at each stage of the carcinogenesis process in CRC patients [31]. Hence, the assessment of the differential expression of BRCP may help to create new approaches for the evaluation of the progression and metastasis of the cancer, and to predict the therapeutic response in CRC. Our results also corroborate the findings of an *in vitro* experiment that demonstrated a deregulated expression of the *ABCG2* gene in GC tissue and cells [32]. In this study, the high expression of *ABCG2*/BRCP was correlated with the advanced stages and poor prognosis of GC. The deregulated expression of the *ABCG2* gene has also been identified as a factor promoting GC that affects cell proliferation and induces resistance to cellular apoptosis. These findings are consistent with our analyses, that confirm the role of the SNPs of the *ABCG2* gene in the initiation and promotion of GC.

Polymorphisms of the *DPYD* gene are also known to play a role in gastric and colorectal carcinogenesis. Our results indicate that the rs1801159 and rs17116806 polymorphisms are associated with a greater susceptibility to GC (**Table 3**). The rs17116806 variant was associated with a reduced risk to colorectal carcinogenesis, demonstrating that the same polymorphism can provoke tumorigenesis in different tissues. International regulatory agencies, such as the United States' Food and Drug Administration (FDA) and the European Medicines Agency (EMA), strongly recommend the monitoring of polymorphisms of the *DPYD* gene for the evaluation of the therapeutic response to treatment based on fluoropyrimidine [33, 34]. Even so, few studies have investigated the role of variations in the *DPYD* gene in susceptibility to cancer, and the clinical manifestations associated with *DPYD* mutations are still poorly understood [32]. To date, there have been no studies investigating the association between the rs1801159 and rs17116806 polymorphisms and susceptibility to the types of cancer investigated in the present study. This study is thus the first to investigate the correlation between *DPYD* polymorphisms and susceptibility to GC and CRC in the population of the Brazilian Amazon region. Previous studies have also shown that modifications of the pyrimidine homeostasis and the products of their degradation can result in several phenotypic manifestations, including neurological disturbances [35] and gastrointestinal disorders [36], like CRC cancer [37]. The rs1801265 variant was significantly associated with the risk of developing six different neoplasia, of the esophagus, stomach, colon, lung, breast, and lymphoma [38]. A recent comprehensive review explains the mechanisms by which the polymorphisms of the *DPYD* gene deregulate the synthesis of pyrimidines and nucleic acid, and thus promote the malignant progression [39]. Finally, a multicenter study concluded that variations in the genes involved in the metabolism of pyrimidines, in particular *DPYD*, may also influence susceptibility to ovarian carcinoma [40]. Our data corroborate all of these find-

ings, which show that *DPYD* has already been extensively identified as a potential candidate gene for several neoplastic types.

It is important to note that our study also demonstrated that some polymorphisms in the transporter genes may have a protective effect against the specific neoplasms, in particular, that the ABCB1 variant presented an approximately 84% decrease in the risk of colorectal carcinogenesis and a 73% decrease in gastric cancer. This gene encodes the Permeability-glycoprotein (P-gp), which belongs to a group of ATP-dependent efflux pumps that selectively transport substances out of the cell. This is the body's first line of defense against oral exposure to potentially toxic and cytotoxic compounds [41-43]. A number of previous studies have shown that the SNPs of the ABCB1 gene may influence susceptibility to a number of gastrointestinal diseases, including CRC and GC, given their role in maintaining intestinal homeostasis [44, 45]. The rs1128503 (1236C>T) and rs2032582 (2677G>T/A) variants when analyzed separately or in haplotypes have shown a differential distribution between the CRC patients and the healthy population which indicated a potential existence of SNPs in the regulatory region of the ABCB1 gene that may influence the expression and function of P-gp, which, in turn, would modulate the risk of CRC [46]. Another investigation demonstrated an apparent gender-related modulation of CRC susceptibility associated with rs1128503 polymorphism. *ABCB1* have also been important candidate gene for influencing the susceptibility to GC [47]. The risk of GC development was significantly higher for the CC genotype as compared to the AA wild genotype (OR = 1.85, 95% CI = 1.15-2.97, P = 0.010) in the Chinese Han population [47].

Based on the existing evidence, we can infer that relevant clinical interventions are possible through the enhancement of our understanding of the basic set of mutations that may induce gastric and colorectal carcinogenesis. These findings provide important insights towards the predictive diagnosis of GC and CRC. The present study has shown that SNPs in the *ABCG2*, *DPYD*, and *ABCB1* genes play an important role in the susceptibility of individuals to both gastric and colorectal cancer in a highly admixed population from the Brazilian Amazon region. To the best of our knowledge, this is the first study to describe an association between susceptibility to CRC and European genetic ancestry in this population. These findings contribute to the understanding of the genetic factors that may underly the prevalence of GC and CRC in the Brazilian Amazon population.

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Disclosure of conflict of interest

None.

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Table S1. Distribution of the genotypes of the polymorphisms investigated in the present study that did not present a statistical association with colorectal cancer in comparison with the cotrol group

Genotype	N (%) case	N (%) control	p^a	OR (95% IC)
ABCC2 (rs717620)	115	127	0.80	TT *vs.* others: 1.11 (0.5-2.37)
CC	83 (72.2%)	86 (67.7%)		
CT	29 (25.2%)	35 (27.6%)		
TT	3 (2.6%)	6 (4.7%)		
Allele C	0.8	0.8		
Allele T	0.2	0.2		
ABCC4 (rs4148551)	115	116	0.30	TT *vs.* others: 1.47 (0.7-3.31)
CC	27 (23.5%)	41 (35.3%)		
CT	66 (57.4%)	50 (43.1%)		
TT	22 (19.1%)	25 (21.6%)		
Allele C	0.5	0.6		
Allele T	0.5	0.4		
ABCC4 (rs3742106)	117	120	0.60	CC *vs.* others: 1.19 (0.57-2.48)
AA	35 (29.9%)	38 (31.7%)		
AC	69 (59.0%)	55 (45.8%)		
CC	13 (11.1%)	27 (22.5%)		
Allele A	0.6	5.5		
Allele C	0.4	0.5		
ABCB1 (rs1045642)	118	105	0.80	AA *vs.* others: 0.93 (0.43-2.02)
GG	33 (28.0%)	28 (26.7%)		
GA	53 (44.9%)	56 (53.3%)		
AA	32 (27.1%)	21 (20.0%)		
Allele G	0.5	0.5		
Allele A	0.5	0.5		
ABCG2 (rs2231142)	116	122	0.20	TT *vs.* others: 1.61 (0.79-3.26)
GG	82 (70.7%)	68 (55.7%)		
GT	32 (27.6%)	51 (41.8%)		
TT	2 (1.7%)	3 (2.5%)		
Allele G	0.8	0.8		
Allele T	0.2	0.2		
CYP2A6 (rs28399433)	113	129	0.50	CC *vs.* others: 0.81 (0.4-1.65)
AA	75 (66.4%)	87 (67.4%)		
AC	31 (27.4%)	34 (26.4%)		
CC	7 (6.2%)	8 (6.2%)		
Allele A	0.8	0.9		
Allele C	0.2	0.1		
CYP2A6 (rs8192726)	114	117	0.60	AA *vs.* others: 0.82 (0.4-1.74)
CC	78 (68.4%)	69 (59.0%)		
CA	21 (18.4%)	39 (33.3%)		
AA	15 (13.2%)	9 (7.7%)		
Alelle C	0.8	0.8		
Allele A	0.2	0.2		
DPYD (rs1760217)	118	99	0.10	GG *vs.* others: 1.69 (0.82-3.47)
AA	79 (66.9%)	51 (51.5%)		
AG	37 (31.4%)	39 (39.4%)		
GG	2 (1.7%)	9 (9.1%)		
Allele A	0.8	0.7		
Allele G	0.2	0.3		

DPYD (rs1801159)	119	134	0.10	CC *vs.* others: 1.7 (0.86-3.38)
TT	53 (44.5%)	49 (36.6%)		
TC	59 (49.6%)	76 (56.7%)		
CC	7 (5.9%)	9 (6.7%)		
Allele T	0.7	0.6		
Allele C	0.3	0.4		
DPYD (rs1801265)	118	133	0.90	GG *vs.* others: 0.93 (0.27-2.96)
AA	66 (55.9%)	76 (57.1%)		
AG	41 (34.7%)	46 (34.6%)		
GG	11 (9.3%)	11 (8.3%)		
Allele A	0.7	0.7		
Allele G	0.3	0.3		
GGH (rs3758149)	94	122	0.90	AA *vs.* others: 1.06 (0.46-2.44)
GG	29 (30.9%)	36 (29.5%)		
GA	50 (53.2%)	62 (50.8%)		
AA	15 (16.0%)	24 (19.7%)		
Alelle G	0.6	0.5		
Allele A	0.4	0.5		
ITGB5 (rs10049380)	112	110	0.90	CC *vs.* others: 0.96 (0.46-3.26)
TT	47 (42.0%)	37 (33.6%)		
TC	51 (45.5%)	56 (50.9%)		
CC	14 (12.5%)	17 (15.5%)		
Allele T	0.6	0.6		
Allele C	0.4	0.4		
SLC22A7 (rs4149178)	118	125	0.50	GG *vs.* others: 1.15 (0.43-2.46)
AA	77 (65.3%)	67 (53.6%)		
AG	34 (28.8%)	48 (38.4%)		
GG	7 (5.9%)	10 (8.0%)		
Allele A	0.8	0.7		
Allele G	0.2	0.3		
SLC22A7 (rs2270860)	116	117	0.90	TT *vs.* others: 0.9 (0.53-2.01)
CC	42 (36.2%)	52 (44.4%)		
CT	56 (48.3%)	48 (41.0%)		
TT	18 (15.5%)	17 (14.5%)		
Allele C	0.6	0.6		
Allele T	0.4	0.4		
SLC29A1 (rs760370)	117	124	0.10	GG *vs.* others: 1.74 (0.85-3.54)
AA	59 (50.4%)	49 (39.5%)		
AG	41 (35.0%)	57 (46.0%)		
GG	17 (14.5%)	18 (14.5%)		
Allele A	0.7	0.6		
Allele G	0.3	0.4		
UMPS (rs1801019)	110	120	0.90	CC *vs.* others: 0.98 (0.48-2)
GG	55 (50.0%)	47 (39.2%)		
GC	48 (43.6%)	55 (45.8%)		
CC	7 (6.4%)	18 (15.0%)		
Allele G	0.7	0.6		
Allele C	0.3	0.4		

[a]Logistic regression adjusted for the confounding variables (age and sex).

Table S2. Distribution of the genotypes of the polymorphisms investigated in the present study that did not present a statistical association with gastric cancer in comparison with the cotrol group

Genotype	N (%) case	N (%) control	p^a	OR (95% IC)
ABCC2 (rs717620)	92	127	0.48	TT *vs.* others: 1.36 (0.57-3.26)
CC	69 (75.0%)	86 (67.7%)		
CT	20 (21.7%)	35 (27.6%)		
TT	3 (3.3%)	6 (4.7%)		
Allele C	0.9	0.8		
Allele T	0.1	0.2		
ABCC4 (rs4148551)	89	116	0.80	TT *vs.* others: 1 (0.42-2.78)
CC	25 (28.1%)	41 (35.3%)		
CT	44 (49.4%)	50 (43.1%)		
TT	20 (22.5%)	25 (21.6%)		
Allele C	0.5	0.6		
Allele T	0.5	0.4		
ABCC4 (rs3742106)	89	120	0.80	CC *vs.* others: 1.09 (0.46-2.57)
AA	26 (29.2%)	38 (31.7%)		
AC	46 (51.7%)	55 (45.8%)		
CC	17 (19.1%)	27 (22.5%)		
Allele A	0.6	0.5		
Allele C	0.4	0.5		
ABCB1 (rs1045642)	92	105	0.20	AA *vs.* others: 0.56 (0.23-1.35)
GG	29 (31.5%)	28 (26.7%)		
GA	44 (47.8%)	56 (53.3%)		
AA	19 (20.7%)	21 (20.0%)		
Allele G	0.6	0.5		
Allele A	0.4	0.5		
CYP2A6 (rs28399433)	86	129	0.20	CC *vs.* others: 1.83 (0.72-4.63)
AA	65 (75.6%)	87 (67.4%)		
AC	18 (20.9%)	34 (26.4%)		
CC	3 (3.5%)	8 (6.2%)		
Allele A	0.9	0.8		
Allele C	0.1	0.2		
CYP2A6 (rs8192726)	88	117	0.90	AA *vs.* others: 0.98 (4.07-2.38)
CC	65 (73.9%)	69 (59%)		
CA	14 (15.9%)	39 (33.3%)		
AA	9 (10.2%)	9 (7.7%)		
Alelle C	0.8	0.8		
Allele A	0.2	0.2		
DPYD (rs1760217)	91	99	0.40	GG *vs.* others: 1.41 (0.64-3.10)
AA	62 (68.1%)	51 (51.5%)		
AG	26 (28.6%)	39 (39.4%)		
GG	3 (3.3%)	9 (9.1%)		
Allele A	0.8	0.7		
Allele G	0.2	0.3		
DPYD (rs1801265)	92	133	0.60	GG *vs.* others: 0.72 (0.22-2.41)
AA	43 (46.7%)	76 (57.1%)		
AG	40 (43.5%)	46 (34.6%)		
GG	9 (9.8%)	11 (8.3%)		
Allele A	0.7	0.7		
Allele G	0.3	0.3		

GGH (rs3758149)	81	122	0.20	AA *vs.* others: 1.65 (0.71-3.86)
GG	31 (38.3%)	36 (29.5%)		
GA	34 (42%)	62 (50.8%)		
AA	16 (19.8%)	24 (19.7%)		
Alelle G	0.6	0.6		
Allele A	0.4	0.4		
ITGB5 (rs10049380)	89	110	0.30	CC *vs.* others: 1.48 (0.65-3.34)
TT	39 (43.8%)	37 (33.6%)		
TC	33 (37.1%)	56 (50.9%)		
CC	17 (19.1%)	17 (15.5%)		
Allele T	0.6	0.6		
Allele C	0.4	0.4		
SLC22A7 (rs4149178)	92	125	0.70	GG *vs.* others: 1.18 (0.54-2.56)
AA	55 (59.8%)	67 (53.6%)		
AG	32 (34.8%)	48 (38.4%)		
GG	5 (5.4%)	10 (8%)		
Allele A	0.8	0.7		
Allele G	0.2	0.3		
SLC22A7 (rs2270860)	93	117	0.90	TT *vs.* others: 0.96 (0.44-2.12)
CC	30 (32.3%)	52 (44.5%)		
CT	42 (45.2%)	48 (41%)		
TT	21 (22.5%)	17 (14.5%)		
Allele C	0.5	0.6		
Allele T	0.5	0.4		
SLC29A1 (rs760370)	93	124	0.80	GG *vs.* others: 1 (0.51-2.35)
AA	42 (45.2%)	49 (39.5%)		
AG	36 (38.7%)	57 (46.0%)		
GG	15 (16.1%)	18 (14.5%)		
Allele A	0.6	0.6		
Allele G	0.4	0.4		
UMPS (rs1801019)	93	120	0.07	CC *vs.* others: 2.02 (0.93-4.39)
GG	54 (58.1%)	47 (39.2%)		
GC	26 (28.0%)	55 (45.8%)		
CC	13 (14.0%)	18 (15.0%)		
Allele G	0.7	0.6		
Allele C	0.3	0.4		

[a]Logistic regression adjusted for the confounding variables (age and sex).