Polymorphisms of xenobiotic-metabolizing and transporter genes and the risk of gastric and colorectal cancer in an admixed population of Brazil.

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Abstract

Introduction

Colorectal (CRC) and Gastric (GC) cancers are responsible for considerable morbidity and mortality worldwide. In the North region of Brazil, these neoplasms are among the three most incident and aggressive types of cancer, constituting a severe problem of public health. Single Nucleotide Polymorphisms (SNPs) of xenobiotic metabolism and transporter genes may play a role in individual response to exposure to some of the compounds implicated in the cancer susceptibility. However, few studies have demonstrated the role of polymorphisms of xenobiotic metabolism and transporter genes in the susceptibility to CRC or GC in admixed populations. In this context, the study of variation in the activation and detoxification processes of xenobiotics may help to clarify the development of either GC or CRC in substructured populations, providing new insights about predictive diagnostic criteria in oncological investigations.

Methods

We performed an association study using 31 SNPs in 15 xenobiotic metabolism and transporter genes. The study was carried out in 121 CRC and 95 GC cases and 140 control individuals from Belém, a city which comprises a population with high levels of miscegenation, located in the Brazilian Amazon. Samples were genotyped using the QuantStudio™12K Flex Real-Time PCR System. Due to the high level of genetic admixture in the studied population, we applied a panel of 61 Ancestry Informative Marker standardized by our research group in an earlier study. Statistical analyses were performed in SPSS v.20.0 and Structure v.2.3.4

Results

The results revealed a significant association between the increased CRC or GC risk and polymorphisms of *ABCG2* (rs2231142) and *DPYD* genes (rs17116806, rs1801159).

Conclusions

Our data suggest that polymorphisms in xenobiotic-metabolizing and transporter genes may be relevant to the susceptibility to both CRC and GC.

Introduction

Gastric and colorectal cancers are the main causes of cancer deaths worldwide [1] [2]. In northern Brazil, the incidence of these types of neoplasm is relatively high in comparison with the average rate observed in other regions of the country [2]. The carcinogenesis of colorectal (CRC) and gastric (GC) cancers is still unclear. A number of studies have indicated that the exposure to several environmental or lifestyle risk factors have been identified for CRC and GC, such as obesity, sedentary behavior, high intake of hypercaloric foods, diets rich in fat and poor in fiber, tobacco smoking and alcohol consumption [3][4][5]

[6]. In addition, the genetic makeup is hypothesized to play an important role in the sporadic form of these neoplasms. Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variations in the human genome, and from a clinical perspective, are potential diagnostic and therapeutic biomarkers in many cancer types. SNPs in xenobiotic metabolizing and transporter genes are known to modify the activity of their encoded enzymes, resulting in an increase or decrease predisposition (depending on the pathway of each enzyme) for the development of gastric or colorectal neoplasms [6][7][8][9]. However, few studies have demonstrated the role of polymorphisms in xenobiotic metabolism and transporter genes in the susceptibility to CRC or GC in admixed populations, such as the Brazilian one. This factor is highly relevant because if subjects of a genetic association study are sampled from two or more subpopulations for which the frequencies of alleles and traits differ; spurious associations may arise due to the population substructure [10].

Given this, the goal of this work was to investigate the role of 31 polymorphisms in 15 xenobiotic metabolizing and transporter genes and the influence of genetic ancestry in CRC and GC susceptibility in a population from the Brazilian Amazon region with a high degree of interethnic admixture.

Patients And Methods

Study participants

In this study, we included three groups: (1) 95 individuals with GC diagnosis; (2) 121 individuals with CRC diagnosis; and (3) 140 cancer-free individuals. The cancer-free individuals did not have personal or familial histories of any type of cancer and they did not show any symptoms or signs of cancer. All individuals resided in Belém, which is a city located in the northern region of Brazil. All patients were treated at two local hospitals: Ophir Loyola Hospital and University Hospital João de Barros Barreto. All selected participants were from the same geographical area and groups of similar socioeconomic status.

Ethics, consent and permissions

All participants gave written informed consent to participate and publish this investigation. The protocol used in the study was approved by the Ethics Committee of the University Hospital João de Barros Barreto (protocol number 231.244/2013) and Ophir Loyola Hospital (298.994/2013).

DNA extraction and quantification

Peripheral blood samples were collected from all individuals of the study and the DNA extraction was performed with commercial kit Biopur KitPlus Mini Spin Extract–250 (Biopur, Brazil) according to the manufacturer's instructions. DNA quantification was performed with NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States).

Analysis of the polymorphisms

Samples were genotyped using QuantStudio™12K Flex Real-Time PCR System by TaqMan Open Array Genotyping (Applied Biosystems, Life Technologies, Carlsbad, USA), according to the protocol published by Applied Biosystems.

Analysis of genetic ancestry

To avoid misinterpretations caused by a high level of genetic admixture in the studied population, we applied a panel of 61 ancestry-informative markers as described previously [11]. The ancestral populations included representatives of three major ethnic groups, including Amerindian from the Brazilian Amazon, African and European populations. More details on these populations can be found in [12]. The genomic ancestry was performed in Structure v.2.3.4 software.

Statistical analysis

The association analyses were performed in SPSS v.20.0 (IBM Corp., Armonk, NY, United States) using Student's t-test, Pearson's χ^2 test, Mann-Whitney test and logistic regression. The genotype distribution was assessed as established by Hardy-Weinberg equilibrium (HWE). A p -value ≤ 0.05 was considered statistically significant.

Results

Demographic characteristics

A total of 356 individuals were analyzed in the present study. Demographic data about case and control groups are presented in Table 1 and Table 2, for GC and CRC, respectively. Regarding sex, the CG case group was predominantly composed of men. Differently, the CRC case group had a predominance of women. Significant differences were found between the two groups (GC and CRC) related to age and sex, therefore, these variables were controlled for in the logistic regression. Genomic ancestry analysis for the CG group showed 45% of European, 22% African and 33% Amerindian ancestries.

The same analysis for CRC patients demonstrated 50% European, 30% Amerindian, and 20% African ancestries. No significant association was found in the genomic ancestry analysis for GC or CRC.

Quality control and Genotyping

A total of 31 polymorphisms were investigated in this study (Table S1). Six variants were excluded from our analysis: nine polymorphisms were out of Hardy-Weinberg Equilibrium and three marker was above the limit of 15% of missing data in Minor Allele Frequency (MAF) analysis. The remaining 19 SNPs were analyzed. Table S2 shows the HWE and MAF data of the 31 polymorphisms and their study status (included or excluded).

Analysis of associations with susceptibility to GC and CRC

Significance associations were found with the investigated polymorphisms in relation to an increased GC risk (Table 3). The results indicated that rs2231142 variant of the *ABCG2* transporter gene increases in about 3 times the risk for developing GC ($p = 0.013$; OR = 3.01, 95% CI = 1.26–7.13). Regarding the *DPYD* polymorphisms, the rs1801159 variant demonstrated to increase the risk to develop GC ($p = 0.03$, OR = 2.35, 95% CI = 1.14–5.05). Similarly, the rs17116806 polymorphism demonstrated to confer greater susceptibility for the development of this cancer type ($p = <0.0001$; OR = 23.25; 95%CI = 66.6–8.33). Individuals homozygous for AA mutant genotype in the rs17116806 of the *DPYD* gene have an approximately 23-fold increased risk for GC development over those with other genotypes. Interestingly, the same polymorphism has been shown the same effect to colorectal carcinogenesis ($p = <0.0001$; OR = 1.31; 95% CI = 30.3–5.5). The data of the other polymorphisms investigated that did not present statistical significance are shown in the supplementary material (Table S3 and Table S4).

Discussion

Colorectal and gastric cancers are among the leading causes of death worldwide [1]. In Brazil, estimates for 2018–2019 from the National Cancer Institute (INCA) indicate more than 417000 new cases of these malignant neoplasms [2]. In the northern region of the country, where our target population resides, GC is the second most prevalent type of neoplasia in men and the fourth in women, whereas the CRC is the fourth most incident in men and the third in women [2].

Although the causes of cancer have not yet been completely elucidated, studies have shown that a large group of mutagen-carcinogenic agents require metabolic activation to allow them to bind to DNA, RNA and proteins; therefore, several environmental components are strong risk factors for GC and CRC development [13][14]. Tomasetti (2017) demonstrated that the percentage of influence of external factors on GC and CRC susceptibility is 55% and 26%, respectively [15], thus, association studies in genetic pathways related to the metabolism and transport of environmental risk factors have aided to better understand the carcinogenesis process in several organs [16][17][18]. Most genetic association studies on cancer are investigations about tumor suppressors or oncogenes. Our analysis, however, proposes the study of xenobiotic-metabolizing and transporter genes, which can also modulate the susceptibility to different types of cancer. Additionally, few of these studies have been performed in admixed populations such as the Brazilian one. The Brazilian population is composed, mainly, by the admixture of Amerindian, European, and African ancestral populations [11][12]. Case-control studies in such admixed populations may be influenced by the variation of allelic frequencies of markers found in each different ethnic groups, which may create biases in the outcomes, especially in investigations of susceptibility to complex diseases, such as cancer [19][20]. In this case, estimates of ethnic admixture must be taken into consideration. Our investigation of genomic ancestry analysis was based on the set of 61 AIMs used in previous genetic studies of complex human diseases[11]. Our research group have performed some studies that demonstrated the influence of population substructure present in the northern region of Brazil with several types of cancer, for example childhood B-cell Leukemia [20][21]and breast and gastric cancer [7]. In the present study, however, no significant difference was found in the ethnic profiles between the case and control groups.

In this study, the polymorphisms shown to be associated with colorectal or gastric cancers are related to xenobiotic metabolism. A major group of mutagenic-carcinogenic agents requires metabolic activation to enable them to bind to DNA, RNA, and proteins. Therefore, genetic polymorphisms in these xenobiotic-metabolizing and transporter genes may account for the individual variation observed in the individual response to exposure [18][19][20].

In our analysis, the rs2231142 variant of the *ABCG2* gene increased approximately 3 times the risk for GC development. The *ABCG2* gene encodes the Human Breast Cancer resistance protein (BCRP)/ATP-binding cassette subfamily G member 2 (*ABCG2*), which is an ATP-binding cassette (ABC) transporter responsible for the active transport of several compounds through extra and intracellular membranes [22]. This protein expression occurs predominantly in the liver and in the apical membrane of the intestinal epithelium, playing an important role in intestinal absorption and mediation of hepatobiliary excretion of its substrates (such as potentially carcinogenic xenobiotics and anticancer drugs) [23]. The BCRP is known as a molecular cause of multidrug resistance (MDR) in several cancer cells, but recently some research has focused on understanding its role as a susceptibility biomarker of human carcinoma cells [24][25]. Gupta et al. found a decrease in mRNA expression of *ABCG2* in colorectal and cervical cancer, suggesting a role of this gene in tumorigenesis through the accumulation of genotoxins and the excess of nitric oxide production, which the author suggests being a common phenomenon in other tissues where this gene is hypo-expressed [26]. Supporting this finding, the study by Liu et al. (2010) demonstrated a differential expression of the BCRP at each carcinogenesis stage [27]. For the promotion of carcinogenesis, the expression of BCRP would be decreased to allow the accumulation of genotoxins and nitric oxide, but in the more advanced stages, BCRP can be positively expressed to efficiently transport chemotherapeutic drugs out of the cancerous cells, protecting them. Therefore, the assessment of differential expressions of BCRP may lead the scientific community to create a new method to evaluate progression, metastasis and to predict therapeutic response of colorectal cancer. The results also corroborate with the findings of Wang and colleagues, who, through an in vitro experiment, demonstrated that the *ABCG2* gene shows dysregulated expression in GC tissues and cells [28]. In this study, the high expression of *ABCG2*/BCRP was correlated with advanced stages and poor prognosis of GC. Deregulated expression of the *ABCG2* gene has further been pointed as a promoter factor to GC that affects cell proliferation and induces resistance to cellular apoptosis. These results corroborate with our analyses, confirming the role of SNPs of the *ABCG2* gene in the initiation and promotion of GC.

Polymorphisms in the *DPYD* gene have also been shown to play a role in gastric or colorectal carcinogenesis. Our data demonstrated that the rs1801159 and rs17116806 polymorphisms are associated with a higher risk of GC susceptibility. Additionally, the rs17116806 has been also associated to an increased susceptibility to colorectal carcinogenesis, demonstrating that the same polymorphism can act on tumorigenesis in different tissues. International regulatory agencies, such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), strongly recommend the monitoring of polymorphisms on the *DPYD* gene for evaluation of therapeutic response in fluoropyrimidine-based treatment [29][30]. Nevertheless, few investigations have studied genetic variations in the *DPYD* gene regarding cancer susceptibility, thus, the variety of clinical manifestations

resulting by mutations in *DPYD* is still not well understood [31]. To date, there are no genetic association studies with the rs1801159 and rs17116806 polymorphisms and the susceptibility to the neoplasms investigated hereby. Therefore, this study is the first to investigate the correlation between *DPYD* polymorphisms and the susceptibility to GC and CRC in the Brazilian Amazon population.

Previous studies have also shown that modifications of the pyrimidines homeostasis and the products of their degradation can result in a number of phenotypic manifestations, including neurological disturbances [32] and gastrointestinal disorders [31]. Tanaka et al. analyzed the rs1801265 variant and the risk to develop six different neoplasms: esophagus, gastric, colon, lung, breast, and lymphomas, suggesting an influence of this variant on the development of these types of cancer [33]. Matáková et al. (2017) demonstrated a significant association with the rs1801160 SNP of the *DPYD* and an increased risk for the CRC development ($p = 0.003$, OR = 3.264, 95% CI = 1.425–7.475) [34]. Edward et al. (2016) showed a comprehensive view of how the polymorphisms in the *DPYD* gene deregulate the pyrimidine and nucleic acid synthesis, consequently, promoting malignant progression of melanoma [35]. A multicenter study concluded that variations in genes involved in the metabolism of pyrimidines, particularly the *DPYD*, may also influence the susceptibility to ovarian carcinoma [36].

From the findings to date, we can infer that relevant clinical interventions is possible by enhancing the knowledge of the basic set of mutations that can lead to gastric and colorectal carcinogenesis. This outcomes creates a new expectation regarding the progress of studies related to the predictive diagnosis of cancer.

Conclusion

In conclusion, our data demonstrate that SNPs in the *ABCG2* and *DPYD* play an important role in gastric and colorectal susceptibility in a highly substructured population from the Brazilian Amazon. The obtained results may help to clarify the genetic factors underlying the GC or CRC development in the studied population.

List Of Abbreviations

- CRC: Colorectal Cancer
- GC: Gastric Cancer
- SNP: Single nucleotide polymorphisms

Declarations

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Authors' contributions

ANCLC, MRF and NPCS designated the study. ANCLC, MRF, JCGR and AACM conducted the molecular genetic study. ANCLC, MRF, DCC and RBA participated in the statistical analyses. ANCLC and MRF wrote the manuscript. DCC and TPS reviewed the manuscript and citations. PPA, SEBS and NPCS were project's coordinators. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

All participants gave written informed consent. The protocol used in the study was approved by the Ethics Committee of the University Hospital João de Barros Barreto (protocol number 231.244/2013) and Ophir Loyola Hospital (298.994/2013).

Consent for publication

All participants consented to the study consented for publication.

Competing interests

All authors have no competing interests with regards to the study. No potential conflicts of interest.

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Tables

Table 1. Demographic variables for patients with gastric cancer and control group.

Variable	Case	Control	<i>p</i> -value
No.	95	140	
Age, years ^a	55.59±9.94	69,44±6,89	0.000 ^b
Gender (Female/Male)	36/59	101/39	<0.001
Genetic Ancestry ^a			
European	0.45±0.16	0.45±0.17	0.87 ^b
African	0.22±0.12	0.23±0.14	0.68 ^b
Amerindian	0.33±0,13	0.32±0.15	0.21 ^b

^aValues are expressed as mean ($\pm$ SD = standard deviation); ^bSignificance determined by Mann-Whitney test.

Table 2. Demographic variables for patients with colorectal cancer and control group.

Variable	Case	Control	<i>p</i> -value
No.	121	140	
Age, years ^a	54.05 $\pm$ 12.06	69.44 $\pm$ 6.89	0.000 ^b
Gender (Female/Male)	67/54	101/39	0.006
Genetic Ancestry ^a			
European	0.50 $\pm$ 0.14	0.45 $\pm$ 0.17	0.015 ^b
African	0.20 $\pm$ 0.10	0.23 $\pm$ 0.14	0.11 ^b
Amerindian	0.30 $\pm$ 0.12	0.32 $\pm$ 0.15	0.77 ^b

^aValues are expressed as mean ($\pm$ SD = standard deviation); ^bSignificance determined by Mann-Whitney test.

Table 3. Genotype distribution of the investigated polymorphisms in patients with gastric or colorectal cancer in comparison with the control group.

Cancer type	Genotype	No. (%) Case	No. (%) Control	p-value ^a	OR (95% IC) ^a
GC	<i>ABCG2 (rs2231142)</i>	93	122	0.013	
	GG	65 (69.9%)	68(55.7%)		GG vs. others:
	GT	25 (26.9%)	51(41.8%)		3.01 (1.26-7.13)
	TT	3 (3.2%)	3 (2.5%)		
	<i>Allele G</i>	0.8	0.77		
	<i>Allele T</i>	0.2	0.23		
GC	<i>DPYD (rs1801159)</i>	94	134	0.03	
	TT	55(58.5%)	49 (36.6%)		TT vs. others:
	TC	35(37.2%)	76 (56.7%)		2.35 (1.14-5.05)
	CC	4(4.3%)	9 (6.7%)		
	<i>Allele T</i>	0.8	0.6		
	<i>Allele C</i>	0.2	0.4		
GC	<i>DPYD (rs17116806)</i>	92	121		
	CC	35 (38%)	95 (78.5%)	<0.0001	AA vs. others:
	CA	41 (44.6%)	26(21.5%)		23.25 (66.6-8.33)
	AA	16 (17.4%)	0 (0.0%)		
	<i>Allele C</i>	0.6	0.9		
	<i>Allele A</i>	0.4	0.1		
CRC	<i>DPYD (rs17116806)</i>	112	121		
	CC	29 (25.9%)	95 (78.5%)	<0.0001	AA vs. others:
	CA	57 (50.9%)	26 (21.5%)		1.31 (30.3-5.5)
	AA	26 (23.2%)	0 (0.0%)		
	<i>Allele C</i>	0.5	0.9		
	<i>Allele A</i>	0.5	0.1		

^aLogistic regression adjusted for confounders: age and sex.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableSup3gastriccancerallelefrequency.docx](#)
- [TableSup4colorectalcancerallelefrequency.docx](#)

- [TableSup1CharacteristicsOfthe31SNPs.docx](#)
- [TableSup2HWEeMAF.docx](#)