Population Stratification Effect on Cancer Susceptibility in an Admixed Population from Brazilian Amazon

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Abstract. *Background/Aim: Many efforts have been made to identify candidate genes involved in cancer susceptibility. The present study aimed to investigate the association between Arg194Trp (XRCC1), Ala222Val (MTHFR) and Arg521Lys (EGFR) polymorphisms (SNPs) and their susceptibility to gastric and breast carcinoma cancer in patients from Brazilian Amazon, controlling population structure interference. Materials and Methods: The SNPs were genotyped by TaqMan® SNP Genotyping Assays. Ancestry was estimated by analysis of a panel with 48 ancestry informative markers. Results: Logistic regression analysis showed an inverse association with a 10% increase in African and European ancestry and cancer risk (odds ratio (OR)=1.919 and 0.676, respectively). In a preliminary Chi-square analysis a positive association between Arg521Lys (EGFR) polymorphism and carcinoma susceptibility was found (p=0.037); however, when two different methodologies to control population structure bias were utilized, this association was lost (p=0.064 and p=0.256). Conclusion: Genetic ancestry influence gastric and breast cancer risk and highlight the importance of population structure inference in association studies in highly admixed populations, such as those from Brazilian Amazon.*

Cancer is the second cause of death worldwide (1). Many efforts have been made in order to identify candidate genes that are possibly involved in cancer susceptibility (2). The X-ray repair cross-complementing group 1 (*XRCC1*), the methylenetetrahydrofolate reductase (*MTHFR*) and the epidermal growth factor receptor (*EGFR*) genes have important functions for DNA synthesis, repair and cellular proliferation making them key candidates to cancer susceptibility studies (3-5).

The *XRCC1* gene, located on chromosome 19 (19q13.2), encodes a crucial scaffold protein that is closely associated with the base excision repair (BER) pathway (3). Single-nucleotide polymorphisms (SNPs) in DNA repair genes have been described to impair their repair capacity, increasing the risk of cancer development (6). One of the most extensively studied SNPs is the Arg194Trp (rs1799782), on exon 6 of *XRCC1* gene (7), which occurs in a highly conserved linker region. This polymorphism could alter the interaction of XRCC1 with other DNA repair proteins within the BER complex, thus increasing the chances of DNA damage (8).

The *MTHFR* gene, located on chromosome 1 (1p36.3) (9), catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides a methyl group to convert homocysteine to methionine. This step is important for DNA synthesis and gene regulation through the methylation process and on the availability of uridylates and thymidylates for DNA synthesis and repair (4). The SNP Ala222Val (rs1801133), on exon 4, is a functional variant, which results in diminished enzyme activity (10).

The epidermal growth factor receptor (EGFR), also known as *ERBB1* or *HER-1*, is a member of the human epithelial receptor tyrosine kinase family, encoded by a gene located on chromosome 7 (7p12.1-12.3) (5). The EGFR molecule is a type I transmembrane glycoprotein with intrinsic tyrosine kinase activity that contributes to signaling cascades with multiple pro-carcinogenic effects, including cell proliferation, inhibition of apoptosis, angiogenesis and invasion (5, 11, 12). The polymorphic variant Arg521Lys (rs2227983), on exon 13, is one of the key polymorphisms within the EGFR signaling pathway and is arising from a

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Table I. *Age and genetic ancestry of cancer and control groups.*

Characteristics	Cases	Controls	*p*-Value*
N	136	127	
Age	69.07±14.7	23.10±4.62	<0.001
Genetic Ancestry			
African ancestry	0.26±0.11	0.20±0.08	<0.001
European ancestry	0.44±0.13	0.52±0.13	<0.001
Native American	0.29±0.12	0.28±0.11	0.170

Values are expressed as mean±SD. *Mann-Whitney test.

single nucleotide change in the extracellular domain within subdomain IV of EGFR (13, 14).

Although several studies have investigated these genes on cancer susceptibility, the results are conflicting among studies in different populations (15-17). To date, polymorphisms in these genes were poorly investigated in the Brazilian population. The Brazilian population is formed by three major ancestral populations, Europeans, Africans and Native Americans and presenting different degrees of admixture from South to North regions (18, 19). The highly admixture nature of the Brazilian population makes the population structure a serious concern to association-studies (20-22). In this way, the aim of this study was to investigate the association between Arg194Trp (*XRCC1*), Ala222Val (*MTHFR*) and Arg521Lys (*EGFR*) polymorphisms with the susceptibility to develop gastric and breast carcinoma cancers in a population from Pará state, Brazil, controlling population structure interference.

Materials and Methods

Study population. A total of 136 patients with gastric and breast cancer (63 gastric carcinomas and 73 breast carcinomas) and 127 healthy subjects (controls) were included in the present study. The samples were collected in João de Barros Barreto University Hospital and Ophir Loyola Hospital, both in Belém, Pará, Brazil. The control group was composed by 127 subjects without cancer, born in Pará, Brazil. Local ethics committee approved this study (protocol numbers 3505/2004 and 043/2008) and written informed consent was obtained from all participating subjects.

Genomic DNA extraction. Genomic DNA was extracted from peripheral blood leukocytes using a phenol-chloroform procedure (23). The DNA concentration and quality was determined by spectrophotometry (Themo Scientific NanoDrop 100; NanoDrop Technologies, Wilmington, DE, USA) at 260/280nm.

Genotyping of SNPs. The XRCC1Arg194Trp (rs1799782), *MTHFR*Ala222Val (rs1801133) and *EGFR*Arg521Lys (rs2227983) gene polymorphisms were genotyped by TaqMan® SNP Genotyping Assays according to the manufacturer's protocol (Applied Biosystems, Foster, CA, USA).

Table II. *Categorical distribution of African and European ancestry in controls and cancer cases.*

Characteristics	Controls n (%)		Cancer n (%)
African ancestry			
0.01-0.10	10 (8.1)		3 (2.3)
0.10-0.20	56 (45.2)		38 (28.8)
0.20-0.30	41 (33.1)		46 (34.8)
0.30-0.40	16 (12.9)		29 (22.0)
0.40-0.50	1 (0.8)		11 (8.3)
0.50-0.60	0 (0)		4 (3.0)
0.60-0.70	0 (0)		1 (0.8)
≥0.70	0 (0)		0 (0)
p-Value		<0.001	
European ancestry			
0.01-0.10	0 (0)		0 (0)
0.10-0.20	1 (0.8)		2 (1.5)
0.20-0.30	7 (5.6)		19 (14.4)
0.30-0.40	18 (14.5)		34 (25.8)
0.40-0.50	23 (18.5)		33(25.0)
0.50-0.60	44 (35.5)		21 (15.9)
0.60-0.70	24 (19.4)		22 (16.7)
≥0.70	7 (5.6)		1 (0.8)
p-Value		<0.001	

Table III. *Odds ratio (OR) and 95% confidence intervals (CIs) of logistic regression model with and without age adjusted for genetic ancestry fractions.*

Characteristic	OR	Cancer 95% CI	*p*-Value
African ancestry			
Risk variation per 10% increase	1.919	(1.455-2.530)	<0.001
Age-adjusted model	6.115	(1.482-25.227)	0.012
European ancestry			
Risk variation per 10% increase	0.676	(0.558-0.820)	<0.001
Age-adjusted model	0.186	(0.051-0.682)	0.011

Estimates of individual ancestry proportions. To estimate the subjects' individual genetic ancestry proportions from ancestral African, European and Native American populations, a panel of 48 ancestry informative markers (IAMs) was performed, as previously described (18).

Statistical analysis. Allele and genotype frequencies were estimated by gene counting. Deviation from Hardy-Weinberg equilibrium was assessed by Chi-square tests with Bonferroni correction. Differences between cases and control samples on age and ancestry were estimated by the *t*-test, Mann-Whitney tests and Fisher's exact test. The individual proportions of European, African and Native American genetic ancestry were estimated using the STRUCTURE software 2.3.3 (24), assuming three parental populations (Europeans, Africans and Native Americans) and running with

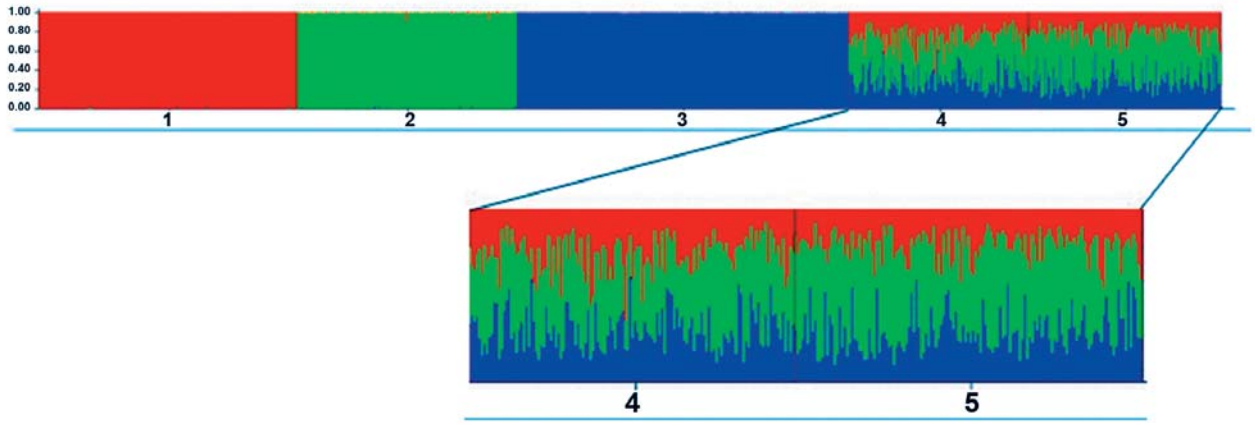

Figure 1. *Genetic ancestry admixture of cancer and control groups. Genetic ancestral composition of 136 patients with gastric and breast cancer (Block 4) and 127 controls (Block 5). Each individual ancestry is depicted as a column, whereas color represents the proportion of ancestry estimated for that individual (African, red; European, green; Native American (NA), blue). Blocks 1, 2 and 3 represent the ancestral populations previously investigated (1=African, 2=European, 3=NA) (20). Genetic ancestry was estimated using the STRUCTURE software.*

100.000 burn-in period and 100.000 Markov Chain Monte Carlo repetitions after burning (25). The STRAT software v. 1.1 (26) was performed to case and control association analysis with 10,000 simulations (27). STRAT utilizes the STRUCTURE output to test for association in the presence of population stratification based on individual ancestry information.

Logistic regression models were performed to investigate African and European genetic ancestry association with cancer and genotypes' association with cancer controlling for genetic ancestry influence.

The Mann-Whitney, *t*-test, Chi-square and logistic regression tests were performed using the SPSS v.18 (SPSS, Chicago, IL, USA). Statistical significance was defined as a two-tailed *p*-value <0.05.

Results

Individual ancestry proportions are shown in Figure 1. Based on these data we determined the mean value of genetic ancestry of all subjects. Age and mean ancestry of cancer patients and healthy subjects are shown in Table I. The case group was older (69.07±14.7 years) than the control group (23.10±4.62 years) ($p<0.001$). The African contribution was more prevalent in the case group ($p<0.001$), whereas the European contribution was more frequent in the control group ($p<0.001$). No statistical difference was found for Native American ancestry.

When comparing the categorical distribution of African and European ancestry in the study population, we observed similar results: different distribution of these ancestries in cancer and control groups (African and European $p<0.001$) (Table II). To determine African and European ancestry influence in cancer susceptibility a logistic regression was performed with and without age as confounder (Table III). In both models, a proportional increase in African ancestry was correlated with cancer risk (odds ratio (OR)=1.919 and 6.115), whereas the increase in European ancestry was inversely correlated with cancer (OR=0.676 and 0.186) (Table III).

Genotype and allelic distribution of Arg194Trp (*XRCC1*), Ala222Val (*MTHFR*) and Arg521Lys (*EGFR*) in control and cancer patients is depicted in Table IV. The genotype frequencies were in Hardy-Weinberg equilibrium ($p>0.05$).

In the Chi-square analysis without ancestry correction, *XRCC1* (Arg194Trp) and *MTHFR* (Ala222Val) polymorphisms were not associated with carcinoma susceptibility ($p=0.241$ and $p=0.134$, respectively); however, *EGFR* (Arg521Lys) polymorphism was associated with cancer susceptibility ($p=0.037$) (Table IV). Two multivariate analyses were performed to control the ancestry influence in association analysis. After the logistic regression model with African and European ancestry as co-variants, no significant association was found (Table IV); however, *MTHFR* and *EGFR* presented borderline *p* values ($p_{ADJUSTED}=0.053$ and 0.064 respectively). When STRAT analysis was performed to correct structure bias, no significant association was found (Table IV).

Discussion

Gastric and breast carcinomas are the two most common cancers in the North region of Brazil. In the present study, the effect of Arg194Trp (*XRCC1*), Ala222Val (*MTHFR*) and Arg521Lys (*EGFR*) polymorphisms on cancer susceptibility was investigated for the first time in a highly admixed Brazilian population.

The admixed population structure is a major concern for genetics studies in American countries (18, 19, 28, 29). Population structure bias could increase the number of false-positive or false-negative results. In studies where allele

Table IV. *Genotype and allelic distribution of XRCC1 (Arg194Trp), MTHFR (Ala222Val) and EGFR (Arg521Lys) variants between Cases and Controls.*

Gene/SNP	Cases n (%)	Controls n (%)	p_{CRUDE}*	$p_{ADJUSTED}$**	p_{PSTRAT}***
XRCC1 (Arg194Trp) rs1799782					
CC	112 (82.4)	98 (77.1)			
CT	24 (17.6)	26 (20.5)			
TT	0	3 (2.4)	0.241	0.126	
C	0.91	0.87			
T	0.09	0.13			0.174
MTHFR (Ala222Val) rs1801133					
CC	56 (41.2)	56 (44.1)			
CT	72 (52.9)	57 (44.9)			
TT	8 (5.9)	14 (11.0)	0.134	0.053	
C	0.68	0.67			
T	0.32	0.33			0.998
EGFR (Arg521Lys) rs2227983					
GG	83 (61.0)	67 (52.8)			
AG	49 (36.0)	48 (37.8)			
AA	4 (2.9)	12 (9.4)	0.037	0.064	
G	0.79	0.72			
A	0.21	0.28			0.256

*p-Value obtained by Chi-square. **p-Value obtained by logistic regression analysis multivariate with African and European genetic ancestry as co-variants. ***p-Value adjusted by STRAT software.

distribution is different among the sub-populations and the general disease risk varies among these sub-populations, population structure acts as a major confounder in case-control associations (30-34).

The present study showed a strong and inverse African and European genetic ancestry influence in cancer risk in the admixed Brazilian population; this influence was carefully taken in account when genetic association was performed. In a preliminary Chi-square analysis a positive association between Arg521Lys (*EGFR*) polymorphism and carcinoma susceptibility was found (p=0.037); however, when two different methodologies to control population structure bias were utilized, this association was lost (p=0.064 and p=0.256). Kittles *et al*. (28) found a similar effect of population structure among African American for prostate cancer. Ignoring potential population stratification within this population, a strong positive association between the CYP3A4 variant and disease was observed (p=0.007). However, after correction of potential population structure, there was an increase in the p value to 0.254 suggesting that this structure may have led to the initial false-positive result (28). Both Arg194Trp (*XRCC1*) and Ala222Val (*MTHFR*) did not show any association to cancer susceptibility in the study population (p>0.05); therefore, several studies investigating these variants present conflicting results as population structure was not taken into account, thus influencing the inconsistency across the results reported (15, 17, 34-36).

There exist several methods to estimate genetic ancestry and identify population stratification (33, 34). In the present study, a panel of 48 IAMs, able to estimate both individual and global ancestry proportions, was performed (18). This panel has already been used in other studies concerning to association with diseases (37-40).

The present work included two unrelated analyses to avoid the population stratification effect in a case control association study. Despite the small sample size, our results demonstrate the potential problem of population structure and highlight the importance to identify and solve this particular issue in association studies, especially in populations with highly ethnic admixture, like the Brazilian Amazon population.

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