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XRCC1 A910G Polymorphism and Gastric Cancer Risk in an Brazilian Population in the Amazon Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to examine the association between the XRCC1 A910G polymorphism in gastric cancer patients in the city of Macapá, State of Amapá, Amazonia, Brazil. DNA samples were obtained from 102 individuals, of which 40 were cancer patients and 62 controls. Polymerase Chain Reaction (PCR) was carried out to detect polymorphism, followed by PCR-RFLP analysis with the restriction enzyme *Hhal*. Of the 40 patients analysed, 22.5% had the Thr910Thr (A/A) genotype, while Ala910Ala (G/G) and Thr910Ala (A/G) genotypes accounted for 25% and 52.5% of samples, respectively. In the control group, of the 62 samples analysed, 74.1% had the Thr910Thr (A/A) genotype, while Ala910Ala (G/G) and Thr910Ala (A/G) represented 9.6% and 16.1% of samples, respectively. Our findings demonstrate that A910G polymorphism was found in most of the patients with gastric cancer in the study population. The G allele was frequently found in the analysed samples, as also observed in the genotype frequency, where AG and GG genotypes were present in cancer patients. This is the first study in Brazil to report the association between A910G polymorphism and gastric cancer.

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1. INTRODUCTION

Gastric cancer is characterised by the growth of abnormal cells in the stomach. It can occur anywhere along its extension, but most cases of this type of tumour are found in the mucous layer, appearing as small and irregular lesions with ulcerations [1]. As cancer progresses, abnormal cells replace normal ones, spreading to other layers of the stomach and affecting peripheral organs [2].

In Brazil, its peak incidence is seen in men of advanced age (nearly 65% of diagnosed patients are over 50 years old), and is the third leading cause of death among men and fifth among women [3].

In 2013, a list of hospitalisation and death rates by gastric cancer in several Brazilian capitals for the years 2005 and 2010 reported that in Macapá, the hospitalisation rate was 3.3 per 100,000 inhabitants in 2005, while lethality was approximately 29%. In 2010, the hospitalisation rate was 8.1 and the lethality was 28% [4].

The occurrence of gastric cancer has been associated with intrinsic factors, as a result of the genetic constitution, and to extrinsic factors, such as consumption of diets with high concentrations of sodium chloride, nitrates, and nitrites contained in smoked and fried foods [5].

Unique polymorphisms of the *XRCC1* gene may affect the expression and function of the *XRCC1* protein. Studies have shown that several polymorphisms of this gene, such as tryptophan (Trp) of arginine (Arg) 194, histidine Arg280 (His), and glutamine Arg399 (Gln), are potentially associated with gastric cancer susceptibility [6].

The DNA repair system consists of a group of proteins encoded by several genes [7] and is a complex, multi-step process involving several proteins and enzymes. Currently, four main pathways for repairing DNA have been identified: base excision, nucleotide excision, double-strand break, and mismatch repair. These DNA repair systems are crucial to maintain the integrity of the human genome [8].

Several studies have suggested that XRCC1 polymorphisms that cause amino acid changes may prevent the interaction of XRCC1 with other enzymatic proteins and consequently alter the process of base excision repair. Since polymorphisms are common and some studies have shown their effect on DNA repair systems, this may increase the susceptibility of some individuals to gastric cancer [9].

In this way, the aim of this work is to investigate whether A910G polymorphism is present in patients diagnosed with gastric cancer in the city of Macapá and analysing its association with this disease for a more accurate diagnosis, along with other possible molecular markers already identified.

2. MATERIALS AND METHODS

The case-control study was carried out in the city of Macapá, state of Amapá, in the Amazon region of Brazil. DNA samples were obtained from 102 individuals, of which 62 were healthy individuals (controls) and 40 were gastric cancer patients of the High Complexity Oncology Unit (Unidade de Alta Complexidade em Oncologia - UNACON) of the Dr. Alberto Lima Clinical Hospital and the Institute of Hematology and Hemotherapy of Amapá (HEMOAP). The study was approved by the Research Ethics Committee (REC) of the Federal University of Amapá (UNIFAP) and was carried out in accordance with the Helsinki Principle Declaration. All individuals signed the Informed Consent Form (ICF).

The protocol used was described in 2013 [10] by a study that analysed the association of A910G polymorphism and its relation with esophageal cancer in a Chinese population. PCR was carried out under the following conditions: 94°C for 5 minutes, 94°C for 30 seconds, 64.2°C for 30 seconds, and 72°C for 30 seconds, 32 cycles and a final extension at 72°C for 8 minutes. The primers used (GenBank reference sequence IDs: NC_000019.9, NM_006297.2, and NP_006288.2) had the following sequence (5'-GACTGCTGGGTCTGAGGGAGG-3', 5'-TCA GCACCACTACCACACCCTG-3').

After amplification of the 238bp PCR product, 10 µl of the product was digested with 1 µL of the restriction enzyme HhaI at 37°C for 16 h in a water bath. Subsequently, 1.5% agarose gel electrophoresis with ethidium bromide was carried out to visualise bands under ultraviolet light. The results of the genotypes followed the

standards: AA (251 bp), AG (251, 169, and 82 bp), and GG (169 and 82 bp).

2.1 Statistical Analysis

All statistical analyses were performed using the software Bio Estat (Ayres, M. Pará, Brazil). Allele and genotype frequencies and general characteristics among gastric cancer patients and controls were analysed using the chi-square test (X2). The odds ratios (ORs) and 95% confidence intervals (95% confidence intervals) of the unconditional logistic regression were used to evaluate the possible associations between genetic variants of XRCC1 and the risk of gastric cancer. Statistical significance was set at $p < 0.05$.

3. RESULTS

Of the 40 gastric cancer patients, 9 were dominant homozygous (AA), 10 were recessive homozygous (GG), and 21 carried the heterozygous mutation (AG). In the control group, of the 62 samples analysed, 46 were normal homozygous (AA), 6 were recessive homozygous (GG) and 10 were mutated heterozygous (AG) for A910G polymorphism. (Table 1). Allele and genotype frequencies and gastric cancer risk are shown in Tables 2 and 3.

4. DISCUSSION

Gastric cancer is a common malignant polygenic disease resulting from complex interactions between several genetic and environmental factors [11,12] with a significant prevalence worldwide [13]. XRCC1 is one of the most important genes implicated in gastric cancer. In recent years, several association studies have been conducted to evaluate the role of XRCC1 polymorphisms, such as Arg194Trp and Arg399Gln, and gastric cancer risk [8,14,15].

The present study was aimed at evaluating XRCC1 A910G polymorphism and gastric cancer risk in the city of Macapá-AP, Brazil. In a study conducted in 2013 [9] that related single nucleotide polymorphisms with the risk of gastric cancer in a Chinese population, a significant association was reported between the polymorphism, more specifically the GG genotype, and a higher susceptibility to gastric cancer in a Chinese population, when compared to the genotypes AA and AG.

Table 1. Distribution of the genotype frequency of *XRCC1* A910G polymorphism in a sample of the population of the city of Macapá-AP

Gastric cancer patients (n=40)					Control group (n=62)				
Gene	**SNP**	**%**	**no SNP**	**%**	**SNP**	**%**	**no SNP**	**%**	***p-value***
XRCC1	31	77.5	9	47.5	10	16.2	52	86.8	P=< 0.0001

SNP: Single nucleotide polymorphism

Table 2. Allele and genotype frequency of XRCC1 A910G polymorphism in gastric cancer patients and controls in a sample of the population of the city of Macapá-AP

	Genotype frequency (%)			Allele frequency (%)	
	AA	**AG**	**GG**	**A**	**G**
Patients (n=40)	09 (22.5)	21(52.5)	10(25)	39(48.7)	41(51.2)
Controls (n=62)	46(74.1)	10(16.1)	06(9.6)	102(82.2)	22(17.7)
Total (n=92)	55(59.7)	31(33.6)	16(17.3)	141(69.1)	63(30.8)
	$X^2 = 26.271$		P=< 0.0001	$X^2 = 25.579$	P=< 0.0001

Table 3. Association between gastric cancer risk and XRCC1 A910G polymorphism

SNPs	**Comparison**	**Association tests**		
		OR (95% CI)	**X^2**	**P-value**
A910G	Homozygous Comparison (GG vs AA)	8.51(2.46-29.40)	13.46	0.0008
	Heterozygous Comparison (AG vs AA)	10.73(3.80-30.03)	23.03	< 0.0001
	Dominant Model (GG/AG vs AA)	9.90(3.88-25.22)	26.15	< 0.0001
	Recessive Model (GG vs AG/AA)	0.32(0.10-0.97)	4.316	0.0721
	Allele Contrast (G vs A)	4.87 (2.58-9.20)	12.03	< 0.0001

Also in 2013 [15] was found an association between the presence of the A910AG SNP and esophageal cancer risk, but these authors pointed out the need to confirm these results in different populations. This was also underlined in another study investigating polymorphisms in XRCC1, MTHFR, and EGFR genes as potential cancer susceptibility markers in a population of Belém-PA [8] and concluded that African and European ancestry are important factors associated to susceptibility to gastric and breast cancers.

In the present study, genotypes and alleles of A910G polymorphism were statistically associated with gastric cancer risk. A significant increase in gastric cancer risk was found in a comparison between homozygous (GG vs AA: OR = 8.51, 95% CI = 2.46-29.40, X^2= 13.46, P= 0.0008), and heterozygous (AG vs AA: OR= 10.73, 95%CI= 3.80-30.03, X^2= 23.03, P= < 0.0001), in a comparison using a dominant model (GG/AG vs AA: OR= 9.90, 95%CI= 3.88-25.22, X^2= 26.15, P= < 0.0001), or the recessive model (GG vs AG/AA: OR= 0.32, 95%CI= 0.10-0.97, X^2 = 4.316, P= 0.0721), and finally in the comparison using the allele contrast model (G vs A: OR= 4.87, 95%CI= 2.58-9.20, X^2 = 12.03, P= < 0.0001) (Table 03).

Table 1 shows significant differences in distribution of the genotype frequency of A910G polymorphism in the analysed samples. The results demonstrate that 77.5% of gastric cancer patients exhibited this polymorphism; 25% of these were associated with the GG genotype and 52.5% with the AG genotype (Table 2). Regarding genotype frequency, in gastric cancer patients the frequency of the G allele (51.2%) was higher than that of A (48.7%).

The allele G was also frequently found in the samples, which is in agreement with the observed in genotype frequency, where AG and GG genotypes were present in gastric cancer patients.

In a study conducted in 2014 [16] that evaluated the A910G XRCC1 polymorphism and the risk of liver cancer in a Chinese population was reported that the frequency of allele A (59.94%) was higher than that of G (40.06%) and that only 17.80% of patients had the GG genotype.

5. CONCLUSION

Gastric cancer is a relatively common genetic disorder in northern Brazil, with a significant mortality rate [17]. In the state of Amapá, this is the third most frequent cancer, which led us to carry out this study. Also, no studies have been conducted in Brazil on the association of A910G polymorphism and gastric cancer. This is first study conducted in Brazil reporting this association. Despite our small sample, especially of gastric cancer patients, future studies should be conducted to evaluate this polymorphism as a genetic marker for gastric cancer risk and contribute to future research aimed at elucidating the facilitation of the acquisition of stomach infection by Helicobacter pylori in patients with polymorphism such as A910G in the XRCC1 gene in a Brazilian context.

CONSENT

All authors declare that written informed consent was obtained from all the patient.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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