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XRCC2 and XRRC4 Gene Polymorphism and Risk of Gliomas

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XRCC genes (X-ray cross complementing group) were found mainly for their roles in protecting mammalian cells against damage caused by ionizing radiation. Studies determined that these genes are important in the genetic stability of DNA. Although the loss of some of these genes does not necessarily confer high levels of sensitivity to radiation, they have been found to represent important components of various pathways of DNA repair. To ensure the integrity of the genome, a complex system of DNA repair was developed. The gene XRCC2 (X – ray cross complementing group 2) is required to repair damage caused when recombination occurs between homologous chromosomes. The gene XRCC4 (X – ray cross complemeting group 4) played an important role in repairing double-strand breaks in DNA at the ends of homologous chromosomes. PCR-RFLP in 80 astrocytomas and glioblastomas brain tumor sample. Patients who had the allele H of the XRCC2 Arg188His and allele T of the XRCC4 G1394T polymorphism had an increased risk of tumor development (OR=13,33; confidence interval at 95%, 95% CI = 6,22 – 28,57; p<0,001) and (OR=6,45; confidence interval at 95%, 95% CI = 2,90 – 14,33; p<0,001). We suggest that XRCC2 Arg188His and XRCC4 G1394T polymorphisms are involved in susceptibility for developing gliomas.

Keywords: Polymorphism; XRCC2; XRCC4; Astrocytoma; Glioblastoma

INTRODUCTION

Tumors of the central nervous system (CNS) represent approximately 2% of all cancers, with an estimated 4,2 to 5,4 per 100.000 individuals per year (Ohgaki and Kleihues, 2005; Tews et al., 2006). Although the incidence of CNS is small compared with other cancers, these among the most serious human malignancies, since they affect the body responsible for coordination and integration of all organic activities. Moreover, as each region of the brain has a vital function the therapy used in other cancers (total surgical removal of the organ or tumor with a generous margin of normal tissue) can´t be applied to cure brain tumors, which undertakes much quality and patient survival (Louis et al., 2002, Ohgaki and Kleihues, 2005; Riemenschneider et al., 2010). Gliomas are the most common tumors of the CNS. Despite the remarkable progress in the characterization of the molecular pathogenesis of gliomas, these tumors remain incurable and, in most cases, refractory to treatment due to their molecular heterogeneity (Kleihues et al., 2002; Riemenschneider et al., 2010).

Astrocytomas account for the large majority of gliomas, making up 70% of the total, and can be divided into: pilocytic astrocytoma (grade I) astrocytomas, incluing low-grade astrocytomas (grade II), anapestic (grade III) and glioblastoma (grade IV) (Kleihues et al., 2002). The relevance of the graduate scheme of malignancy based

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on histopathology is indicated by the patient´s survival.

Patientes with low-grade astrocytomas (grade II) have a median survival of about seven years, patients with anaplastic astrocytomas (grade III) have a mean survival of half of that time (McCormack et al., 1992), while patients with glioblastoma have average 9 to 11 months (Simpson et al., 1993).Unlike astrocytomas astrocytomas, is well documented progression of tumors of grades II and III for most malignant tumor (Ino et al., 2001; Collins, 2004; Hartmann et al., 2004; Ichimura et al., 2004; Ohgaki, 2005; Riemenschneider et al., 2010). Ng and Lam (1998) suggestd the division of glioblastomas in two clinical and molecular entities distinct primary glioblastomas or new, that occur in elderly patients and are clinically very aggressive, and secondary glioblastomas, wich develop from low astrocytomas degree pre-existing and have a more prolonged clinical course.

Human tumors may develop through DNA damage caused by ultra violet rays, ionizing radiation and environmental chemicals. This causes accumulation of DNA damage leading to tumor development and various cellular dysfunctions. The DNA repair system is crucially important for cellular life (Kawabata et al., 2005). To ensure the integrity of the genome, a complex system of DNA repair was developed.

The gene XRCC (X-ray cross complementing group 2) is needed to repair damage caused when recombination occurs between homologous chromosomes (Loizidou et al., 2008; Andreassi et al., 2009). The repair of recombination between homologous is a mechanism that fixes various DNA damages. The large number of repeated sequences can potentially lead to a large number of undesirable interactions between chromosomes. The repair of recombination between homologues plays an important role in maintaining genome integrity. Although it is known that double-strand breaks are the main inducers of chromosomal aberrations, the mechanisms by which these are formed, are still unresolved (Griffin and Tracker, 2004).

Deficiencies in protein XRCC2 lead to an increase in errors in chromosome segregation due to defects in centrosome, resulting in aneuploidy and other chromosomal aberrations as small increases in telomeres (Thacher, 2005). XRCC2 is an important component in the machinery repair between homologous recombination. Changes in this machine due to presence of polymorphisms, lead to damages that result in tumor development (Jiao et al., 2008).

The gene XRCC2 is located on chromosome 7 at 7q36.1 region. A rare polymorphism is located at nucleotide position 31479 in exon 3 Arg188His (rs3218536). This polymorphism is substitution of wild-type allele G by mutant allele A (Jiao et al., 2008).

The DNA repair gene X-ray cross-complementing group 4 (XRCC4), a member of the non-homologous end-joining (NHEJ) repair system, plays a major role in the repair of the double-strand breaks of the DNA sequence.

This gene is critical to the maintenance of overall genome stability, and is also thought to play a key role in human carcinogenesis (Alberts, 2002; Zaha, 2003; Sankaranarayanan, 2006; Chang et al., 2009). XRCC4 is an important member of the non-homologous end-joining (NHEJ) repair system, not only working in conjunction with Ku70/Ku80 and ligase 4, but also playing a major role in the precision end joining of blunt DNA double strand breaks (Taylor e Lehmann, 1998; Xia et al., 2001; Sankaranarayanan, 2006; Mahaney et al., 2009).

The gene XRCC4 (X-ray cross complementing group 4) is located on chromosome 5q14.2 in the region 5. Their protein fulfills an important role in the repair of double strand breaks in DNA at the ends of chromosomes homologous (Hsieh et al., 2008, Yano et al., 2009). Polymorphisms in promoter regions may result in decreased or increased expression of genes. The XRCC4 gene has a polymorphism in the promoter region. This polymorphism G1394T (rs6869366) is characterized by replacement of a guanine for a thymine (Hsieh et al., 2008).

MATERIAL AND METHODS

Study Population

Eighty gliomas were analyzed, which had been surgically resected from previously untreated patients under the care of the Neurosurgery Department of Fundação Pio XII, Cancer Hospital of Barretos (Barretos, SP, Brazil). The samples, classified according to WHO criteria, were: 43 astrocytomas and 37 glioblastomas. The clinical outcome, including length of survival, was obtained from patient records and by contacting each patient's general practioner. For SNP studies, blood samples of 100 healthy individuals were collected as control. Because of the highly heterogeneous ethnic composition of the Brazilian population, the individuals of the control group were selected from the general population of São Paulo State, with no family history of cancer in first-degree relatives.

DNA extraction and primer construction

DNA extraction was performed using proteinase K and phenol-chloroform according to routine molecular biology protocols. Primers were constructed using the Gene Runner 3.05 program (Hasting Software, Inc.) from gene sequence of the XRCC2 Arg 188 His and XRCC4 G1394T polymorphism, obtained in the dbSNP of NCBI (accession numbers: rs3218536 and rs6869366, respectively). Table 1 shows the primers and PCR products sizes.

Table 1. Polymerase chain reaction primer

	Primer	Sequence	Length (bp)	Pcr product (bp)
SNP XRCC1Arg188His	ARG188HIS-F	ATG GAG GAG AAA GTG TGA AC	20	278
	ARG188HIS-R	TGG TTG CTG CTT TGA GAA TC	20	
SNP XRCC4G1394T	F	TAC CTC ACA AAT AAA CTA AG	20	175
	R	GAA AAC GCA AAC AAC TGG CA	20	

PCR = polymerase chain reaction; SNP = single nucleotide polymorphism

PCR was carried out in a final volume of 25 µL containing 50 ng genomic DNA template, 1xPCR buffer with 2 mM MgCl2, 0.4 µM of each primer (Invitrogen), 50 µM dNTPs (Amersham Biosciences) and 0.5 U DNA polymerase (Biotools). The PCR cycling conditions were: 94°C for 5 min, followed by 35 denaturation cycles of 30 s at 94°C, 30 s of annealing at 60°C, and 30 s of extension at 72°C, and a final elongation cycle at 72°C for 5 min. For RFLP, the PCR products were digested by *Sex A* (4 U at 37°C for 16 h – XRCC2 Arg188His) and HpyCh4 III (1,25U at 37°C for 4 h – XRCC4 G1394T). For XRCC2 Arg188His, SexAI recognizes a restriction site at His188 allele (GT/AC) and generates two fragments of different sizes (192 and 84 pb), while Arg188 allele generates only one fragment of 278 bp. For XRCC4 G1394T, HpyCH4 III recognizes a restriction site at G1394 allele (ACN/GT) generates two fragments of different sizes (120 and 56 pb) and T1394 allele generates only one (175 bp). DNA fragments were electrophoresed through a 10% acrylamide:bisacrylamide gel (19:1) and stained with silver nitrate.

PCR products were purified and submitted to bidirectional sequencing, to further confirm the reliability of the genotype analysis. The PCR products were purified with ExoSAP (USB), followed by sequencing with the DYEnamic ET Dye Terminator Kit (Amersham Bioscience), according to instructions.

Statistical analysis

PCR-RFLP. The independence of alleles (Hardy-Weinberg equilibrium) was ensured using the chi-square test. The distribution of genotype and allele frequencies among patients and controls was compared using chi-square and Fisher exact tests. Overall survival curves were obtained using the Kaplan-Meier method and compared with a log-rank test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using a logistic regression model. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with GraphPad InStat 4.0 and GraphPad Prism 5.0 softwares (GraphPad Software, Inc.).

RESULTS

Analysis of tumors and control populations according to the XRCC2 codon Arg188His XRCC4 codon G1394T Eighty patients and 100 control subjects were included in this study. Genotype frequencies in controls and patients were in Hardy-Weinberg equilibrium. Allele and genotype frequencies of XRCC2 Arg188His and XRCC4 G1394T in controls and patients are shown in Table 2. The frequencies of Arg/Arg, Arg/His and His/His among controls were 68, 19 and 13%, while in patients the frequencies Arg/Arg, Arg/His and His/His were 11, 15 and 54%, respectively (P<0,0001). For XRCC4 G1394T the frequencies of G/G, G/T and T/T among controls were 45, 33, 22%, while in patients the frequencies G/G, G/T and T/T were 9, 13 and 58%, respectively (P=0,001).

Logistic regression analysis for the investigation of polymorphism association with risk of astrocitomas and gliobastomas brain tumors is presented in Table 3. Compared to Arg/Arg, the most common genotype of the polymorphism XRCC2Arg188His in the study population, the genotypes with presence of allele His revealed an increased risk of tumor development (OR=13,33; 95% IC, 6,22 – 28,57; p<0,001). When the polymorphism XRCC4 G1394T was analyzed, we observed a increased risk of tumor development for the presence of the allele T (OR=6,45; 95% IC, 2,90 – 14,33; p<0,001).

Comparison of overall survival of patients according to XRCC2 Arg188His genotypes show significant difference (p=0,348) and XRCC4 G1394T genotype show significant differences (p=0,07). In the XRCC2 Arg188His genotype, the median survival of patients with Arg/Arg, Arg/His and His/His was 68 weeks and in XRCC4 G1394T genotype the median survival of patients with G/G, G/T and T/T was 69 weeks. (Figures 1 and 2).

Table 2. Allele and genotype frequencies in case and control groups.

SNP	Genotype	Case Group	Control Group	P
XRCC2 Arg188His	Arg/Arg	11 (13,8)	68 (68,0)	<0,001
	Arg/His	15 (18,8)	19 (19,0)	
	His/His	54 (67,5)	13 (13,0)	
	His	0,77	0,22	<0,001
XRCC4 G1394T	G/G	9 (11,3)	45 (45,0)	0,001
	G/T	13 (16,3)	33 (33,0)	
	T/T	58 (72,4)	22 (22,0)	
	T	0,8	0,38	0,001

Data are reported as number with percent in parentheses. SNP = single nucleotide polymorphism

Table 3. Association ofXRCC1 Arg194Trp and ARG399Gln single nucleotide polymorphisms with risk of cancer

SNP	Genotype	Case/control	OR (95%CI)	P
XRCC2 Arg188His	Arg/Arg	11/68	1	-
	Arg/His	15/19	4,88 (1,93 - 12,36)	<0,001
	His/His	54/13	25,68 (10,66 - 61,83)	<0,001
	Arg/His ou His/His	69/32	13,33 (6,22 - 28,57)	<0,001
XRCC4 G1394T	G/G	9/45	1	-
	G/T	13/33	1,97 (0,75 - 5,15)	0,163
	T/T	58/22	13,18 (5,53 - 31,39)	<0,001
	G/T ou T/T	71/55	6,45 (2,90 - 14,33)	<0,001

SNP = single nucleotide polymorphism.

DISCUSSION

SNPs are recognized as important tools in human genetics and medicine and have been widely used in genetic association studies of various complex diseases, such as cardiovascular, psychiatric and autoimmune disease, obesity, osteoporosis, diabetes and cancer (Curran et la., 2001; Miller and Kwok, 2001; Lin et al., 2003; Tamura et al., 2003; Yamada et al., 2003; Hirai et al., 2005). In humans, several reviews of SNPs have also been conducted throughout the genome with the intention of determining the patterns of the haplotypes in the populations (Daly et al., 2001; Jeffreys et al., 2001; Patil et al., 2001; Reich et al., 2001; Gabriel et al., 2002). Data from these tests are extremely useful for studying the genetic basis common complex diseases (Phillips et al., 2003). Polymorphisms in DNA repair genes may be associated with differences in the efficient repair of DNA damage and may influence the risk for developing tumors. This can result in subtle changes in the structure of proteins from genes and alter their functions (Sreeja et al., 2007).

In this study we determined the relationship between XRCC2Arg188His and XRCC4G1394T and susceptibility to cancer and patient survival in 80 gliomas. The present case-control study showed that the XRCC2 His188 was more frequent in the cancer population than in non-cancer populations (0,77 and 0,22 respectively P<0,001), and that the presence of this genotype may increased the risk of developing astrocytomas and glioblastomas (OR=13,33; 95% IC, 6,22 – 28,57; p<0,001). For XRCC4 G1394T the T1394 was more frequent in the cancer population than in non-cancer populations (0,8 and 0,38 respectively P=0,001), and that the presence of this genotype may increased the risk of developing astrocytomas and glioblastomas T (OR=6,45; 95% IC, 2,90 – 14,33; p<0,001).

Loizidou et al (2008) investigated the association of

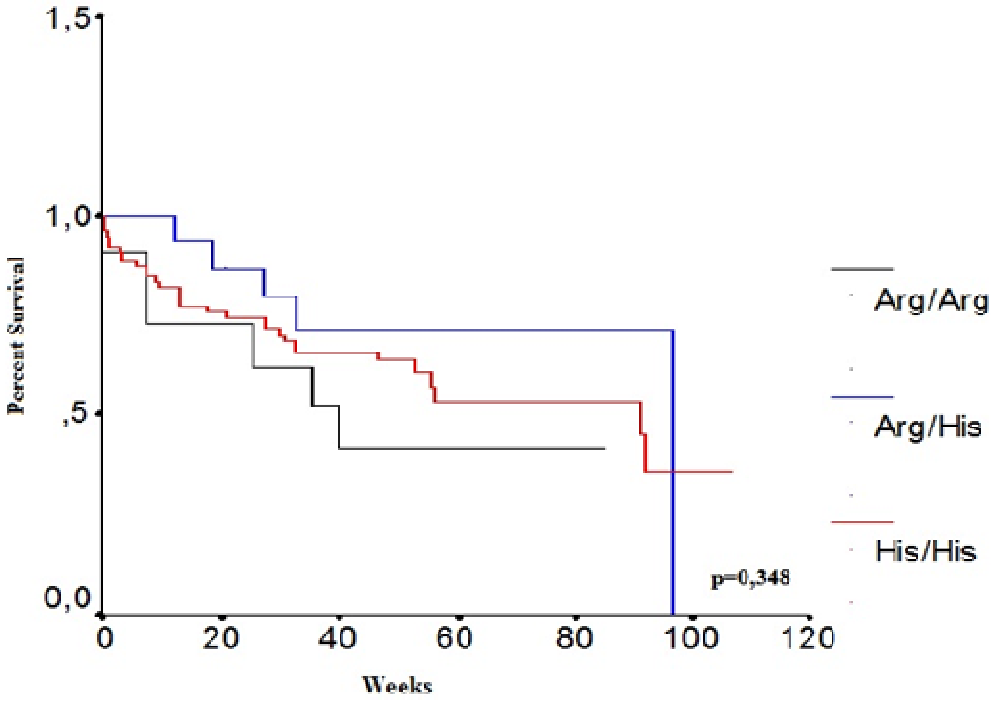

Figure 1. Overall survival in patients according to XRCC2 Arg188His single nucleotide polymorphism.

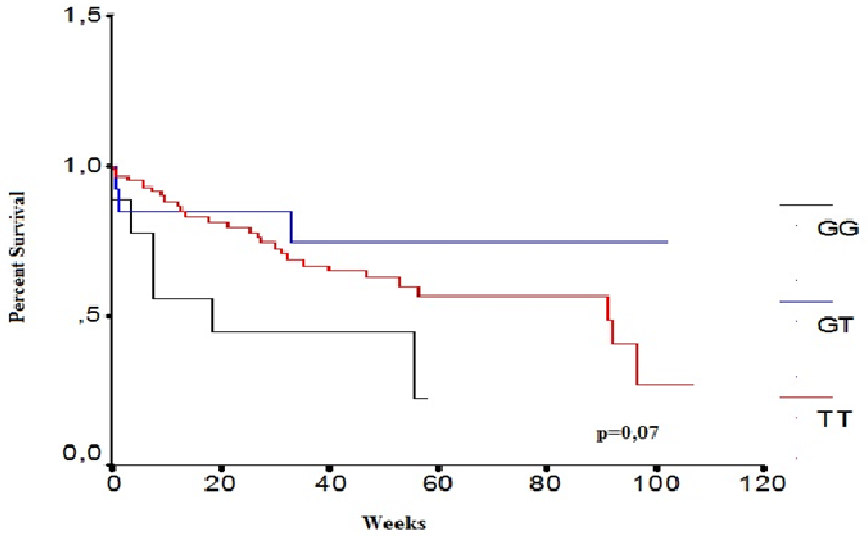

Figure 2. Overall survival in patients according to XRCC4 G1394T single nucleotide polymorphism.

XRCC2 Arg188His polymorphisms in 1109 women with breast cancer and 1,177 controls. The results were: 972 patients had the genotype Arg / Arg 135 genotype Arg / His and one patient had the genotype His / His, while the control group, 999 individuals had the genotype Arg / Arg 177 genotype Arg / His and one individual presented the genotype His / His. The allelic frequency of His allele was 0.06% in case group and 0.08 in the control group. In this study the researchers concluded that individuals who possess the allele polymorphism of the XRCC2 His have

a "protective effect" against the development of breast tumors.

Han et al (2004) analyzed the presence of XRCC2 Arg188His polymorphisms in 220 patients with endometrial cancer and 666 controls. The researchers concluded that the presence of polymorphism in women does not alter the risk for these tumors. In another study by Brooks et al (2008), the presence of XRCC2 Arg188His polymorphism was analyzed in 612 patients with breast cancer and 612 controls. The genotype frequencies of polymorphisms found between cases and controls showed no variations. With these results the authors concluded that the presence of XRCC2 Arg188His polymorphism is not involved in the etiology of breast tumors.

Tranah et al (2004) studied 556 cases of colorectal adenoma and 557 controls. In this study the researchers analyzed whether the presence of XRCC2 Arg188His polymorphism was involved in the etiology of these tumors. The genotypic frequencies for the polymorphism found between cases and controls was similar, noting only that the genotype His / His, was not found between case and control groups. With these results the researchers concluded that this polymorphism is not involved in the etiology of these tumors.

Bau et al (2010) analyzed the presence of XRCC4 polymorphisms G1394T in 370 patients with colorectal cancer and 370 controls. The researchers observed differences in genotype frequencies between case and control groups. For the case group if the observed frequencies were 0.8% for the G / G genotype, 21.9% for genotype G / T and 77.3% for genotype T / T. In the control group the frequencies were 11.9% for genotype G / T, 88.1% for T / T genotype, and for the G / G genotype no individual was detected. The allele frequencies in case group for the T allele were 88.2% and in the control group were 94%, while for the G allele frequency in case group was 11.8% in the control group was 6.0 %. With the data obtained in the study the researchers concluded that the presence of the G allele of the G1394T polymorphism is a risk factor for colorectal cancer etiology.

In the study by Wu et al (2010), XRCC4 G1394T polymorphism was analyzed in 266 children with leukemia and 266 controls. The G / G genotype was not found in the control group nor in the case group. The frequencies of genotype G / T in the case group were 33.1% and in the control group were 20.3%, while for the T / T genotype the pitch in case group was 66.9% in the control group was 79.7%. The researchers concluded that the presence of only one allele G, as in the case of genotype G / T could influence the etiology of childhood leukemia. Hsu et al (2009) studied the presence of XRCC4 polymorphisms G1394T in 164 patients with lung cancer and 640 controls. The researchers concluded that the presence of G allele is a risk factor and that those with genotype G / T exhibit susceptibility to developing lung cancer.

XRCC4 G1394T polymorphism was studied in 158 patients with bladder cancer and 158 controls. The researchers concluded that the presence of genotype G / T and G allele may be involved in the etiology of bladder cancer (Chang et al, 2009). Hsieh et al (2008) analyzed the polymorphism G1394T XRCC4 in 136 patients with endometriosis and 112 controls. The genotype frequencies for the case group were 91.1% for genotype T / T, 5.2% for genotype T / G and 0.7% for genotype G / G. For the control group the frequencies were 79.4% for T / T genotype, 17.9% for genotype T / G and 2.7% for genotype G / G. The researchers concluded that the presence of XRCC4G1394T polymorphism may be associated with susceptibility to endometriosis.

In another study by Hsieh et al (2008), XRCC4 G1394T polymorphism was analyzed in 120 patients with leiomyoma and 112 controls. The genotype frequencies obtained for the case group were 91.7% for genotype T / T, 6.7% for genotype G / T and 1.7% for the G / G genotype, and the frequency of allele T 95 % And 5% for allele G. In the control group the frequencies were 79.4% for T / T genotype, 17.9% for genotype G / T and 2.7% for genotype G / G while the frequencies for the T allele were 88.4% for the G allele 11.6%. The researchers concluded that the 1394T polymorphism present in the XRCC4 gene is associated with high susceptibility to the development of leiomyoma. Chiu et al (2008) analyzed the polymorphism G1394T gene XRCC4 in 636 oral cancer cases and 636 controls. The G / G genotype was not detected between case and control groups, the frequencies for the T / T genotype in case and control groups were 91.5% and 94% respectively while for the genotype G / T of these frequencies were 8.5 in case group and 6% in the control group. With these results the authors concluded that the polymorphism G1394T XRCC4 gene is not involved in the etiology of oral cancer.

In summary, our study provides evidence that the XRCC2Arg188His and XRCC4 G1394T polymorphism may contribute to the etiology of human astrocytomas and glioblastomas, since the allele His188 and T1394 was found more frequently in patients than in controls and its presence is associated to the genotype and to the patients survival

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