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Polymorphism of *XRCC7* Gene and Risk of Glioma: A Prospective Case–Control Study

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Abstract

Objective The aim of this study is to determine association between polymorphism of XRCC7 gene and glioma.

Materials and Methods A case–control study was designed to analyze the prevalence of the various XRCC7 genotypes in 30 cases of histologically proven glioma and 30 age- and sex matched controls.

Result There is significantly higher prevalence of the GT and the TT genotypes of XRCC7 gene in cases of glioma as compared with control. The prevalence was significantly pronounced in two subgroups–middle aged and male gender. The presence of the XRCC7 GT or TT genotype conferred a significantly higher risk of developing glioma (odds ratio: 13.021,2.114–80.213).

Conclusion The presence of the T allele in XRCC7 polymorphism may increase the susceptibility to glioma.

Keywords
- glioma
- XRCC7 polymorphism

Introduction

Gliomas are increasing in incidence and its occurrence is multifactorial. Many genetic studies have been conducted to relate with its cause. Any mutation in DNA repair genes such as p53, Rb, Xray Repair Cross-Complementing (XRCC), and RAD is considered as one of the causes of neoplastic pathology. The *XRCC7* gene is one of the DNA repair gene involved in the repair of nonhomologous end joining double-strand breaks.[1] Mutation in this gene can lead to genomic instability.[2–4] In this study, we studied the association of *XRCC7* gene polymorphisms in 30 patients of glioma and 30 age- and sex-matched controls who were admitted in the neurosurgery department for pathologies other than brain tumors such as aneurysm and disc prolapse.

Materials and Methods

This prospective observational study was done in blood samples taken from 30 cases suffering from glioma. Age- and sex-matched controls are selected from the patients with noncancerous central nervous system pathologies and who were biologically unrelated to cases.

The cases were interviewed and various parameters including demographic profile, clinical symptoms, neuro-imaging, histological subtype, and gene expression were recorded. For the controls, only demographic variables and the XRCC7 genotype were recorded. All newly diagnosed, histologically confirmed glioma cases were included in the study. Recurrent gliomas were excluded from our study.

Blood (2 mL) was collected in ethylenediaminetetraacetic acid tubes was kept at 4°C and one aliquot were stored at

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−80°C. Quantification of DNA was done by measuring the optical density at 260 nm in a nanodrop or spectrophotometer (Hitachi). Genotypes for the XRCC7 G6721T polymorphism were determined using purified DNA. We used the primers mentioned by Wang et al.[1] 5′-CGGCTGCCAACGTTCTTTCC-3′ (nucleotides 6626–6645) was the sense primer, with 5′-TGCCCTTAGTGGTTCCCTGG-3′ (complementary to nucleotides 6974–6993; GenBank accession no. L27425) being the antisense primer.[5]

The primers amplified 368-bp fragment containing the G/T variant in intron 8, which were then subjected to digestion with *Pvu*II (New England BioLabs, Inc., Beverly, Massachusetts, United States) at 37°C overnight. The homologous GG allele had only one band of 368-bp; the heterozygous allele (GT) had three bands of 368, 274, and 94 bp; and the homozygous TT allele had two bands of 274 and 94 bp because of the gain of the restriction site. The 20 µL polymerase chain reaction (PCR) mixture had 50 ng of genomic DNA, 5.0 p-mol of each primer, 0.1 mM of each deoxy-nucleoside triphosphate, 1 x PCR buffer [50 mM KCl, 10 mM Tris HCl (pH 9.0 at 25°C), and 0.1% Triton X-100], 1.5 mM MgCl2, and 1.0 units of Taq polymerase (Sigma-Aldrich Biotechnology, Saint Louis, Missouri, United States). The PCRs were performed using PTC-200 DNA Engine (MJ Research, Inc., Watertown, Massachusetts, United States), with profile consisting of an initial melting step of 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 45 seconds, followed by a final elongation step of 72°C for 10 minutes with a minor modification for *XRCC7* gene.

Agarose gel (1%) was used to check the PCR products, and 2% agarose gel stained with ethidium bromide was used to visualize restriction digested products, which were photographed using the Digital Imaging System.

We measured the frequency of the G and T allele of the *XRCC7* gene in cases and controls (►**Fig. 1**).

Lane1 = 100 Bp ladder

Lane 2, 3,6,7,8 = heterozygous (GT)

Lane 4 = mutant type (TT)

Lane 5 = homozygous wild (GG)

Lane 10= ladder (50bp)

After running PCR, 10 lanes on agarose gel were created. First and last lanes were designated as L lane and loaded DNA marker into these L lanes. These first and last DNA marker lanes have bands of 100 and 50bp, respectively.

GG genotype—1 band (368bp)

GT genotype—3 band (94bp, 274bp, 368bp)

TT genotype—2 band (94bp, 274bp)

Statistical Analysis

Data was stratified into subgroups based on demographic variables and histopathological findings. Fisher's exact test and chi-squared test were used to evaluate the differences in the frequency distribution of age, gender, and the XRCC7 (G6721T) genotypes. To study the association between XRCC7 (G6721T) genotype and the risk of glioma, a multivariate logistic analysis was done. For statistical analysis, the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM) was used.

Results

The frequency distributions of various demographic parameters such as age, gender, and XRCC7 genotype have been summarized in ►**Table 1**. The vast majority of cases were in the age groups of 20 to 40 years ($n = 14$, 46.67%) and 40 to 60 years ($n = 10$, 33.33%). Only one case (3.33%) was below the age of 20 years and five (16.67%) cases were above the age of 60 years. The controls also had a similar distribution. Most of them were in the age groups of 40 to 60 years ($n = 11$, 36.67%) and 20 to 40 years ($n = 10$, 33.33%). Three (10%) controls were below 20 years and 6 (20%) were above 60 years of age. The distribution of cases and controls in different age groups was analyzed

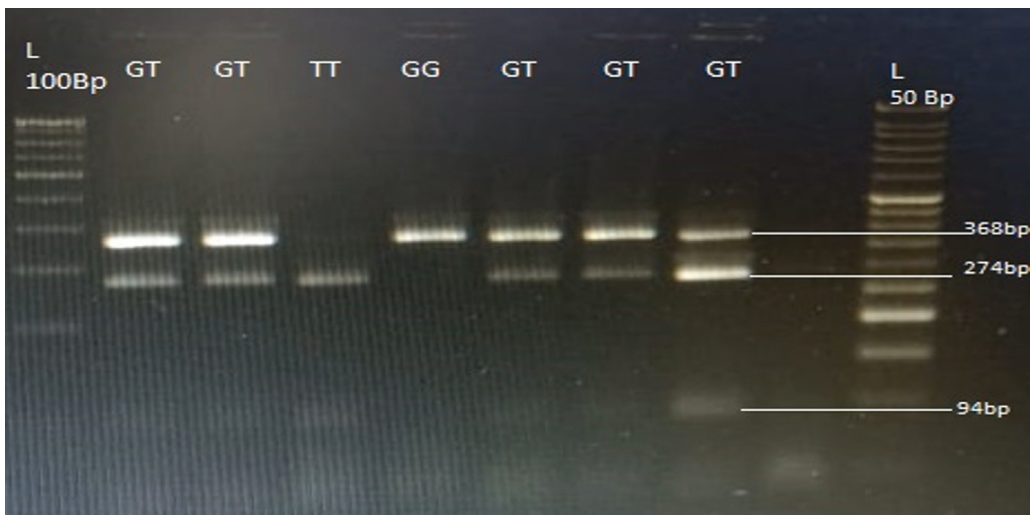

Fig. 1 Polymerase chain reaction amplicon electrophoresis on agarose gel after restriction digestion.

Table 1 Comparison of the age, gender, and XRCC7 genotype in cases and controls

	Case (*n*, %)	Control (*n*, %)	*p*-Value	Test performed
Age group				
<20	1 (3.33)	3 (10)	0.614	Chi-squared test
20–40	14 (26.67)	10 (33.33)		
40–60	10 (33.33)	11 (36.67)		
>60	5 (16.67)	6 (20)		
Gender				
Female	12 (40)	18 (60)	0.196	Chi-squared test
Male	18 (60)	12 (40)		
XRCC7 (G6721T) genotype				
GG	2 (6.66)	12 (60)	0.009	Fisher's exact test
GT	13 (43.33)	9 (30)	0.007	Fisher's exact test
TT	15 (50)	9 (30)	0.003	Fisher's exact test
GT + TT	28 (96.67)	18 (80)	0.002	Fisher's exact test
Frequency of G and T allele of XRCC7(G6721T)				
T allele frequency	43	27	0.003	Chi-squared test
G allele frequency	17	33		

using chi-squared and no statistically significant difference (p-value = 0.614) was found between them.

Out of a total of 30 cases, 12 (40%) subjects were female and the remaining 18 (60%) subjects were male. On the other hand, the control group had 18 (60%) females and 12 (40%) males. The chi-squared test was used to test for significance and no statistically significant difference was found between the distribution of gender in the two groups (p-value = 0.196). Thus, the cases and controls were well matched with respect to age (p-value = 0.658) and gender (p-value = 0.196).

Out of a total of 30 cases, two (6.67%) subjects were having the GG genotype. The remaining 28 (93.33%) cases were having at least one T allele. Out of these 28 cases, 13 (46.43%) subjects were of the GT genotype and the remaining 15 (53.57%) subjects were having the TT genotype. The control group consisted of 12 (40%) patients of GG genotype and 9 (30%) subjects each of GT and TT genotypes. Thus, 60% ($n = 18$) of the control subjects had at least one T allele. The chi-squared test was used to test for differences in the overall distribution and a statistically significant difference (p-value = 0.009) was found between the groups.

In the cases, there were 43 (71%) T alleles and 17 (28%) instances of G alleles. On the other hand, the controls consisted of 27 counts of T alleles (45%) and 33 instances of G allele (55%). The frequency of the XRCC7 T allele was significantly (p-value = 0.003) higher in cases ($n = 43$) as compared with controls ($n = 27$). Furthermore, as compared with the GG genotype, the prevalence was higher for the GT genotype (p-value = 0.007) as well as the TT genotype (p-value = 0.003) in patients with glioma. Findings are suggestive of the fact that the presence of the T allele in the *XRCC7* (G6721T) gene may be a risk factor for glioma.

The cases and controls were further stratified into subgroups on the basis of age group and gender as summarized in ►**Table 2**. Of the 15 cases in the age group less than 40 years, 1 subject had the GG genotype, 7 had GT, and the remaining 11 had TT genotype. The corresponding number in controls in the same age group was 6, 3, and 4, respectively. No significant difference was found between the groups (p-value = 0.063).

In the age group 40 to 60 years, there were 0, 6, and 4 cases, respectively, with the GG, GT, and TT genotypes. The corresponding number for the controls was 6, 3, and 2, respectively. Significant difference was found between the two groups (p-value = 0.029).

In the age group more than 60 years, there were 1, 2, and 2 cases, respectively, with the GG, GT, and TT genotypes. The corresponding number for the controls was 0, 3, and 3, respectively. No significant difference was present between the two groups (p-value = 0.98).

In the males, in the control group, there were 8, 2, and 2 subjects, respectively, who had GG, GT, and TT genotypes, respectively. The corresponding number in the cases was 0, 7, and 11, respectively. There was a significant (p-value <0.001) difference in the frequency distribution of the genotypes between cases and controls in males. As for females, the controls consisted of 4, 7, and 7 subjects each with GG, GT, and TT genotypes, respectively. The corresponding number for cases was 2, 6, and 4, respectively. No significant difference was present between the cases and controls in female groups (p-value = 0.828).

Table 2 The subgroup analysis of XRCC7 genotype frequency in cases and controls

XRCC7 (G6721T) genotypes	Cases			Control			*p*-Value	Test performed
	GG	GT	TT	GG	GT	TT		
Gender								
Males	0	7	11	8	2	2	<0.001	Fisher's exact test
Females	2	6	4	4	7	7	0.828	Fisher's exact test
Age group								
Age <40 years	1	5	9	6	3	4	0.063	Fisher's exact test
Age 40–60 years	0	6	4	6	3	2	0.029	Fisher's exact test
Age >60 years	1	2	2	0	3	3	0.98	Fisher's exact test

Thus, the subgroup analysis revealed that as compared with controls, male patients (p-value < 0.001) and in the age group 40 to 60 years in glioma group (p-value $= 0.029$) had a significantly higher number of cases with GT or TT genotype than the GG genotype.

►**Table 3** shows the stratification of cases into subgroups on the basis of histopathological subtype: histological grade and neuroimaging parameters (T2 fluid attenuated inversion recovery [FLAIR] and postcontrast enhancement). Of the five cases who had a histopathological grade 2 glioma 1, 2, and 2, respectively, had the genotypes GG, GT, and TT. The corresponding number in grade 3 and grade 4 was 0, 3, 6 and 1, 8, and 7 subjects, respectively. Using the GG genotype as reference, there were no significant differences in the prevalence of the GT or TT genotypes of cases (p-value $= 0.63$).

On classifying the cases on the basis of histological subtype, there were 0, 1, and 4 cases of astrocytoma with the genotypes GG, GT, and TT. The corresponding number for glioblastoma and oligodendroglioma was 1, 8, 7 and 1, 4, 4, respectively. There was no significant (p-value $= 1.0$) difference in the genotypes of cases based on the histological subtype.

In patients whose imaging revealed a contrast enhancement, there were 1 (5%), 10 (50%), and 9 (45%) patients with genotypes GG, GT, and TT. On the other hand, in patients without any contrast enhancement, the number was 1 (10%), 3 (30%), and 6 (60%), respectively. Even though the proportion of patients with GT/TT genotype was higher in those with contrast enhancement (95%) versus those who did not show contrast enhancement (90%), the difference was statistically not significant (p-value $= 0.492$).

In patients whose imaging revealed a T2 FLAIR mismatch, there were 1 (5%), 9 (45%), and 10 (50%) patients with genotypes GG, GT, and TT, respectively. On the other hand, in patients whose imaging revealed no T2 FLAIR mismatch, there were 1 (10%), 4 (40%), and 5 (50%) patients with genotypes GG, GT, and TT, respectively. Thus, the

Table 3 The stratification analysis of XRCC7 genotype in cases

XRCC7 (G6721T) genotypes	Cases			*p*-Value	Test performed
	GG	GT	TT		
Histological grade					
Grade 2	1	2	2	0.63	Fisher's exact test
Grade 3	0	3	6		
Grade 4	1	8	7		
Histological subtype					
Astrocytoma	0	1	4	1	Fisher's exact test
Glioblastoma	1	8	7		
Oligodendroglioma	1	4	4		
T2 FLAIR mismatch					
Absent	1	9	10	1	Fisher's exact test
Present	1	4	5		
Contrast enhancement					
Absent	1	3	6	0.492	Fisher's exact test
Present	1	10	9		

Abbreviation: FLAIR, fluid attenuated inversion recovery.

Table 4 Logistic regression analysis of XRCC7 genotype and the risk of glioma using age, gender, and XRCC7 genotype as input variables

	Degrees of freedom	*p*-Value	Odds ratio	95% confidence limit for odds ratio	
				Lower	Upper
Age group	1	0.560	1.221	0.624	2.391
Male gender	1	0.059	3.165	0.958	10.458
XRCC7G6721T—GG	2	0.015			
XRCC7G6721T—GT/TT	1	0.006	13.021	2.114	80.213
Constant	1	0.131	0.201		

proportion of patients having different genotype is roughly the same in both the groups and there is no significant (p-value = 1) difference between them.

As summarized in ►**Table 4**, to ascertain the effects of age, gender, and XRCC7 genotypes on the likelihood of developing a glioma, a logistic regression was performed. The logistic regression model was statistically significant ($\chi 2 = 14.365$, $p = 0.006$). The model explained 28.4% (Nagelkerke R^2) of the variance in occurrence of glioma and correctly classified 73.3% of the subjects. Though males were 3.1 times more likely to develop glioma as compared with females, the p-value for the coefficient was not significant (0.059). Cases with the XRCC7 genotype of type GT or TT were 13.02 times more likely to develop a glioma and the difference was statistically significant (p-value = 0.006).

Discussion

Our main aim was to study whether an association exists between the various genetic polymorphisms of XRCC7 that is a DNA repair gene, and the risk of developing glioma. A case–control study was done to analyze the prevalence of the various XRCC7 genotypes in 30 cases of histologically proven glioma and 30 controls matched for age and sex. This is the first study done on *XRCC7* gene in glioma in Indian population.

As compared with the controls, we found a significantly higher prevalence of the GT and the TT genotypes in cases of glioma. The prevalence was significantly pronounced in two subgroups—males and middle age (40–60 years).

The presence of the XRCC7 GT or TT genotype conferred a significantly higher risk of developing glioma (odds ratio: 13.021,2.114–80.213).

Our results are in concordance with the findings of Wang et al,[2] who also found a significantly higher prevalence of the XRCC7 GT/TT genotype in cases as compared with controls, especially in males in the age group 46 to 60 years.

Wang et al also reported a significant difference in the distribution of XRCC7 genotype based on histopathological grades (low as well as high grade). However, our study did not find any difference on grouping the cases by histological subtype or histopathological grade perhaps due to small sample size.

The fact that *XRCC7* gene polymorphism was also present in a significant number in control group points to the multifactorial etiology of the disease. Furthermore, some studies have found that human chromosome number 8q11 (which also contains the *XRCC7* gene) can functionally correct the hyper-radiosensitivity of severe combined immunodeficiency cells, which are otherwise extremely prone to radiation induced DNA damage,[5–7] helping guide the treatment for different types of gliomas.

Even though the exact significance of *XRCC7* gene polymorphisms is not known and other DNA repair genes must surely be playing a significant role in the pathogenesis of glioma, we hope that our study might prove useful in[8]

Contributing to a better understanding of the disease pathogenesis.
Identifying the subjects who are at an increased risk of developing a glioma.

Limitations

The single most important limitation of our study was the small sample size. To put things in perspective, our sample size was 10 times smaller than that of Wang et al (30 controls in our study versus 342 by Wang et al). Additionally, the controls in our study were not healthy controls from the community but admitted in the neurosurgery department for other indications such as aneurysms and stroke. This could be significant if the XRCC7 polymorphisms also play a significant role in these diseases. This is single-center study; perhaps multicenter study would provide better insight in the role of *XRCC7* gene in gliomas.

Conclusion

Certain polymorphisms of the XRCC7 may predispose subjects to glioma, especially in middle-aged males. Though, larger studies are required to investigate our findings. It is imperative that we also investigate the role of other genes involved in DNA repair and their role in the etiopathogenesis of glioma.

Conflict of Interest
None declared.

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