

Evaluation of the association of ERCC2 rs13181 polymorphism with different types of gliomas in patients in Northeast Brazil

Cléciton Braga Tavares

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Larysse Cardoso Campos-Verdes

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Pedro Vitor Lopes-Costa

Postgraduate Program in Health Sciences, Federal University of Piauí, Teresina, Brazil

Frsancisca das Chagas Sheyla Almeida Gomes-Braga

Department of Oncology, São Marcos Hospital, Teresina, Brazil

Elmo Jesus Nery Junior

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Carla Solange Melo Escórcio-Dourado

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Alesse Ribeiro Santos

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Emerson Brandao Sousa

Department of Oncology, São Marcos Hospital, Teresina, Brazil

Renato Oliveira Pereira

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Luiz Henrique Gebrim

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Benedito Borges da Silva (✉ beneditoborges@globocom)

Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), Department of Health, Northeast, Brazil

Research Article

Keywords: ERCC2 gene rs13181 polymorphism, glioma, Brazil

Posted Date: April 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-403362/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Gliomas are the most common primary tumors of the central nervous system with unclear etiology. However, hereditary factors may play an important role in glioma development, with mutations and single nucleotide polymorphisms (SNPs) being prominent among the genetic changes. This study aimed to evaluate the association of the *ERCC2* gene rs13181 variant polymorphism between high- and low-grade gliomas in patients from Brazil's Northeast region. Samples from glioma patients stored in paraffin blocks were used. DNA extraction was performed using the MagMAX™ FFPE DNA/RNA Ultra kit, and for genotyping, the Taqman assay probe C_3145033_10 corresponding to the SNP rs13181 of the *ERCC2* gene was selected. Quantitative and categorical variables were analyzed using the *t*-test and Fisher's exact test or Chi-square test ($p < 0.05$). Patients with low-grade gliomas were younger than those with high-grade gliomas ($p = 0.003$). Statistically significant differences were not observed in the expression of the GG, GT, and TT genotypes between low- and high-grade gliomas. The *ERCC2* rs13181 genotypes found were TT, GG, and GT; however, no difference in the expression between different glioma types was observed.

Introduction

Tumors of the central nervous system (CNS) account for approximately 2% of all cancers, with a worldwide incidence of 4.2 to 5.4 cases per 100,000 individuals per year, with gliomas being the most common primary brain tumor [1]. In Brazil, more than 10,270 new cases were registered in 2016 [1, 2]. These tumors were classified into low-grade (grades I and II) and high-grade (grades III and IV) gliomas and this classification was important for patient prognosis since low-grade gliomas, such as pilocytic astrocytoma (grade I) and diffuse astrocytoma (grade II), exhibit a better prognosis than high-grade gliomas, such as anaplastic astrocytoma (grade III) and glioblastoma (grade IV)[1].

Environmental and lifestyle factors, such as exposure to ionizing radiation and smoking, have been speculated to be risk factors for the incidence of these neoplasms [3]. However, hereditary factors also play an important role in glioma development, and among the different genetic alterations, mutations and single nucleotide polymorphisms (SNP) are crucial [4–6]. Therefore, these tumors are considered to be caused by cumulative DNA damage, which is secondary to the interaction between the environment and genetic predisposition, making the existence of a DNA repair system critically important for cell life, as it guarantees the integrity of the genome by preventing mutagenesis [7–9].

The *ERCC2* is a gene located on chromosome 19q13.3 and has been found to be responsible for DNA repair via the nucleotide excision repair (NER) pathway. The most frequently identified polymorphism in *ERCC2* is Lys751Gln (rs13181), characterized by the replacement of thymine (T) by guanine (G) at locus 751, which can alter the enzymatic activity of some encoded proteins and is associated with several types of cancer, including gliomas [10]. However, in literature, findings on this SNP and its association with gliomas are contradictory. Accordingly, a study conducted by Chen et al. [11] reported increased risk for glioma development, while McKean-Cowdin et al. [12] showed decreased risk associated with gliomas. In

addition to the conflicting results, the above-mentioned studies are primarily confined to the Asian population.

To the best of our knowledge, there are no such studies in literature based on the Brazilian population, consisting of a rich racial miscegenation, that evaluate the presence of *ERCC2* rs13181 variant polymorphism among patients with gliomas, and this led us to design the current study in patients with different glioma types in Northeast Brazil.

Results

Epidemiological characteristics. Patients with low-grade gliomas had a significantly lower mean age than high-grade glioma patients, 20.05 ± 15.9 and 44.47 ± 18.93 years [mean \pm standard deviation of the mean (SDM)], respectively ($p < 0.001$). Patients with low- and high-grade gliomas were considered homogenous with respect to gender, smoking, alcohol use, and cancer familiar history (Table 1).

Genotypic Expression. Statistically significant differences were not observed in the expression of the GG, GT, and TT genotypes of the SNP *ERCC2* rs13181 between high- and low-grade gliomas (Table 2).

Discussion

Several studies have demonstrated the existence of DNA repair SNPs responsible for the induction and/or progression of neoplasms [10]. Among these polymorphisms, the rs13181 variant of the *ERCC2* gene, also known as *ERCC2* Lys751Gly, can alter the enzymatic activity of some encoded proteins, such as helicase, and may be associated with several types of cancer, including gliomas [10, 13].

To the best of our knowledge, this is the first study to evaluate the expression of SNP rs13181 of the *ERCC2* gene between high- and low-grade gliomas using DNA samples isolated from neoplastic tissue stored in paraffin blocks in Brazilian patients. In the current study, the patient groups with high- and low-grade gliomas were homogeneous regarding sex, smoking, alcohol use, and family cancer history. However, the mean age of patients with high-grade gliomas was significantly higher than those with low-grade gliomas, while no statistically significant difference was observed in the expression of GG, GT, and TT genotypes.

As for the epidemiological characteristics of the patients, some studies reported a higher prevalence of gliomas in males and no association between sex and the histological grade of these tumors [14–16], corroborating the results of the present study. There was no difference between family history of cancer and gliomas, perhaps because this association is more common in certain types of syndromes, such as Li Fraumeni, Turcort, neurofibromatosis, among others [17], which did not occur in this study. There was also no association between gliomas and smoking or alcohol consumption; however, some authors reported a higher risk for high-grade gliomas in cases of excessive smoking [18] and alcohol use [19, 20]. High-grade gliomas occur in older patients [14–16], corroborating the present study results, as cellular aging increases immunological senescence, telomere shortening, chronic inflammation with antigenic

stimulation, genomic instability, and mutations forming more aggressive neoplasms such as high-grade gliomas [17].

Some studies have shown an association between the rs13181 variant of the *ERCC2* gene and the risk of developing gliomas [21, 22, 23]. However, most of these studies were conducted only in Asian populations and showed contradictory results regarding the influence of genotypes of this variant in the development of glial tumors [24–28]. In the present study, the rs13181 SNP of the *ERCC2* gene was found in all samples. However, no statistically significant differences in the expression of the GG, GT, and TT genotypes between high- and low-grade gliomas were observed, consistent with the findings of Qian et al. [24] and Zhou et al. [25], and divergent from those of Huang et al. [26], Cui et al. [27] and Jia et al. [28], who showed an increased risk, particularly in relation to the GT genotype in the Asian population.

The absence of an association between genotypes and the degree of malignancy of glial tumors in the current study may be due to its small sample size and the lack of a consistent genetic pattern in the Brazilian population. Brazil has a rich mixture of Europeans, Africans, and Indians, presenting different results from those found in other countries, where the populations are predominantly Caucasian, Asian, or African [29].

In conclusion, in the current study, patients with low-grade gliomas had a significantly lower mean age than high-grade glioma patients, and the *ERCC2* rs13181 genotypes found were TT, GG, and GT; however, no difference in the expression between high- and low-grade gliomas was found in patients in Northeast Brazil. Nevertheless, further studies should be carried out with a larger sample size due to the rich Brazilian racial miscegenation.

Methods

Patients

This study was approved by the Internal Review Board of the Federal University of Piauí, Brazil and was conducted according to the Declaration of Helsinki. All patients or their legal guardians gave written informed consent. Fifty-one paraffin-embedded tissue samples from patients with histologically confirmed gliomas were obtained from the pathology department's archives. The selected samples were in storage for less than five years. The inclusion criteria considered a histological glioma diagnosis without any previous treatment. An informed consent form was not necessary since the material had been previously collected, being part of the disease's surgical treatment.

DNA Extraction

The previously cataloged biopsy specimens stored in paraffin blocks were cut into 60- μ m thick slices using a microtome and placed in sterile 1.5-mL Eppendorf tubes. The samples were treated with xylene and heated at 50 °C overnight to increase DNA yield and then heated again at 90 °C for 1 h to remove the

paraffin. The material was then washed thrice using 100% ethanol and dried using a vacuum centrifuge to completely remove contaminants.

For DNA extraction, MagMAX™ FFPE DNA/RNA Ultra kit supplied by Applied Biosystems™ (Foster City, CA, USA) was used, wherein magnetic microbeads were used to enable DNA extraction with high performance for sturdy samples. The protocol diligently followed the manufacturer's recommendations, and the degree of purity and concentration of the extracted DNA was assessed using a Nanodrop 2000 spectrophotometer (Thermo-Fisher Scientific, Waltham, MA, USA), wherein the ratio between the 260/280 nm wavelength was measured, and the values considered ideal ranged from 1.8 to 2.0. The samples were normalized to a concentration of 30 ng/μL for later analysis through quantitative PCR.

qPCR for SNP genotyping

For SNP genotyping, a Taqman assay probe C_3145033_10 corresponding to the SNP rs13181 of the *ERCC2* gene was selected (Table 3). The 20-μL reaction comprised 10 μL of TaqMan™ GTXpress™ Master Mix (Applied Biosystems), 4 μL of DNA, and 6 μL of ultrapure water treated with DEPC.

The reaction was conducted using an Applied Biosystems qPCR 7500 FAST thermocycler in fast mode for 40 cycles. The results were analyzed using the Applied Biosystems TaqMan Genotyper Software ver. 1.6.

Statistical analysis

For comparative analysis between the groups, t-test and Fisher's exact or chi-square test were used for quantitative and categorical variables, respectively. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using the adjusted conditional logistic regression for gender and age. The level of significance was set at $p < 0,05$. The statistical data were analyzed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated in this analysis are available from the corresponding

author upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was obtained to conduct this study.

Author Contributions

T CB and *dSBB* conceived and designed the study; *NJEJ*, *E-DCSM* provided study materials and tools; *TCB* and *dSBB* were responsible for the collection and assembly of data, data analysis, and interpretation; *C-VLC*, *G-BFCSA*, *L-CPV*, *dSAR*, *PRO* was involved in writing the manuscript; *TCB*, *dSBB*, and *GLH* revised the manuscript.

Acknowledgments The authors thank the Postgraduate Program of the Northeast Network of Biotechnology (RENORBIO), the largest network Program in Brazil for the qualification of doctors in Biotechnology.

References

1. De Leeuw, B. I., Van Baarsen, K., Snijders, M., Robe, P. A. & T., J. & J. T. Interrelationships between molecular subtype, anatomical location, and extent of resection in diffuse glioma: a systematic review and meta-analysis. *Neurooncol. Adv.* **1**, <https://doi.org/10.1093/noajnl/vdz032> (2019).
2. Calastri, M. C. J. *et al.* Genetic variants related to cell cycle and stability of telomere in patients with glioma. *Asian. Pac. J. Cancer. Prev.* **20**, 2345–2351 <https://doi.org/10.31557/APJCP.2019.20.8.2345> (2019).
3. Gao, X., Tang, Y. J., Zhang, G. F., Yu, L. & Qi, S. T. ERCC2 rs13181 polymorphism association with glioma susceptibility in a Chinese population. *Genet. Mol. Res.* **15**, <https://doi.org/10.4238/gmr.15027585> (2016).
4. Xin, Y., Hao, S., Lu, J., Wang, Q. & Zhang, L. Association of ERCC1 C8092A and ERCC2 Lys751Gln polymorphisms with the risk of glioma: a meta-analysis. *PLoS One.* **9**, e95966 <https://doi.org/10.1371/journal.pone.0095966> (2014).
5. Fan, S. C., Zhou, J. G. & Yin, J. Z. Investigation of the role of XRCC1 genetic polymorphisms in the development of gliomas in a Chinese population. *Genet. Mol. Res.* **15**, <https://doi.org/10.4238/gmr.15038268> (2016).
6. Xu, G. Z., Liu, Y., Zhang, Y., Yu, J. & Diao, B. Correlation between VEGFR2 rs2071559 polymorphism and glioma risk among Chinese population. *Int. J. Clin. Exp. Med.* **8**, 16724–16728 (2015).
7. Li, J. *et al.* Association between XRCC1 polymorphisms and glioma risk among Chinese population. *Med. Oncol.* **31**, 186–190 <https://doi.org/10.1007/s12032-014-0186-2> (2014).

8. Rodriguez-Hernandez, I. *et al.* Analysis of DNA repair gene polymorphisms in glioblastoma. *Gene*. **536**, 79–83 <https://doi.org/10.1016/j.gene.2013.11.077> (2014).
9. Xu, G. Z., Liu, Y., Zhang, Y., Yu, J. & Diao, B. Correlation between VEGFR2 rs2071559 polymorphism and glioma risk among Chinese population. *Int. J. Clin. Exp. Med.* **9**, 16724–16728 (2015).
10. Tavares, C. B. *et al.* Association between single nucleotide polymorphisms and glioma risk: a systematic literature review. *Cancer. Invest.* **38**, 169–183 <https://doi.org/10.1080/07357907.2020.1719502> (2020).
11. Chen, D. Q., Yao, D. X., Zhao, H. Y. & Yang, S. J. DNA repair gene ERCC1 and XPD polymorphisms predict glioma susceptibility and prognosis. *Asian Pac. J. Cancer Prev.* **13**, 2791–2794 <https://doi.org/10.7314/apjcp.2012.13.6.2791> (2012).
12. McKean-Cowdin, R. *et al.* Associations between polymorphisms in DNA repair genes and glioblastoma. *Cancer Epidemiol. Biomarkers Prev.* **18**, 1118–1126 <https://doi.org/10.1158/1055-9965.EPI-08-1078> (2009).
13. Balkan, E. *et al.* ERCC2 Lys751Gln rs13181 and XRCC2 Arg188His rs3218536 gene polymorphisms contribute to susceptibility of colon, gastric, HCC, lung and prostate cancer. *J. BUON.* **25**, 574–581 (2020).
14. Ohgaki, H. & Kleihues, P. Epidemiology and etiology of gliomas. *Acta Neuropathol.* **109**, 93–108 <https://doi.org/10.1007/s00401-005-0991-y> (2005).
15. Posti, J. P. *et al.* Presenting symptoms of glioma in adults. *Acta Neurol. Scand.* **131**, 88–93 <https://doi.org/10.1111/ane.12285> (2015).
16. Rasmussen, B. K. *et al.* Epidemiology of glioma: clinical characteristics, symptoms, and predictors of glioma patients grade I-IV in the the Danish Neuro-Oncology Registry. *J. Neurooncol.* **135**, 571–579 <https://doi.org/10.1007/s11060-017-2607-5> (2017).
17. Pellerino, A., Bruno, F., Internò, V., Rudà, R. & Soffietti, R. Current clinical management of elderly patients with glioma. *Expert Rev. Anticancer Ther.* **12**, 1037–1048. doi: 10.1080/14737140.2020.1828867. Epub 2020 Oct 8. PMID: 32981392.
18. Ahn, S. *et al.* Cigarette smoking is associated with increased risk of malignant gliomas: a nationwide population-based cohort study. *Cancers.* **12**, 1343 <https://doi.org/10.3390/cancers12051343> PMID: 32466105; PMCID: PMC7280985 (2020).
19. Howell, A. E. *et al.* Testing for causality between systematically identified risk factors and glioma: a Mendelian randomization study. *BMC Cancer.* **20**, 508 (2020).
20. Baglietto, L. *et al.* Alcohol consumption and risk of glioblastoma; evidence from the Melbourne Collaborative Cohort Study. *Int. J. Cancer.* **128**, 1929–1934 (2011).
21. Gao, X., Tang, Y. J., Zhang, G. F., Yu, L. & Qi, S. T. ERCC2 rs13181 polymorphism association with glioma susceptibility in a Chinese population. *Genet. Mol. Res.* **15**. doi: 10.4238/gmr.15027585. PMID: 27323065 (2016).
22. Rodriguez-Hernandez, I. *et al.* Analysis of DNA repair gene polymorphisms in glioblastoma. *Gene*. **536**, 79–83 <https://doi.org/10.1016/j.gene.2013.11.077> Epub 2013 Dec 8. PMID (2014).

23. Gao, K., Mu, S. Q. & Wu, Z. X. Investigation of the effects of single-nucleotide polymorphisms in DNA repair genes on the risk of glioma. *Genet. Mol. Res.* **13**, 1203–1211 (2014).
24. Qian, T., Zhang, B., Qian, C., He, Y. & Li, Y. Association between common polymorphisms in ERCC gene and glioma risk: A meta-analysis of 15 studies. *Medicine.* **96**, e6832 <https://doi.org/10.1097/MD.00000000000006832> (2017).
25. Zhou, C. X. & Zhao, J. H. Systematic review on the association between ERCC1 rs3212986 and ERCC2 rs13181 polymorphisms and glioma risk. *Genet. Mol. Res.* **14**, 2868–2875 <https://doi.org/10.4238/2015.March.31.17> (2015).
26. Huang, L. M. *et al.* Association between ERCC2 polymorphisms and glioma risk: a meta-analysis. *Asian Pac. J. Cancer Prev.* **15**, 4417–4422 <https://doi.org/10.7314/apjcp.2014.15.11.4417> (2014).
27. Cui, Q. K., Zhu, J. X., Liu, W. D., Wang, Y. H. & Wang, Z. G. Association of ERCC1 rs3212986 & ERCC2 rs13181 polymorphisms with the risk of glioma. *Pak. J. Med. Sci.* **30**, 1409–1414 <https://doi.org/10.12669/pjms.306.5221> (2014).
28. Jia, T. L., Wu, H. J., Wang, H. B., Ma, W. B. & Xing, B. Association between the ERCC2 rs13181 polymorphism and the risk of glioma: a meta-analysis. *Genet. Mol. Res.* **14**, 12577–12584 <https://doi.org/10.4238/2015.October.19.1> (2015).
29. Costa-Silva, D. R. *et al.* Insulin-like growth factor 1 gene polymorphism in women with breast cancer. *Med. Oncol.* **34**, 59 <https://doi.org/10.1007/s12032-017-0915-4> Epub 2017 Mar 17. PMID (2017).

Tables

Table 1. Epidemiological characteristics of the studied gliomas.

Characteristics	High-grade gliomas	Low-grade gliomas	p-value
Age (mean / SD)	44.47 ± 18.93	20.05 ± 15.90	<0.001*
GENDER	<i>n</i> (%)	<i>n</i> (%)	
Female	13 (43.3%)	11 (52.4%)	0.524
Male	17 (56.7%)	10 (47.6%)	
Smoking	<i>n</i> (%)	<i>n</i> (%)	
Yes	3 (10%)	1 (4.8%)	0.493
On	27 (90%)	20 (95.2%)	
Use of alcohol	<i>n</i> (%)	<i>n</i> (%)	
Yes	6 (20%)	3 (14.3%)	0.598
On	24 (80%)	18 (85.7%)	
Family CA	<i>n</i> (%)	<i>n</i> (%)	
Yes	7 (23.3%)	4 (19%)	0.714
On	23 (76.7%)	17 (81%)	

Table 2. Expression of *ERCC2* rs13181 polymorphism genotypes among gliomas.

Genotypes	Gliomas		OR (95%CI)
	High-grade	Low-grade	
	<i>n</i> (%)	<i>n</i> (%)	
GG	2 (6.7%)	3 (14.3%)	
GT	12 (40%)	8 (38.1%)	1.083 (0.345-3.402)
TT	16 (53.3%)	10 (47.6%)	1.257 (0.411-3.842)

Table 3. Gene code and SNP used in our TaqMan® assay.

Gene variant VDR	Sequence context VIC/FAM
rs13181 G > T (Chr.19:45351661) TaqMan Assay ID: C_3145033_10	TGCTGAGCAATCTGCTCTATCCTCTCTCT[G/T]CAGCGTCTCCTCTGATTCTAGCTGC