



# Polymorphisms in *TPT1* Pathways in Pediatric Astrocytomas

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ASN Neuro  
Volume 15: 1–10  
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DOI: 10.1177/17590914231153481  
journals.sagepub.com/home/asn  


## Abstract

Central nervous system tumors, especially astrocytomas, are the solid neoplasms with the highest incidence and mortality rates in childhood. The diagnosis is based on histopathological characteristics, but molecular methods have been increasingly used. Translationally controlled tumor protein (TCTP) protein, encoded by the tumor protein, translationally controlled 1 (*TPT1*) gene, is a multifunctional protein with an important physiological role in the cell cycle. Expression of this protein has been associated with several neoplasms, including astrocytomas in adults. However, the role of this protein in pediatric astrocytomas is largely unknown. We aim to evaluate in cases of pediatric astrocytomas, the frequency of polymorphisms in the *TPT1* gene and other genes associated with its molecular pathways, such as *MTOR*, *MDM2*, *TP53*, and *CDKN1A*, correlating it with protein expression and clinical variables, in formalin-fixed, paraffin-embedded (FFPE) samples. These samples were submitted to genotyping and immunohistochemistry analyses. The most revealing results refer to the *MDM2* gene, rs117039649 [G/C], in which C polymorphic allele was observed only in the glioblastomas ( $p = .028$ ). The *CDKN1A* gene, rs3176334 [T/C] presented a homozygous polymorphic genotype only in high-grade astrocytomas, when infiltrating tumors were compared ( $p = .039$ ). The immunohistochemical expression of cytoplasmic MDM2 correlated with better survival rates in patients with glioblastoma ( $p = .018$ ). The presence of polymorphisms in the *MDM2* and *CDKN1A* genes, as well as a specific correlation between MDM2 expression, suggests a likely association with risk in pediatric astrocytomas. This study sought the probable role involved in the TCTP pathway, and associated proteins, in the tumorigenesis of pediatric astrocytomas, and some could have potential impact as prognostic markers in these patients.

## Keywords

astrocytomas, polymorphisms, *TPT1*, TCTP, *MDM2*, biomarkers

Received October 28, 2022; Revised January 10, 2023; Accepted for publication January 11, 2023

## Introduction

Translationally controlled tumor protein (TCTP) is a protein, highly conserved in eukaryotic cells, encoded by tumor protein, translationally controlled 1 (*TPT1*) gene (Acunzo et al., 2014; Bommer & Thiele, 2004). TCTP is usually expressed in mitotically active tissues, and it is involved physiologically in cell proliferation, protein synthesis, and immune response (Bommer & Thiele, 2004). Through interaction with other proteins, such as *MTOR* and *MDM2*, TCTP can affect the expression of p53 and p21 proteins, impacting apoptosis and cell proliferation (Acunzo et al., 2014; Amson et al., 2012; Bommer et al., 2015). All these proteins have physiological interactions but can also acquire pathological status, which

has associated TCTP with several neoplasms, including gliomas (Amson et al., 2012; Chen et al., 2015; Gu et al., 2014; Miao et al., 2013; Rocca et al., 2015). Studies have shown that TCTP was more expressed in high-grade gliomas

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and indicated worse prognosis, in the adulthood, whereas its expression decreased in tumor reversion phenomena, a biological process in which neoplastic cells lose its malignant phenotype (Amson et al., 2013; Tuynder et al., 2004). Moreover, TCTP has been studied as a therapeutic target, and sertraline, an antidepressant, has been shown to inhibit TCTP levels, thus increasing p53 expression, and inducing tumor reversion (Amson et al., 2013; Baú-Carneiro et al., 2022; Boia-Ferreira et al., 2017).

Genetic variations are quite common throughout the human genome. The simplest form of variation is the exchange of a single nucleotide (single-nucleotide polymorphisms—SNPs). It is estimated that there are around 8 million SNPs with an allelic frequency greater than 1%. Although most SNPs do not have important functional associations, there are certain SNPs that can substantially alter the ultimate expression or function of a gene product (Chanock, 2001). These functional, or unfunctional, variants can affect the final observed phenotype and thus change the risk classification of the disease, contribute to the understanding of early diagnosis and the possible impacts on changing the response to treatment (Lehrnbecher et al., 2005; Pertovaara et al., 2006). Polymorphic genetic variants are the basis of genetic association studies in research on genetic risk factors for disease susceptibility. The expression of molecules from the transcription factor superfamily can be altered by the presence of a certain polymorphism, which makes this project attractive since it seeks for possible genetic markers that may be associated with worse prognosis in pediatric astrocytomas, the most common solid malignant neoplasia in children (Ostrom et al., 2020). Thus, the present study aims to investigate the association between SNPs in *TPT1*, *MTOR*, *MDM2*, *TP53*, and *CDKN1A* genes in pediatric astrocytoma samples, as well as protein expression, to try to evidence potential genetic biomarkers that would help predict the patients' prognosis.

## Methods

### Study Population

This study was approved by the Human Research Ethics Committee of the Hospital Pequeno Príncipe, in Curitiba, Paraná (CAAE: 13240619.5.3001.0020/2019). This institution was founded in 1919 and today it is a renowned pediatric hospital in Brazil, a region with a particular colonization, compared to the other regions of our country. Most of its population descends from European immigrants, such as Italian, German, Polish, Ukrainian, among others. One hundred eighty-six primary pediatric astrocytomas diagnosed between 2003 and 2015 were selected, reviewed, and anonymized and with unrelated parentage.

The following variables were collected from the medical records: sex (male, female); age at diagnosis (years); outcome (death); and survival (months), and these subjects

were grouped according to the World Health Organization (WHO) classification (Louis, 2016).

One hundred-one cases of the 186 cases previously reviewed still had remaining neoplastic tissue in their formalin-fixed, paraffin-embedded (FFPE) samples. The final sample consisted of 56 pilocytic astrocytomas (WHO grade 1), 23 diffuse astrocytomas (WHO grade 2), 3 anaplastic astrocytomas (WHO grade 3), and 19 glioblastomas (WHO grade 4).

Attempting to homogenize the phenotype to improve the analyses, groupings based on the four histological grades were used. This grouping was carried out based on morphological, molecular, and prognostic differences between the astrocytomas, and the samples were divided into circumscribed astrocytomas, which included only grade 1, and infiltrating astrocytomas, referring to grades 2, 3, and 4.

Seeking the most appropriate analyses from a clinical point of view, three forms of analysis were organized. The astrocytomas were compared in isolated histological groups (1, 2, 3, and 4) and in two different groupings: low-grade×high-grade (grades 1 + 2×grades 3 + 4), and low-grade×high-grade in infiltrating astrocytomas (grade 2×grades 3 + 4). Table 1 shows the baseline characteristics in the study population.

### DNA Extraction and Genotyping

The DNA was obtained from paraffinized cuts of the samples using a commercially available paraffin DNA extraction kit (Qiagen®). Markers for coverage (target SNP—tag SNP) of five genes' loci (*TPT1*, *MTOR*, *MDM2*, *TP53*, and *CDKN1A*) were selected according to available information in the SNPinfo website (SNPinfo, 2022). After this search three tag SNPs for *TPT1* gene (rs2234222 [G/C]; rs9595305 [T/C] and rs1062420 [T/C]), two for *MTOR* gene (rs1034528 [G/C]; rs12732063 [G/A]), two for *MDM2* gene (rs2279744 [T/G]; rs117039649 [G/C]), one for *TP53* gene (rs121912664 [C/T]), and two for *CDKN1A* gene (rs3176326 [G/A]; rs3176334 [T/C]) were selected. The patients' purified DNA was amplified by real-time PCR (Applied Biosystems 7500 Real-Time PCR System) using TaqMan® system for genotyping discrimination. Tag SNPs used in the study met the following two quality control criteria: overall call rate >90%, and Hardy–Weinberg Equilibrium (HWE).

### Tissue Microarray (TMA) and Immunohistochemistry

From each of the 101 cases, hematoxylin-eosin slides were prepared. Two different neoplastic areas free from tissue processing artifacts or degeneration, such as necrosis or calcification, were selected for TMA block construction. Each TMA block included eight cases, with two samples from each case; each sample was 3 mm in diameter. The FFPE TMA blocks were cut into sections and placed on microscopic slides, which were submitted to immunohistochemistry following the procedure described by da Silva-Camargo et al. (2018). The TMA slides were subjected to antigen retrieval

**Table 1.** Baseline Characteristics in the Study Population.

Variables	Pilocytic (grade 1) (n=56)	Diffuse (grade 2) (n=23)	Anaplastic (grade 3) (n=3)	Glioblastoma (grade 4) (n=19)
Age at diagnosis <sup>a</sup>	5.2 ± 3.7	4.8 ± 4.3	13.3 ± 1.6	9.7 ± 3.6
Sex <sup>b</sup>				
Male	27 (48.2)	11 (47.8)	1 (33.3)	13 (68.8)
Female	29 (51.8)	12 (52.2)	2 (66.4)	6 (31.5)
Number of deaths	0 (0.0)	3 (13.0)	0 (0.0)	8 (42.1)
Survival (months)*	N/A	11.2 ± 7.0	N/A	8.8 ± 4.8

Note. <sup>a</sup>Mean ± SD. <sup>b</sup>Absolute number (percentage). Astrocytomas WHO classification (grades 1/2/3/4). N/A = not applicable.

and incubated with the following monoclonal mouse antibodies: anti-TCTP antibody (1:100, Abcam®, Cambridge, MA, USA), anti-human MTOR antibody (1:100, Cloud®, Katy, TX, USA), anti-MDM2 antibody (1:200, Affinity®, Jiangsu, China), anti-human p53 protein antibody (1:200, Dako®, Glostrup, Denmark) and anti-human p21 protein antibody (1:400, Cloud®, Katy, TX, USA). The reveal HRP detection system from Spring Bioscience® (Fremont, CA, USA), which included the secondary antibody, was used. For visualization, slides were incubated with a diaminobenzidine complex and substrate and counterstained with Harris Hematoxylin. As positive controls, samples of tissues expressing each protein were used. As a negative control, the primary antibody was omitted, as shown in Supplementary Figure 1 (Silva-Camargo et al., 2018).

### Morphological Analysis of Protein Expression

The evaluation of protein immunostaining was performed as follows. For quantitative analysis, TCTP and MTOR slides were digitized by Axio Scan.Z1® scanner (Carl Zeiss, Oberkochen, Germany), resulting in 300 to 500 images for each sample. Approximately 50 images from each sample were selected, avoiding necrotic areas or the presence of tissue processing artifacts. Then, these images were analyzed using Image Proplus® software, which performs morphometric analysis by color according to a previously established color reference. The software distinguishes the color brown, which is positive, from other colors and provides the mean tissue positive area in each sample in square micrometers ( $\mu\text{m}^2$ ) (Silva-Camargo et al., 2018). Semiquantitative morphological analyses of TCTP and MTOR, as well as MDM2, p53, and p21 proteins were also carried out. The Allred score, as described for hormone receptors immunostaining evaluation in breast cancer, was used (Allred et al., 1998). The advantage of the Allred score compared to the morphometric analyses was to evaluate the intensity of the immunostaining, as well as its subcellular location.

### Statistical Analysis

Nominal variables (sex, number of deaths, survival, and genotype) were expressed by absolute and relative frequency and

non-nominal (age at diagnostic and morphometric expression) by means and SD. Genetic distribution analysis was made by logistic regression *p*-value. HWE was observed when the *p*-value was not significant (*p*-value for HWE cut-off = .001). For the immunohistochemical study, Pearson correlation analyses between the proteins were carried out. Data were analyzed using the computer program by IBM® SPSS Statistics v.20.0 software and considered significant *p* ≤ .05. Haploview 4.2 was used to estimate the HWE.

## Results

### Polymorphisms in Astrocytomas for all Groups

The distribution of genotypic analysis for all tag SNPs in the four groups (n = 101) can be observed in Table 2.

*MDM2* rs117039649 [G/C] showed homozygosis (CC) for polymorphic allele only in glioblastomas (*p* = .028). An interesting observation can be made for the SNP of the *TP53* gene, despite not having shown statistical difference. *TP53* rs121912664 [C/T] showed no homozygosis for wild C allele. Almost all patients were heterozygous (CT), except for one patient homozygous for polymorphic T allele.

### Polymorphisms in low-Grade Versus High-Grade Astrocytomas (Grades 1 + 2 × grades 3 + 4)

In this analysis, the astrocytoma samples were separated into two groups (n = 101) following this grouping (Table 3): low grade (1 + 2) and high grade (3 + 4). The rs117039649 [G/C] for *MDM2* showed statistically significant result (*p* = .005) like the ones described in Table 2.

### Polymorphisms in Infiltrating Astrocytomas (Grade 2 × grades 3 + 4)

We analyzed only infiltrating astrocytomas (n = 45), excluding grade 1 pilocytic astrocytomas, divided into low and high-grade tumors (grade 2 × grades 3 and 4). The results are shown in Table 4. In this evaluation, *CDKN1A* rs3176334 [T/C] had a homozygous (CC) genotype for C polymorphic allele only in high-grade astrocytomas (*p* = .039). *MDM2* rs117039649

**Table 2.** Genotypic Frequencies Distribution and Analysis Between all WHO Groups.

Gene—reference SNP <sup>a</sup> allelic variation [1/2] <sup>b</sup>	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	P-value**
<i>TPT1</i> —rs2234222 [G/C]	GG	GC	CC	
WHO grade 1	48 (85.8)	7 (12.5)	1 (1.7)	.908
WHO grade 2	21 (91.3)	2 (8.7)	0 (0.0)	
WHO grade 3	3 (100.0)	0 (0.0)	0 (0.0)	
WHO grade 4	18 (94.7)	1 (5.3)	0 (0.0)	
<i>TPT1</i> —rs9595305 [T/C]	TT	TC	CC	
WHO grade 1	15 (28.9)	27 (51.9)	10 (19.2)	.444
WHO grade 2	4 (21.1)	8 (42.1)	7 (36.8)	
WHO grade 3	0 (0.0)	1 (50.0)	1 (50.0)	
WHO grade 4	4 (22.2)	12 (66.7)	2 (11.1)	
<i>TPT1</i> —rs1062420 [T/C]	TT	TC	CC	
WHO grade 1	47 (90.4)	5 (9.6)	0 (0.0)	.542
WHO grade 2	20 (90.9)	2 (9.1)	0 (0.0)	
WHO grade 3	3 (100.0)	0 (0.0)	0 (0.0)	
WHO grade 4	18 (100.0)	0 (0.0)	0 (0.0)	
<i>MTOR</i> —rs1034528 [G/C]	GG	GC	CC	
WHO grade 1	32 (57.1)	21 (37.5)	3 (5.4)	.661
WHO grade 2	8 (34.8)	14 (60.9)	1 (4.3)	
WHO grade 3	2 (66.7)	1 (33.3)	0 (0.0)	
WHO grade 4	9 (47.4)	9 (47.4)	1 (5.2)	
<i>MTOR</i> —rs12732063 [G/A]	GG	GA	AA	
WHO grade 1	54 (96.4)	2 (3.6)	0 (0.0)	.515
WHO grade 2	21 (91.3)	2 (8.7)	0 (0.0)	
WHO grade 3	3 (100.0)	0 (0.0)	0 (0.0)	
WHO grade 4	19 (100.0)	0 (0.0)	0 (0.0)	
<i>MDM2</i> —rs2279744 [T/G]	TT	TG	GG	
WHO grade 1	12 (34.3)	20 (57.1)	3 (8.6)	.194
WHO grade 2	8 (57.1)	5 (35.7)	1 (7.2)	
WHO grade 3	0 (0.0)	1 (50.0)	1 (50.0)	
WHO grade 4	9 (60.0)	5 (33.3)	1 (6.7)	
<i>MDM2</i> —rs117039649 [G/C]	GG	GC	CC	
WHO grade 1	37 (92.5)	3 (7.5)	0 (0.0)	.028
WHO grade 2	22 (100.0)	0 (0.0)	0 (0.0)	
WHO grade 3	2 (100.0)	0 (0.0)	0 (0.0)	
WHO grade 4	15 (83.3)	0 (0.0)	3 (16.7)	
<i>TP53</i> —rs121912664 [C/T]	CC	CT	TT	
WHO grade 1	0 (0.0)	53 (98.1)	1 (1.9)	.839
WHO grade 2	0 (0.0)	23 (100.0)	0 (0.0)	
WHO grade 3	0 (0.0)	3 (100.0)	0 (0.0)	
WHO grade 4	0 (0.0)	19 (100.0)	0 (0.0)	
<i>CDKN1A</i> —rs3176326 [G/A]	GG	GA	AA	
WHO grade 1	3 (7.1)	14 (33.4)	25 (59.5)	.834
WHO grade 2	0 (0.0)	6 (33.3)	12 (66.7)	
WHO grade 3	0 (0.0)	0 (0.0)	2 (100.0)	
WHO grade 4	1 (6.6)	4 (26.7)	10 (66.7)	
<i>CDKN1A</i> —rs3176334 [T/C]	TT	TC	CC	
WHO grade 1	25 (48.1)	20 (38.5)	7 (13.4)	.178
WHO grade 2	11 (55.0)	9 (45.0)	0 (0.0)	
WHO grade 3	1 (50.0)	0 (0.0)	1 (50.0)	
WHO grade 4	5 (27.8)	9 (50.0)	4 (22.2)	

Note. <sup>a</sup>Tag SNP based on NCBI dbSNP website. <sup>b</sup>Allelic variation 1/2: 1 = wild allele, 2 = polymorphic allele. Absolut number (percentage). SNP = single-nucleotide polymorphism; TPT1 = tumor protein, translationally controlled 1; WHO = World Health Organization.

\*\*p-value test for logistic regression.

**Table 3.** Genotypic Frequencies Distribution and Analysis Between Astrocytomas (Grades 1 + 2×Grades 3 + 4).

Gene—reference SNP <sup>a</sup> allele variation [1/2] <sup>b</sup>	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	P-value**
<i>TPT1</i> —rs2234222 [G/C]	GG	GC	CC	
Grades 1 + 2	69 (87.3)	9 (11.4)	1 (1.3)	.543
Grades 3 + 4	21 (95.4)	1 (4.6)	0 (0.0)	
<i>TPT1</i> —rs9595305 [T/C]	TT	TC	CC	
Grades 1 + 2	19 (26.8)	35 (49.3)	17 (23.9)	.454
Grades 3 + 4	4 (20.0)	13 (65.0)	3 (15.0)	
<i>TPT1</i> —rs1062420 [T/C]	TT	TC	CC	
Grades 1 + 2	67 (90.5)	7 (9.5)	0 (0.0)	.143
Grades 3 + 4	21 (100.0)	0 (0.0)	0 (0.0)	
<i>MTOR</i> —rs1034528 [G/C]	GG	GC	CC	
Grades 1 + 2	40 (50.6)	35 (44.3)	4 (5.1)	.992
Grades 3 + 4	11 (50.0)	10 (45.6)	1 (4.4)	
<i>MTOR</i> —rs12732063 [G/A]	GG	GA	AA	
Grades 1 + 2	75 (94.9)	4 (5.1)	0 (0.0)	.281
Grades 3 + 4	22 (100.0)	0 (0.0)	0 (0.0)	
<i>MDM2</i> —rs2279744 [T/G]	TT	TG	GG	
Grades 1 + 2	20 (40.8)	25 (51.0)	4 (8.2)	.531
Grades 3 + 4	9 (52.9)	6 (35.3)	2 (11.8)	
<i>MDM2</i> —rs117039649 [G/C]	GG	GC	CC	
Grades 1 + 2	59 (95.2)	3 (4.8)	0 (0.0)	.005
Grades 3 + 4	17 (85.0)	0 (0.0)	3 (15.0)	
<i>TP53</i> —rs121912644 [C/T]	CC	CT	TT	
Grades 1 + 2	0 (0.0)	76 (98.7)	1 (1.3)	.591
Grades 3 + 4	0 (0.0)	22 (100.0)	0 (0.0)	
<i>CDKN1A</i> —rs3176326 [G/A]	GG	GA	AA	
Grades 1 + 2	3 (5.0)	20 (33.3)	37 (61.7)	.743
Grades 3 + 4	1 (5.9)	4 (23.5)	12 (70.6)	
<i>CDKN1A</i> —rs3176334 [T/C]	TT	TC	CC	
Grades 1 + 2	36 (50.0)	29 (40.3)	7 (9.7)	.119
Grades 3 + 4	6 (30.0)	9 (45.0)	5 (25.0)	

Note. <sup>a</sup>Tag SNP based on NCBI dbSNP website. <sup>b</sup>Allelic variation 1/2: 1 = wild allele, 2 = polymorphic allele. Absolut number (percentage).

SNP = single-nucleotide polymorphism; TPT1 = tumor protein, translationally controlled 1.

\*\* p-value test for logistic regression.

[G/C] had *borderline* (marginal) statistical significance ( $p = .059$ ).

### Immunohistochemistry in Astrocytomas

For the immunohistochemistry markers, the results were analyzed considering the four groups but Figure 1 only indicates groups 1 and 4. We found more TCTP morphometric expression in WHO grade 4 group ( $15,664,0\mu\text{m}^2 \pm 6418,9\mu\text{m}^2$ ), but without statistical significance (Supplemental Table S1).

Pearson correlation was made between TCTP and other molecules, and when we compared the expression of TCTP and p21 proteins, we have obtained interesting results. In grade 1 astrocytomas, there was a moderate positive Pearson correlation ( $r = 0.301$ ;  $p = .025$ ) between nuclear p21 and TCTP expression. As for grade 2 astrocytomas, we found a moderate positive Pearson correlation ( $r = 0.439$ ;  $p = .036$ ) between cytoplasmic p21 and TCTP expression.

### Association Between Immunohistochemistry and Survival

This analysis was conducted between protein expression and survival of patients with glioblastoma (grade 4) who died. The mean survival of these patients was 8.8 ( $\pm 4.8$ ) months and, using this value as a cut-off of reference, the patients of group 4 were classified into two groups: below average survival (BAS) and above average survival (AAS). Four proteins had the potential to be analyzed considering Allred score and survival: TCTP, p53, p21, and MDM2 (Figure 2). mTOR protein was not included in this analysis, as their evaluation showed the same Allred score result in both groups, making it impossible to compare them.

When we compared protein expression and survival rates in patients diagnosed with glioblastomas, considering the Allred score, we found that patients who survived longer had more expression of cytoplasmic MDM2 ( $p = .018$ ) and p53 ( $p = .020$ ).

**Table 4.** Genotypic Frequencies Distribution and Analysis Between Astrocytomas (Grades 2 × 3 + 4).

Gene—reference SNP <sup>a</sup> allele variation [1/2] <sup>b</sup>	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	P-value**
<i>TPT1</i> —rs2234222 [G/C]	GG	GC	CC	
Grade 2	21 (91.3)	2 (8.7)	0 (0.0)	.577
Grades 3 + 4	21 (95.4)	1 (4.6)	0 (0.0)	
<i>TPT1</i> —rs9595305 [T/C]	TT	TC	CC	
Grade 2	4 (21.1)	8 (42.1)	7 (36.8)	.251
Grades 3 + 4	4 (20.0)	13 (65.0)	3 (15.0)	
<i>TPT1</i> —rs1062420 [T/C]	TT	TC	CC	
Grade 2	20 (90.9)	2 (9.1)	0 (0.0)	.157
Grades 3 + 4	21 (100.0)	0 (0.0)	0 (0.0)	
<i>MTOR</i> —rs1034528 [G/C]	GG	GC	CC	
Grade 2	8 (34.8)	14 (60.9)	1 (4.3)	.572
Grades 3 + 4	11 (50.0)	10 (45.6)	1 (4.4)	
<i>MTOR</i> —rs12732063 [G/A]	GG	GA	AA	
Grade 2	21 (91.3)	2 (8.7)	0 (0.0)	.157
Grades 3 + 4	22 (100.0)	0 (0.0)	0 (0.0)	
<i>MDM2</i> —rs2279744 [T/G]	TT	TG	GG	
Grade 2	8 (57.1)	5 (35.7)	1 (7.2)	.907
Grades 3 + 4	9 (52.9)	6 (35.3)	2 (11.8)	
<i>MDM2</i> —rs117039649 [G/C]	GG	GC	CC	
Grade 2	22 (100.0)	0 (0.0)	0 (0.0)	.059
Grades 3 + 4	17 (85.0)	0 (0.0)	3 (15.0)	
<i>TP53</i> —rs121912664 [C/T]	CC	CT	TT	
Grade 2	0 (0.0)	23 (100.0)	0 (0.0)	N/A
Grades 3 + 4	0 (0.0)	22 (100.0)	0 (0.0)	
<i>CDKN1A</i> —rs3176326 [G/A]	GG	GA	AA	
Grade 2	0 (0.0)	6 (33.3)	12 (66.7)	.503
Grades 3 + 4	1 (5.9)	4 (23.5)	12 (70.6)	
<i>CDKN1A</i> —rs3176334 [T/C]	TT	TC	CC	
Grade 2	11 (55.0)	9 (45.0)	0 (0.0)	.039
Grades 3 + 4	6 (30.0)	9 (45.0)	5 (25.0)	

Note. <sup>a</sup>Tag SNP based on NCBI dbSNP website. <sup>b</sup>Allelic variation 1/2: 1 = wild allele, 2 = polymorphic allele. Absolut number (percentage). N/A = not applicable; SNP = single-nucleotide polymorphism; *TPT1* = tumor protein, translationally controlled 1.

\*\*p-value test for logistic regression.

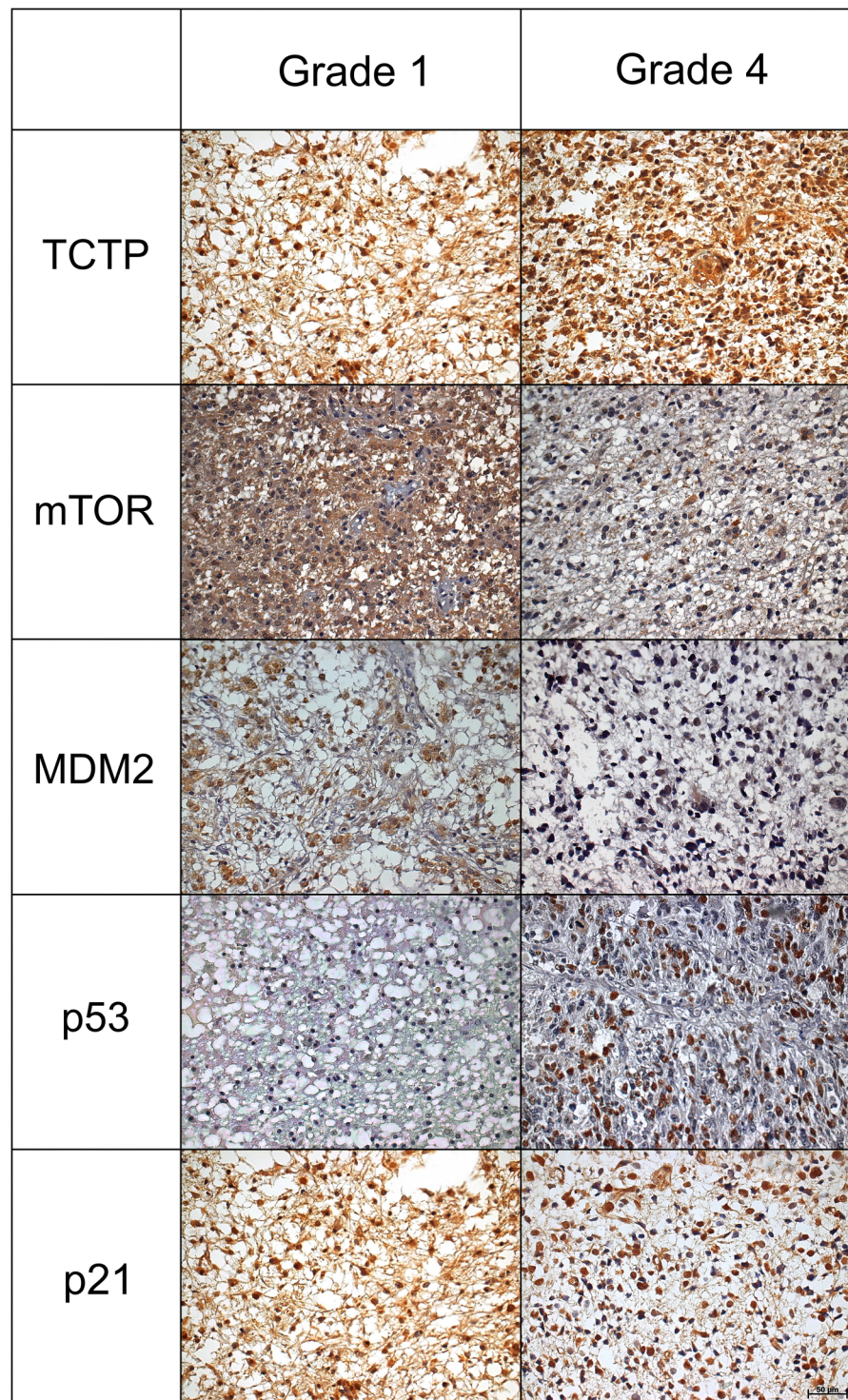
## Discussion

Tumors of the central nervous system (CNS) are the most common solid neoplasms in children, with the highest mortality rates and among the histological subtypes, astrocytomas are the most common (Ostrom et al., 2020). They are classified by the WHO in grades 1 to 4, which correlates directly to aggressiveness and poor prognosis. Grade 1 tumors are low-grade circumscribed astrocytomas, whereas grades 2, 3, and 4 are infiltrating astrocytomas (Louis, 2016). They can be subdivided into low-grade astrocytomas (grades 1 and 2) and high-grade astrocytomas (grades 3 and 4). These two groups of astrocytomas present different molecular pathways of gliomagenesis (Ryall et al., 2020). Several proteins have distinct roles in neoplasms, including astrocytomas, acting as either prognostic or therapeutic biomarkers. *TCTP* is one of these proteins, and through direct or indirect interaction with other proteins such as *MTOR*, *MDM2*, *p53*, and *p21*,

it has been considered an independent prognostic factor in astrocytomas in adults (Gu et al., 2014). Our objective was to analyze SNPs in *TPT1* and *TCTP* expression, as well as associated molecular pathways, in pediatric astrocytomas.

Regarding SNPs in *TPT1*, we did not observe an association with pediatric astrocytomas. Two genes showed significant results (*MDM2* and *CDKN1A*) in all analyzes performed and all genes are in HWE (Supplemental Table S2) with the exception of *MDM2* (rs117039649) and *TP53* (rs121912664), and this aspect could be because the samples are from tumors.

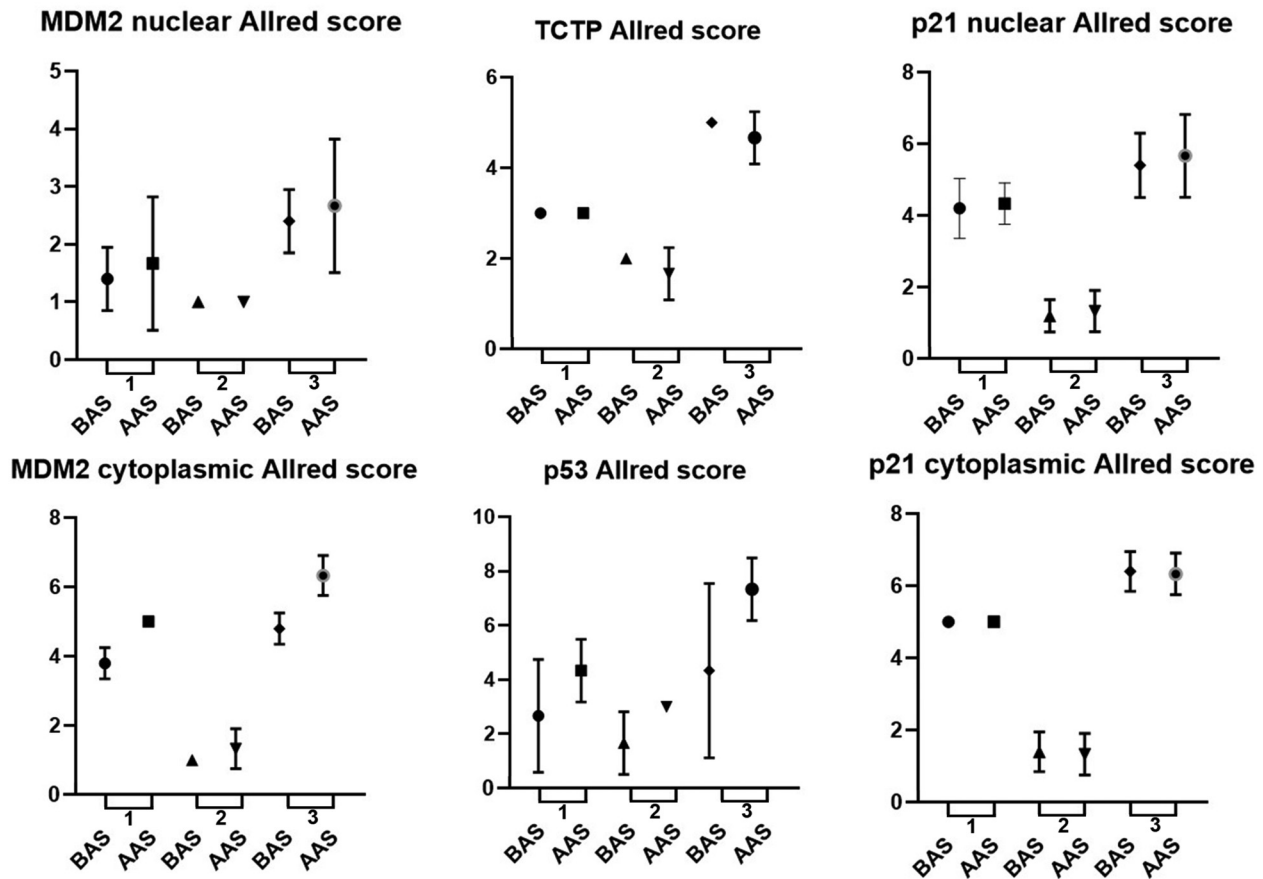
*MDM2* rs117039649 [G/C] showed homozygosis (CC) for polymorphic allele only in glioblastomas (WHO grade 4), both in isolated and low-grade×high-grade comparisons, which could indicate risk. Furthermore, in the specific analysis of the subgroup of infiltrative astrocytomas, this SNP showed a possible importance despite its marginal significance. *MDM2* gene codifies *MDM2* protein which inhibits



**Figure 1.** Immunoexpression for the five proteins TCTP, mTOR, MDM2, p53 and p21. Left column shows astrocytomas grade 1 and right column shows astrocytomas grade 4 (IHC, 200x).

p53 protein, impacting apoptosis (Moll & Petrenko, 2003). Perhaps, this polymorphism could provide a greater escape from apoptosis in glioblastomas. Previous studies have demonstrated an association between this SNP and neoplasms. In gynecological cancer, polymorphic C allele has shown a

protective role (Gansmo et al., 2015; Roszak et al., 2015; Zhang et al., 2018). Different mechanisms of carcinogenesis, and different levels of gene expression, between gliomas and female genital tumors could explain this opposite role, compared to our results. When we compare with 1000 genome



**Figure 2.** Survival and immunoexpression proteins (TCTP, MDM2, p53 and p21) distribution in glioblastomas (group 4). Survival was classified into two groups considering the mean of survival: below average survival (BAS) and above average survival (AAS). The proteins were evaluated according to the Allred value (distribution, intensity, Allred score). Each graphic has 3 analyses: 1 for distribution in below and above average survival; 2 for intensity in below and above average survival; 3 for Allred score in below and above average survival.

databases (NCBI, 2022), the frequency of the polymorphic C in this population is 1.3%, much lower than the frequency in our cohort, which was 5.5%. This could point to a risk factor of the polymorphic allele in pediatric astrocytomas.

*TP53* rs121912664 [C/T] represents R337H mutation, which has high incidence in the south of Brazil, compared to other populations (Custodio et al., 2011; Ribeiro et al., 2001) perhaps due to the founder effect reported by the authors. We have found that almost every patient, except for one, showed heterozygosity for this SNP. Even though there was no statistical significance in this analysis, this result could indicate an important potential role for T polymorphic allele in pediatric astrocytomas. Furthermore, other studies have shown the impact of SNP in *TP53* gene on prognosis of pediatric astrocytomas (Mascelli et al., 2016).

When we compared only infiltrating astrocytomas, we found that *CDKN1A* rs3176334 [T/C] presented a homozygous (CC) polymorphic genotype only in high-grade astrocytomas, which could also indicate risk. The 1000Genomes databases (NCBI, 2022) shows that the frequency of the polymorphic C in this population is 36.5%, a little higher than the frequency

in our cohort, which was 33.7%. We have not found previous studies associating this SNP and gliomas or other neoplasms. But since *CDKN1A* is responsible for encoding p21 protein, which controls cell cycle, we could speculate that this polymorphism could enhance tumor cell proliferation.

Immunohistochemistry analysis did not show statistically significant results when we compared protein expression to WHO tumor grade. But when we correlated TCTP and other proteins, p21 showed positive Pearson correlation. Interestingly, p21 had nuclear expression in grade 1 astrocytomas, whereas grade 2 astrocytomas showed cytoplasmic expression. p21 protein sub-cellular location has been linked to different functions. When nuclear, it controls cell cycle; when cytoplasmic, it has been associated with a carcinogenic role (Kreis et al., 2019; Shamloo & Usluer, 2019). This could indicate that in low-grade astrocytomas, interaction between TCTP and p21 proteins is different: in grade 1, p21 protein develops its canonical function whereas in grade 2, it plays an oncogenic role.

Finally, we have found a correlation between cytoplasmic expression of MDM2 protein and better outcome in patients with glioblastomas. MDM2, when in the nucleus, is



responsible for degrading the p53 protein and inhibiting apoptosis. But previous studies have observed a better outcome when cytoplasmic expression was assessed, as evidenced by Park et al. in patients with breast cancer (Park et al., 2013). Perhaps, cytoplasmic MDM2 could play a similar role in glioblastomas. The p53 protein also showed direct correlation between nuclear expression and survival. Mutations in *TP53* gene indicate high-grade astrocytomas (Louis, 2016). However, the role of p53 protein expression regarding prognosis is still unclear. Studies have shown conflicting results, such as no association and association with poor prognosis (Chaurasia et al., 2016; Drach et al., 1996).

Our study has some limitations that should be highlighted in relation to the preliminary results. Even though the previous edition of WHO classification of tumors of the CNS has been used, the authors are aware that the new nomenclature distinguishes pediatric from adult-type gliomas. It also acknowledges the clinical and molecular differences between pediatric-type diffuse gliomas (Louis et al., 2021). Thus, molecular features, such as *BRAF* fusions and *H3K27* and *H3G34* mutations, should be included in future studies seeking a more accurate diagnosis, including morphological and molecular features. Our results mark an early study on pediatric astrocytomas and TPT1 and their associated molecular pathways. The authors suggest carrying out future studies that include larger and ethnically different populations. In addition to seeking to increase the sample number and carry out the classic case-control study design. Even though a new WHO classification of tumors of the CNS has been proposed in 2021, the previous classification was maintained in our study, considering that this was the reference at the time of the cohort's diagnosis. In addition, in order to update it, molecular testing (even though of extreme importance, currently unavailable in our institution), would be necessary.

## Conclusions

To date, there are no studies evaluating the role of *TPT1* and its molecular associations in pediatric astrocytomas, and in this article, two SNPs (*MDM2* rs117039649 [G/C] and *CDKN1A* rs3176334 [T/C]) showed a suggestive risk association in glioblastomas. As for MDM2 protein expression, cytoplasmic expression indicated better overall survival. The results could suggest that this gene and its protein could become potential prognostic markers in pediatric astrocytomas.

## Acknowledgments

The authors are grateful to Pequeno Príncipe Complex for their support.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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## Supplemental Material

Supplemental material for this article is available online.

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