

## ORIGINAL ARTICLE

# Glutamate Transporter 1 expression in human glioblastomas

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## Summary

**Purpose:** The purpose of our study was to investigate the mRNA expression profile of glutamate transporter 1 (GLT-1) in different types and grades of brain tumors, such as glioblastoma multiforme, astrocytomas (pilocytic, diffuse, anaplastic), oligodendrogliomas, ependyomas, medulloblastomas, and meningiomas using Real Time Quantitative PCR technique (qRT-PCR).

**Methods:** A total of 66 surgically removed primary brain tumors were collected retrospectively and the total RNA was isolated from each tumor sample. cDNA was generated and GLT-1 mRNA expression was evaluated with quantitative qRT-PCR.

**Results:** The mRNA expression of GLT-1 was significantly lower in primary brain tumors when compared to control brain tissues. GLT-1 expression was inversely correlated with the tumor grade, implicating its potential role in tumor progression. GLT-1 mRNA expression was lowest in

grade 4 tumors, such as glioblastoma multiforme and medulloblastomas. The tumors with grade 3 and 4 combined displayed lower expression compared to tumors with grades 1 and 2. In grade 4 tumors, female patients displayed lower GLT-1 expression compared to male patients. In addition, glioblastoma multiforme patients older than 65 years of age showed lower GLT-1 expression when compared to the patients younger than 65.

**Conclusion:** qRT-PCR was found to be a sensitive method in detecting GLT-1 expression in brain tumors. This study may lay the foundation for the future research about the excitotoxicity and brain tumors and GLT-1 might be a potential biomarker. Targeted therapies based on excitotoxic molecular pathways against gliomas should be designed to effectively combat these diseases.

**Key words:** glutamate, GLT-1, glioma, tumor

## Introduction

Primary brain tumor cells are mainly derived from glia and called glioma. Glioblastoma, or glioblastoma multiforme, is known as being the most common malignant primary brain tumor type [1]. It is really hard to cure with an average survival of 12-18 months after being detected. With high-grade tumors, the patient loses his/her life in 6-12 months [1]. The biology and pathogenesis of gliomas is an area of intense research in order to develop molecular therapies [2]. It is not exactly known

how the glia cells turn into gliomas. Even though gliomas display similar properties to other tumors, they also have their own special biology [3].

The tumors are graded according to the WHO (World Health Organization) system, which is based on appearance of certain characteristics of the tumor such as atypia, mitosis, endothelial proliferation, and necrosis. The WHO 2016 system not only contains the morphological and histological findings but also includes the molecular pathology.

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According to the WHO data, the lowest grade astrocytomas (grade I) make up only 2% of recorded astrocytomas, whereas grade II astrocytomas make up 8%, and the higher grade anaplastic astrocytomas (grade III) make up 20% of the recorded cases. The highest graded astrocytoma (grade IV GBM) is the most common primary nervous system cancer and the second most frequent brain tumor after brain metastasis. Despite the low incidence of astrocytomas compared to other human cancers, the mortality is significant.

The growth of glioma is prevented by the chromium and for this reason gliomas grow by destroying the peritumoral normal brain tissue around them. This process is an excitotoxic mechanism achieved by secreting excessive glutamate which will destroy neurons [4-6]. Excitotoxicity is caused by the excess release of glutamate into the synaptic cleft after the over-induction of glutamate receptors. Excitotoxicity is observed in age-dependent neurodegenerative diseases and also in stroke, epilepsy and brain trauma [7,8]. The prevention of excitotoxicity will help to prevent or slow down these diseases. Glutamate might follow two paths after it is taken into the astrocyte: 1) It might be metabolized by glutamate dehydrogenase (GDH) and enter the tricarboxylic acid (TCA) cycle. 2) It might be converted to glutamine by glutamine synthetase (GS) [9,10]. Excessive glutamate is absorbed by the glutamate transporters on the perisynaptic astrocytes, mainly by the majority of glutamate transporter 1 (GLT-1) [11]. The dysfunction of glutamate transport, which is important for differentiation, survival, proliferation and neural development, is observed in malignant gliomas [12].

Elevated extracellular glutamate levels have been shown in glioma [13]. Glutamate release depends on *de novo* synthesis of glutamate from glutamine, which is released in exchange for cystine via a glutamate-cystine exchanger termed "system X<sup>c</sup>" [14]. In following studies, it was shown that glioma cells in culture or in xenograft models release toxic levels of glutamate for neurons and glia [15]. It was shown in a previous study with glioma cell lines and nude mice that GLT-1 levels were decreased in glioma cells overexpressing GLT-1 and nude mice slowed down the progression of gliomas via inducing apoptosis [16]. A previous study with a tissue microarray showed a reverse correlation of EAAT-2 (GLT-1) expression using brain tumors of varying grades. Tissue microarray is based on protein expression conducted via antibody staining. However, no mRNA expression study of GLT-1, which basically indicates gene expression, has been conducted yet. In addition, our study includes a larger variety of primary brain tumors with different grades.

The purpose of this study was to identify GLT-1 mRNA expression using Real Time Polymerase Chain Reaction (RT-PCR, quantitative PCR) and compare among different types of brain tumors of varying grades. Primary brain tumors were grouped as glioblastomas, astrocytomas (pilocytic, diffuse, anaplastic), medulloblastomas, meningiomas, oligodendrogliomas, ependymomas [17] and classified into four grades (grade 1, 2, 3, 4) according to World Health Organization 2016.

## Methods

### Ethics

This study was approved by the Adnan Menderes University, Faculty of Medicine, Clinical Research Ethics Committee. Since the study was retrospective, no approval form was obtained from the patients.

**Table 1.** World Health Organization 2016 classification of central nervous system

Central nervous system tumors	
Grade	Tumor types
G1	Pilocytic Astrocytoma, Meningioma, Ependymoma
G2	Diffuse Astrocytoma, Oligodendroglioma, Ependymoma
G3	Anaplastic Astrocytoma, Anaplastic Oligodendroglioma
G4	Glioblastoma Multiforme (GBM), Medullablastoma

**Table 2.** Patient characteristics

Patient characteristics	n (%)
Sex	
Male	40 (60.6)
Female	26 (39.4)
Total	66 (100)
Type	
GBM	27 (40.9)
Medullablastoma	8 (12.1)
Astrocytoma	9 (13.6)
Pilocytic Astrocytoma	4 (44.4)
Diffuse Astrocytoma	3 (33.3)
Anaplastic Astrocytoma	2 (22.2)
Ependymoma	9 (13.6)
Oligodendroglioma	4 (6.1)
Meningioma	9 (13.6)
WHO 2016	
G1	14 (21.2)
G2	13 (19.7)
G3	4 (6.1)
G4	35 (53)

n: number of patients, WHO: World Health Organization, G: grade, GBM: Glioblastoma Multiforme

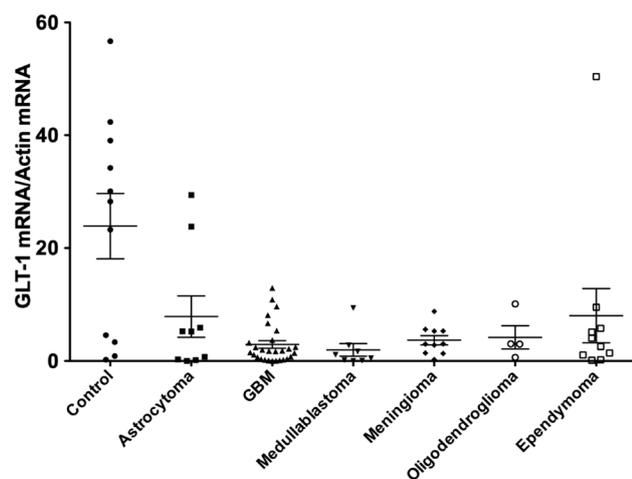
### Patients and samples

Formalin-fixed, paraffin-embedded archival tissue blocks belonging to patients with diagnosis of primary brain tumors from 2012 to 2017 were obtained from Izmir Tepecik Training and Research Hospital, Turkey. A total of 66 surgically removed primary brain tumors were collected retrospectively. Eleven healthy brain tissues were used as control. Tumor grade was determined according to WHO 2016. The WHO grading scheme is based on the appearance of certain characteristics of the tumor such as atypia, mitosis, endothelial proliferation, and necrosis. Patients between 19 and 77 years of age were included in the study. Medulloblastoma patients were between 2 and 39 years of age. The information regarding age, gender, primary site, tumor type and grade were collected for all patients.

Briefly, formalin-fixed, paraffin-embedded tissue blocks for primary brain tumors were obtained. Tissue cylinders with a 0.6 mm diameter were punched from representative tissue areas of each donor tissue and brought on to a recipient paraffin block. Each tissue microarray (TMA) spot included at least 50% tumor cells.

### RNA isolation and cDNA synthesis

Total RNA was isolated from tumor-rich areas of formalin-fixed paraffin-embedded (FFPE) tissue blocks



**Figure 1.** Glutamate Transporter 1 (GLT-1) mRNA expression in different primary brain tumors (astrocytoma, GBM, medulloblastoma, meningioma, oligodendroglioma, ependymoma) vs. control tissues. GLUT-1 expression is significantly lower in each tumor type when compared to control, but not significantly different among tumor types. GLUT-1 mRNA expression is represented by data points in each tumor type. Mean and standard error of the mean (SEM) of each group is indicated by long horizontal bar and black vertical bars, respectively. Two-tailed unpaired t-test with Welch's correction were performed,  $p < 0.05$  was accepted as significant. Astrocytoma vs. control  $p = 0.0326$ , GBM vs. control  $p = 0.0049$ , medulloblastoma vs. control  $p = 0.0040$ , meningioma vs. control  $p = 0.0062$ , oligodendroglioma vs. control  $p = 0.0076$ , ependymoma vs. control  $p = 0.0491$ . One way analysis of Variance (One Way ANOVA) test was performed,  $p < 0.05$  was accepted as significant. P value was determined to be  $< 0.0001$ ,  $R^2 = 0.3537$ .

using the Invitrogen (New York, USA) RNA FFPE Kit reagents following the manufacturer's standard protocols. RNA concentration and purity were determined and confirmed via spectrophotometry. One  $\mu\text{g}$  of RNA was reverse-transcribed using high capacity RNA to cDNA kit (WizBio, Seongnam, South Korea) according to the guidelines of the manufacturer's protocol and used in RT-PCR reactions.

### Real time PCR

The cDNA was subjected to quantitative PCR analysis with GLT-1 (GLT-1 fwd: AACAAATATGCCCAAGCAGGT, GLT-1 rev: CTCCCAGGATGACCCAAAC) and  $\beta$ -actin specific primers ( $\beta$ -actin fwd: AACTGGGACGACATGGAGAA,  $\beta$ -actin rev: GAAGGTCTCAAACATGATCTGG) using Syber Green Gene Expression Assay from GeneAll (Seoul, Korea) and quantifications were performed according to the manufacturers' instructions. Relative abundance of mRNA was obtained by normalization to  $\beta$ -actin mRNA levels.

### Statistics

All the statistical analyses were performed using the statistical software GraphPad Prism 5 Software, Inc., San Diego, USA. Namely, for the analysis of GLT-1 mRNA expression, two-tailed, unpaired t-test with Welch's correction and one-way ANOVA tests were conducted using Prism 5 software. Significant differences are shown by asterisks indicating  $p^{*,\#,\alpha,\beta} < 0.05$ . Error bars in figures represent standard error of the mean (SEM).

## Results

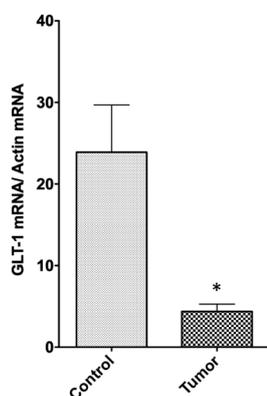
### Patient characteristics

Sixty-six patients who were pathologically diagnosed with primary brain tumors in Tepecik Training and Research Hospital, Turkey between 2012 and 2017, were analyzed for GLT-1 expression. The tumors were defined and graded according to WHO 2016 classification (Table 1). The characteristics of the patients are summarized in Table 2. Forty (60.6 %) patients were male and 26 (39.4 %) female. Patients between 19 and 77 years of age were included in the study. Medulloblastoma patients were between 2 and 39 years of age. According to WHO 2016 classification, 14 patients had grade 1 (pilocytic astrocytoma, meningioma, ependymoma), 13 patients had grade 2 (diffuse astrocytoma, oligodendroglioma, ependymoma), 4 patients had grade 3 (anaplastic astrocytoma, anaplastic oligodendroglioma) and 35 patients had grade 4 tumors (GBM) and medulloblastoma) tumors (Table 2).

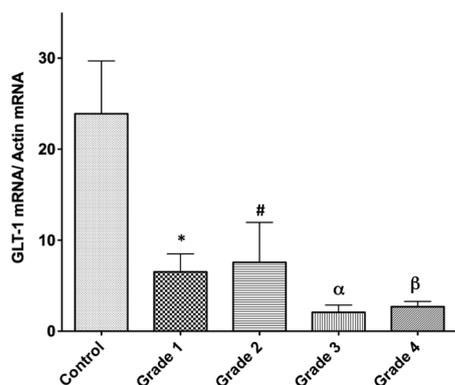
### GLT-1 mRNA expression is decreased in primary brain tumors

In order to determine the GLT-1 mRNA expression in primary tumors and in healthy control brain

tissues, we isolated the total RNAs from paraffin-embedded tissue samples. We then prepared cDNA from these total RNAs and determined mRNA expression of GLT-1 gene by qRT-PCR. The GLT-1 mRNA expression was normalized to the endogenous actin mRNA levels. The expression levels of GLT-1 mRNA in different primary brain tumors including astrocytoma (pilocytic, diffuse, anaplastic), GBM, medulloblastoma, meningioma, oligodendroglioma and ependymoma) were compared with the GLT-1 mRNA expression in healthy brain tissues as control (Figure 1). Mean and SEM of each group was indicated by long horizontal bar and black vertical bars, respectively. GLT-1 mRNA expression was decreased significantly in each type of primary brain tumors when compared to control healthy brain tissues (Figure 1). The GLT-1 mRNA



**Figure 2.** GLT-1 mRNA expression in all primary brain tumors combined vs. control. GLT-1 expression was significantly lower in tumors combined when compared to control. Two-tailed unpaired t-test with Welch's correction was performed,  $p < 0.05$  was accepted as significant. Tumor vs. control  $p = 0.0077$ .



**Figure 3.** GLT-1 mRNA expression in each tumor grade (1, 2, 3, 4) vs. control. GLT-1 expression was significantly lower in each grade when compared to control, but not significantly different among grades. One way analysis of variance (One way ANOVA) test was performed,  $p < 0.05$  was accepted as significant.  $p^{*,\#,\alpha,\beta}$  value was determined to be  $< 0.0001$ ,  $R^2 = 0.3504$ .

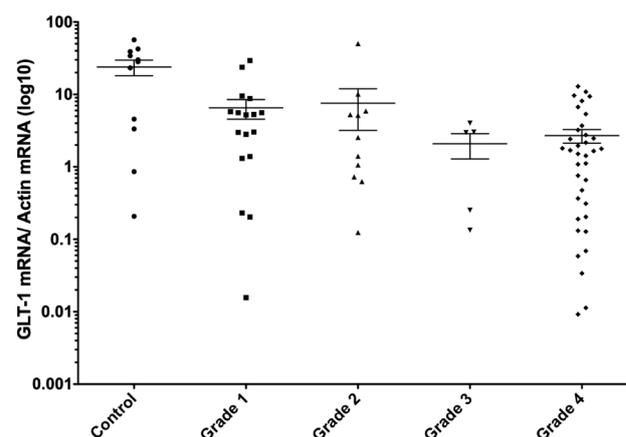
expression seems to be lowest in GBM and medulloblastoma, which are grade 4 tumors (Table 1 and Figure 1). However, GLT-1 mRNA expression in different tumor types was not significantly different from each other according to one-way ANOVA (Figure 1).

We next combined the GLT-1 mRNA expression in all different primary brain tumors compared with the control brain tissue and observed that the GLT-1 expression was significantly lower in brain tumors when compared to control (Figure 2).

#### *GLT-1 mRNA expression is inversely correlated with the tumor grade*

We next analyzed the association between the GLT-1 mRNA expression and the tumorigenicity. Central nervous system tumors were grouped according to their histological tumor grades following the WHO 2016 classification guidelines by the neuropathologists of the pathology department. The WHO grading scheme is based on the appearance of certain characteristics of the tumor such as atypia, mitosis, endothelial proliferation, and necrosis. These features reflect the malignant potential of the tumor in terms of invasion and growth rate. Tumorigenicity increases from grade 1 to grade 4 and grade 1 might be accepted as "benign".

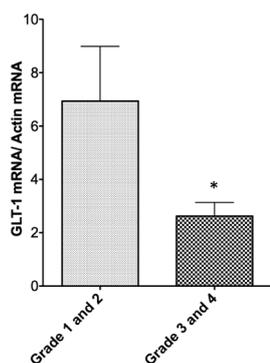
We determined the GLT-1 mRNA expression in each grade (grade 1, 2, 3, or 4) and compared with the control tissues. The GLT-1 mRNA expression in each grade was significantly lower than the expression in control tissue but not different among the grades according to one-way ANOVA



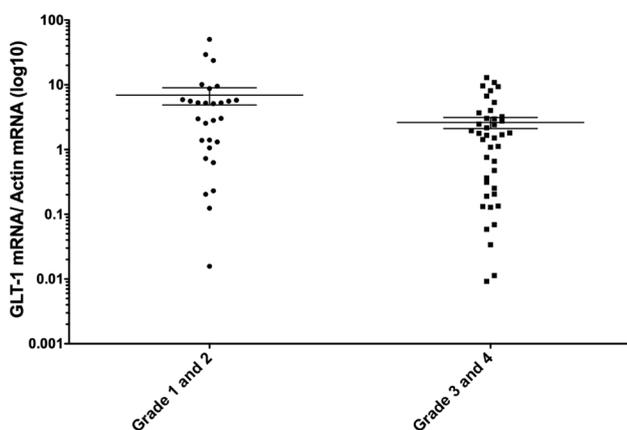
**Figure 4.** GLT-1 mRNA expression in each tumor grade (1, 2, 3, 4) vs. control. GLT-1 mRNA expression is represented by data points in each grade. Mean and SEM of each group was indicated by long horizontal bar and black vertical bars, respectively. Post-test for linear trend was performed,  $p < 0.05$  was accepted as significant. P value was determined to be  $< 0.0001$ ,  $R^2 = 0.2664$ . A significant negative association was found with a slope =  $-4.684$ .

(Figure 3). When we applied the post test for linear trend, we again observed that the GLT-1 expression was decreased in all grades following a linear trend. Mean and SEM of each group was indicated by long horizontal bar and vertical black bars, respectively. We found that GLT-1 expression tends to be significantly lower with the progression of cancer, demonstrating an inverse correlation with the increase of the grade of the tumor. Post-test for linear trend also showed a significant negative association with a slope of -4,6864 ( $R^2=0.2664$  and  $p<0.0001$ ) (Figure 4).

We categorized the tumor samples in two categories (group of grade 1 and 2 tumors and group of grade 3 and 4 tumors) and compared the GLT-1 mRNA expression between the groups (Figure 5). We observed that GLT-1 mRNA expression was significantly lower in grades 3 and 4 groups when



**Figure 5.** GLT-1 mRNA expression in grade 1 and 2 tumors vs. grade 3 and 4 tumors. GLT-1 mRNA expression was significantly lower in grade 3 and 4 tumors when compared to grade 1 and 2 tumors. Two-tailed unpaired t-test with Welch's correction was performed,  $p^*<0.05$  was accepted as significant. Grade 3 and 4 vs. Grades 1 and 2  $p=0.0193$ .



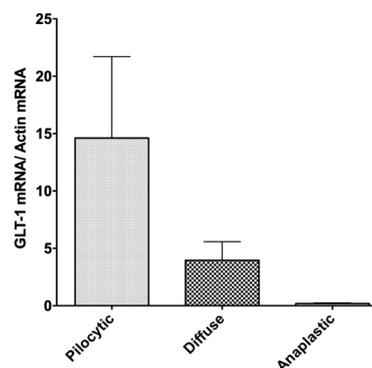
**Figure 6.** GLT-1 mRNA expression in grade 1 and 2 tumors vs. grade 3 and 4 tumors. The figure shows the plotted data points of the graph in Figure 5. The mean and the SEM of each group was demonstrated by long horizontal bar and vertical black bars, respectively.

compared to grades 1 and 2 group using unpaired t-test (Figure 5). This data confirms the fact that GLT-1 mRNA expression is inversely correlated with the tumor grade. Figure 6 only shows the plotted data points of the graph in Figure 5. The mean and the SEM of each group was demonstrated by long horizontal bar and vertical black bars, respectively (Figure 6).

Pilocytic, diffuse and anaplastic astrocytomas are among the various types of astrocytomas and graded as grade 1, 2 and 3, respectively according to the WHO 2016. Some of the samples in our study were defined to be pilocytic, diffuse and anaplastic astrocytomas. Even though the sample size was small (Table 2), we compared the GLT-1 mRNA expression in pilocytic, diffuse and anaplastic astrocytomas and observed that the GLT-1 expression was lowest in anaplastic astrocytoma (grade 3) and highest in pilocytic astrocytoma (grade 1) (Figure 7). Although the differences were not significant due to the small sample size of anaplastic astrocytoma group according to the Student's t-test with Welch's correction, there seems to be a trend showing an inverse correlation of the GLT-1 gene expression with the tumor grade within astrocytomas (Figure 7), which is consistent with the previous data (Figures 3 and 4).

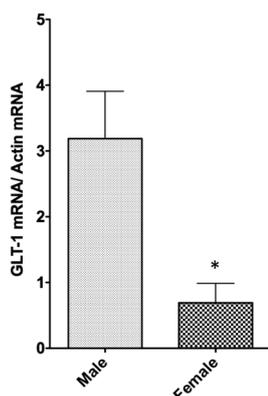
*GLT-1 mRNA expression is higher in male GBM patients than in females*

Low-grade glioma incidence is nearly identical in males and females, whereas malignant brain tumors in general occur more commonly in males, regardless of the patient age or geographical loca-

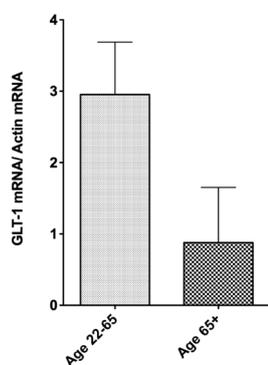


**Figure 7.** GLT-1 mRNA expression in pilocytic, diffuse and anaplastic astrocytomas. GLT-1 mRNA expression seems to be lower in diffuse astrocytomas compared to pilocytic astrocytomas and in anaplastic astrocytomas compared to diffuse or pilocytic. However, these differences are not significant. Two-tailed unpaired t-test with Welch's correction was performed,  $p<0.05$  was accepted as significant. Pilocytic vs. diffuse  $p=0.2398$ , diffuse vs. anaplastic  $p=0.1467$ , pilocytic vs. anaplastic  $p=0.1352$ .

tion [18-20]. As shown in recent reports, GBM occurs with a male-to-female ratio of 1.6:1 [21]. For this reason, we were interested in investigating the gender aspect of GLT-1 mRNA expression. In our study, we had the highest number of patient samples in grade 4 tumor group. Therefore, we analyzed the GLT-1 mRNA expression according to the gender and found that the GLT-1 mRNA expression was higher in males compared to females (Figure 8). This is again consistent with the data mentioned above that male patients carry more malignant brain tumors compared to females. This data may suggest that there is a correlation between GLT-1 expression, the gender, the tumorigenicity and also the influence of sex hormones in GBM. We also analyzed the GLT-1 expression in males and females of grade 2 and grade 1 tumor groups and found no significant difference or a ten-



**Figure 8.** GLT-1 mRNA expression in males and females of grade 4 tumors (GBM and medulloblastomas). GLT-1 mRNA expression was significantly lower in females when compared to males. Two-tailed Unpaired t-test with Welch's correction was performed,  $p < 0.05$  was accepted as significant. Males vs. females  $p = 0.0032$ .



**Figure 9.** GLT-1 mRNA expression in age 22-65 and age 65+ groups. GLT-1 mRNA expression seems to be lower in 65+ age group when compared to age 22-65 group. However, this difference was not significant. Two-tailed unpaired t-test with Welch's correction was performed,  $p < 0.05$  was accepted as significant. Age 22-65 vs. age 65+  $p = 0.0996$ .

dency of GLT-1 mRNA expression among females and males (data not shown). Grade 3 tumors did not have enough female samples to carry out the analysis. These data indicate that a gender difference in GLT-1 mRNA expression occurs only in high-grade tumors (grade 4) and not in lower grade tumors showing that the gender-based difference of GLT-1 expression increases when the tumorigenicity increases.

#### *GLT-1 mRNA expression tends to be higher in grade 4 tumors of aged (+65) patients*

Since age is a risk factor for cancer as well as many other metabolic and neurodegenerative diseases, we were interested in knowing whether the GLT-1 expression differs among the grade 4 tumors of aged patients. We had a higher number of patients in grade 4 tumor group (GBM and medulloblastoma) compared to others (Table 2). Therefore, we compared the patients older than 65 years of age and younger and found that older patients (+65) display lower GLT-1 mRNA expression compared to younger patients (Figure 9). However, this difference is not statistically significant according to Student's t-test with Welch's correction due to the small sample size of the 65+ age group. The 65+ age group had only 3 patients while the younger age group (age 22-65) had 24 patients. Although not significant, there seems to be a tendency of a lower GLT-1 expression in older patients compared to younger ones. This difference would be significant with a larger sample group of 65+ age group (Figure 9).

## Discussion

We determined the GLT-1 mRNA expression in 66 primary brain tumor tissues and 11 healthy brain tissues as control using qRT-PCR. To our knowledge, this is the first study identifying the GLT-1 gene expression in primary brain tumors with varying grades such as astrocytomas (pilocytic, diffuse, anaplastic), meningiomas, ependymomas, oligodendrogliomas, GBMs and medulloblastomas (Tables 1 and 2).

We observed that GLT-1 mRNA expression was decreased significantly in all types of primary brain tumors when compared with the healthy brain tissues (Figure 1). GBM and medulloblastoma, which are grade 4 tumors, seemed to display the lowest GLT-1 mRNA expression (Figure 1), although not significant. Our data is consistent with the previous finding [16] where tissue microarray analyses results showed decreased EAAT-2 (GLT-1) expression in high-grade glial tumors compared with low-grade astrocytomas and normal brain. They

also identified an undetectable level of GLT-1 in glioma cell lines [16]. We then combined all types of tumors and compared them with the control tissues from healthy brains. What we found out was that the GLT-1 mRNA expression was lower in the tumors combined when compared to control (Figure 2). This is consistent with our previous data where the GLT-1 mRNA expression in each tumor type is lower when compared to healthy brain control (Figure 1).

When we analyzed the GLT-1 expression according to the grades of the tumors, we identified an inverse correlation between the GLT-1 expression and the tumor grade (Figure 3 and 4). Additionally, when the grade 3 and 4 tumors were grouped together and compared with the grade 1 and 2 tumors, the GLT-1 mRNA expression was found to be lower in grade 3 and 4 tumor group (Figure 5 and 6). Finally, within astrocytomas, the GLT-1 expression was again inversely correlated with the tumor grades, anaplastic astrocytomas showing the lowest expression and the pilocytic astrocytomas the highest, although not significant due to the small sample size of anaplastic astrocytomas (Figure 7). If the sample size of anaplastic astrocytomas was higher, the result would probably reach significance.

Many cancers have a gender aspect including glioblastomas [22]. A recent study showed that the sex of the patient is correlated with the prognosis and also with the responses to different treatments, suggesting that it may be important to consider the gender when optimizing the therapeutic regimen for each patient [22]. Therefore, we were interested in knowing whether GLT-1 expression differs according to gender in primary brain tumors. We analyzed the GLT-1 mRNA expression in samples of male and female patients of grade 4 tumors, since it contains the highest number of samples in our study and found that the males express higher GLT-1 expression compared to females (Figure 8). We did not observe any difference in GLT-1 expression among genders in grade 1 and 2 tumors. These data show that the gender-based difference of GLT-1 expression occurs when the tumorigenicity increases. Grade 3 tumors did not have an appropriate sample size for females to carry out the comparisons.

Many studies in the literature reported that malignant brain tumors generally occur more commonly in males regardless of patient age or geographical location. However, the incidence of low-grade glioma was similar in males and females [18-20]. These previous studies show that the gender difference of the incidence is correlated with the tumorigenicity. This is consistent with our find-

ing in this study since we observed the gender difference of GLT-1 expression only in grade 4 tumors. Although the malignant tumors are more common in males than in females, we found that the GLT-1 mRNA expression, which inversely correlates with tumorigenicity, was higher in males compared to females. We expected females to express higher levels of GLT-1 expression compared to males; however, the correlation of GLT-1 expression with the gender might be playing a role in a more complicated network than we think. A recent study demonstrated that sex influences survival among patients with GBM. Compared with male patients, female patients with GBM have a higher cancer-specific-survival (CSS) after surgery [23]. However, this result of that study failed to reach statistical significance in multivariate Cox regression models of regional and distant stages. As a result, in our study, it is not clear why males display higher GLT-1 expression in grade 4 tumors and the effect of gender on GLT-1 expression warrants further investigation.

Cancer is an age-related disease carrying an increased risk factor for older ages. The incidence of primary brain tumors is highest in elderly patients, and advanced age often is a negative prognostic factor [24]. The efficacy of various treatments on high-grade gliomas is not satisfactory [25], although different combination therapies are being applied [26]. For this reason, we were interested in determining the correlation between GLT-1 expression and aging. We found out that GLT-1 mRNA expression decreases in GBM patients older than 65 years of age (+65) group compared to the younger group (22-65 of age) although statistically not significant with the Student's t-test with Welch's correction due to the small sample size of the 65+ age group (Figure 9). Since age is a risk factor for GBM and GLT-1 is inversely correlated with tumorigenicity according to our results, the reduction of GLT-1 mRNA expression in the tumors of the elderly would be expected. We observed this reduction in our study, although not significant. If the sample size of GBM patients older than 65 years of age (+65) group was larger, this difference would probably reach statistical significance.

Memantine and Rilutek are two drugs suggested for Alzheimer's disease and amyotrophic lateral sclerosis (ALS), respectively [27]. The molecular mechanisms underlying the development of these drugs are based on the prevention of excitotoxicity [27]. These glutamate modulators including others are also candidates for treating other brain diseases such as obsessive-compulsive disorder [28]. Excitotoxicity is one of the molecular pathways underlying brain diseases including gliomas [29]

and exploring excitotoxicity for therapeutics is a very promising field. This study will pave the way for the future research regarding the excitotoxicity and brain tumors and GLT-1 might be a potential biomarker for this disease due to its significant decrease in high-grade gliomas. Targeted therapies based on excitotoxic molecular pathways against gliomas should be designed to effectively combat these diseases.

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## Conflict of interests

The authors declare no conflict of interests.