Glutamate Receptor Expression in Astrocytoma Tissue from Patient with and without Seizure

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Abstract

Introduction: Numerous studies have been conducted on the frequency of seizures in astrocytoma patients. This connection underlines how important it is to understand the relationship between astrocytomas and seizures, both for therapeutic as well as diagnostic reasons.

Objectives: To investigate the potential association between somatic gene mutations and the incidence of seizures in astrocytoma patients.

Materials and Methods: We collected 20 fresh frozen tissue surgical specimens from astrocytoma patients. Half of these patients (n = 10) experienced seizure after tumor removal surgery, while the other half (n = 10) did not. DNA extraction and whole-exome sequencing were performed to identify and compare the proportion of affected patients with somatic mutations in each gene among these two groups.

Results: The ionotropic glutamate receptors, categorized into AMPA, kainate, and NMDA receptors were found in 2 and 2, 4 and 2, and 7 and 7 patients between seizure and non-seizure group, respectively. Similarly, for the metabotropic glutamate receptors, the presence of these genes was found to be comparable between the seizure and non-seizure groups, with no statistically significant differences identified ($p > 0.05$).

Conclusion: Our study found no significant difference in somatic mutations between the seizure and the non-seizure group in astrocytoma patients. The lack of difference in somatic mutations between these two groups suggests that the etiology of seizures in this population is complex and not solely determined by this gene.

Keywords : Astrocytoma, Seizure, Somatic mutations

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Introduction

For over a century, there has been evidence of a link between brain tumors and seizures. However, it wasn't until the second half of the 20th century that researchers started to classify and comprehend specific types of tumors, such astrocytomas, that were more likely to cause seizures.[1] Numerous studies have been conducted on the frequency of seizures in people with astrocytomas, and some of these studies have raised the possibility that seizures may be the initial a sign of an underlying astrocytoma.[2] This connection underlines how important it is to understand the relationship between astrocytomas and seizures, both for therapeutic as well as diagnostic reasons. Seizure treatment is a crucial component of comprehensive patient care since it can have an important impact on the quality of life for patients with astrocytomas.[3] While astrocytomas can cause seizures, the recurrent seizures themselves may have an effect on the development of the tumor and the surrounding neuronal microenvironment. The interaction between these factors has important implications for therapeutic strategies since preventing seizures may not only reduce symptoms but also have an impact on how the tumor develops.[4]

Numbers of causal of seizure in astrocytoma are purpose, astrocytomas have the potential to disturb the complex neuronal networks in the brain. Growth of the tumor may put pressure on adjacent brain structures, impairing neurotransmission and changing neural dynamics.[5] Additionally, a tumor itself may start to produce abnormal electrical activity, acting as an epileptogenic focus.[4,5] Research on the precise methods by which astrocytomas cause seizures is currently ongoing. Seizures may be brought on by the release of excitatory neurotransmitters from the tumor or the inflammatory tissue around it, according to some theories. Additionally, alterations in ion channel function and disruptions in the blood-brain barrier associated with tumor growth can also play a role.[6]

In recent years, there has been growing interest in understanding the role of glutamate receptors in gliomas with epilepsy. However, astrocytoma, there is limited studies. In recent years, there has been growing interest in understanding the role of glutamate receptors in astrocytomas with epilepsy. One study conducted by Smith et al. investigated glutamate receptor expression in astrocytoma tissues obtained from patients with comorbid epilepsy. The study found that there was a significant upregulation of glutamate receptor expression in astrocytomas compared to non-epileptic controls.[7]

Glutamate receptors are central to synaptic transmission and plasticity in the brain, and their dysregulation has been implicated in various neuropathological conditions, including epilepsy.[8] The glutamate receptor gene family, consisting of multiple subunits with various isoforms, has emerged as a genetic element of interest in the context of astrocytoma-related seizures. Genetic variations in these receptor genes can alter receptor function, potentially modulating the excitatory neurotransmission landscape and influencing seizure susceptibility.[9]

The focus of this research is to investigate the potential association between genetic variations in the glutamate receptor genes and the incidence of seizures in patients with astrocytoma. Understanding the genotypic underpinnings of seizure activity in these patients could offer new therapeutic approaches, and allow for the development of targeted treatments that cater to the genetic profile of each patient. This research is committed to

contributing to a more personalized medical framework, one that recognizes the role of genetic variations in the neurological sequelae of astrocytoma, with the goal of providing the highest level of clinical care and improving patient outcomes.

Materials and Methods

1. Biological samples and sequencing template preparation

From the surgical specimens of astrocytoma patients, we collected 20 fresh frozen tissue samples. Half of these patients (n = 10) experienced seizure after tumor removal surgery, while the other half (n = 10) did not. DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen Inc.). Subsequently, Nanodrop (Thermo Fisher Scientific, Inc.) and TapeStation (Agilent Technologies, Inc.) were then utilized to assess the quantity and quality of the extracted DNA. The clinical information of all the patients was retrieved from the electronic medical records (EMR) of the hospital. All patients provided written informed consent before recruitment. The study was approved by the ethical committee of Faculty of Medicine, Prince of Songkla University

2. Whole-exome sequencing

We used the library preparation system of Agilent SureSelect XT Human All Exon v8 (Agilent Technologies, Inc.) to conduct whole exome sequencing. A Qubit dsDNA High Sense Assay Kit (Invitrogen, Carlsbad, CA, USA) was used to quantify the library, and the Agilent D1000 ScreenTape assay was used to perform size measurements. An Illumina NovaSeq-6000 platform (Illumina, San Diego, California, United States) with paired-end reads of 150-bp was used to perform sequencing. The average targeted coverage depth was 200x.

We used FastQC (version 0.11.9) to assess the quality of the paired-end sequence files and Trimmomatic (version 0.39) to trim them. The BWA program (version 0.7.17) was used to align the optimally prepared FASTQ files with the human reference genome (version GRCh38.13). The resulting Sequence Alignment Map (SAM) files were converted to a Binary Alignment Map (BAM) format and sorted by the SAMtools (version 1.17)[10]. The sorted BAM files were then regrouped, and Picard (version 3.0.0) was used to mark identical sequences. We used Genomic analysis toolkit (GATK, version 4.4.0) base quality score recalibration to process unduplicated BAM files and adjust the base quality score. We used Mutect2 in tumor-only mode to perform variant calling. A public Panel of Normals (PON) was downloaded from the public repository of GATK at https://storage.googleapis.com/gatk-best-practices/somatic-hg38/1000g_pon.hg38.vcf.gz. We used GATK4 GetPileupSummaries, Calculate Contamination and FilterMutectCalls with a default value of argument to filter the variants generated by Mutect2. The resultant variants were annotated by Funcotator. The annotated mutational data were stored in MAF files. We used maftools package in R to summarize and visualize the data[11].

3. Identification of mutation marker for post-surgical seizure

We divided patients with astrocytoma into two groups based on the occurrence of post-surgical seizure. In our study, post-surgical seizure manifested in 10 patients with astrocytoma, while 10 did not have that symptom. We identified somatic mutations among these two groups. We also found somatic mutations in 22 genes, which were previously associated with seizure, such as GRIN2D, GRIA4, GRIK1, GRIN1, GRM7, GRIK2,

GRM2, GRM3, GRIA1, GRIA2, GRIA3, GRIK4, GRIK5, GRIN2A, GRIN2B, GRIN2C, GRIN3A, GRIN3B, GRM1, GRM4, GRM5, GRM6. Fisher's exact test was used to compare the proportion of affected patients with somatic mutations in each gene across two groups. We considered a p-value < 0.05 as statistically significant.

Results

Our study included a total of 20 patients with astrocytoma, divided into two groups: those with a history of seizures (seizure group) and those without (non-seizure group). The seizure group comprised 10 patients, while the non-seizure group contained 10 patients. Table 1 presents patient characteristics, tumor type, and treatment characteristics for the entire cohort. The mean age of the patients was 46.0 years in seizure group and 45.0 years in non-seizure group at the time of diagnosis. The most common seizure type among the 13 patients was focal to bilateral tonic-clonic seizure (46.2%), followed by focal impair awareness with motor onset seizures (23.1%), followed by focal aware motor onset (15.4%). Mean duration of follow-up after initial diagnosis of 3.2 years. The most common location of the astrocytoma found in this study was in the frontal lobe (60%), followed by the temporal lobe (20%) and the cerebellar lobe (10%). Tumors involving the frontal lobe were more likely to cause seizure (p=0.037, frontal lobe involved vs. not involved).

Table 1 Characteristics of patients with astrocytoma between seizure group and non-seizure group

	Seizure (N=10)	No seizure (N=10)
Age – years (mean, range)	46.0 (36-73)	45.0 (15-74)
Female sex – no. (%)	3 (30)	7 (70)
Number of perform surgery		
- 1	6	4
- 2	2	5
- 3	2	1
WHO classification of brain tumors		
- WHO grade I	0	0
- WHO grade II	3	4
- WHO grade III	4	4
- WHO grade IV	3	2
Location of tumor		
- Frontal	7	5
- Temporal	2	2
- Occipital	0	1
- Insular	1	0
- Cerebellar	0	2
Side of tumor		
- Right	5	6
- Left	4	3
- Bilateral	1	1
Seizure semiology		
- Focal onset without impaired awareness	1	1
- Focal onset with impaired awareness	1	2
- Generalized seizure	6	0
- Unknown onset	2	0

Table 1 Characteristics of patients with astrocytoma between seizure group and non-seizure group

	Seizure (N=10)	No seizure (N=10)
Adjuvant treatment		
- Radiotherapy	9	9
- Chemotherapy	3	3
Other neurological symptoms		
- Headache	5	6
- Weakness	4	5
- Numbness	0	2
- Cognitive impairment	1	3
- Ataxia	0	2
- Blurred vision	1	1
AEDs before surgery		
- Yes	9	5
- No	1	5
Present with seizure		
- Yes	9	2
- No	1	8
Post surgical seizure		
- No	5	8
- At least daily	2	0
- At least weakly	0	0
- At least months	3	2
Tumor size (cm3)	138.6	127.6
Maximum tumor volume (ml)	69.3	63.8
Perilesional edema		
- No	2	0
- Mild	5	6
- Moderate	3	1
- Severe	0	3
Intratumoral hemorrhage		
- Yes	1	4
- No	9	6
Last follow up (months)	45.0	31.8
MRS score		
- 0	5	1
- 1	1	2
- 2	0	3
- 3	1	0
- 4	2	0
- 5	0	4
- 6	1	0
Status at last follow		
- Alive	9	10
- Dead	1	0

Ionotropic Glutamate Receptors

The ionotropic glutamate receptors, categorized into AMPA, kainate, and NMDA receptors, were analyzed for their gene presence in both patient groups. The AMPA receptors (GluA1, GluA2, GluA3, GluA4) in seizure group and non-seizure group were found in 2 and 2 patients, respectively. The kainate receptors (GluK1, GluK2, GluR3, GluK4, GluK5) were found in 4 and 2 patients, respectively. While the NMDA receptors (GluN1, GluN2A, GluN2B, GluN3) were found in 7 and 7 patients, respectively. Statistical analysis demonstrated no significant differences in the presence of these ionotropic receptor genes between the two groups ($p > 0.05$).

Metabotropic Glutamate Receptors

Similarly, for the metabotropic glutamate receptors, which are organized into group 1 (mGluR1 and mGluR5), group 2 (mGluR2 and mGluR3), and group 3 (mGluR4, mGluR6, mGluR7, and mGluR8). The presence of metabotropic receptor genes was found to be comparable between the seizure and non-seizure groups, with no statistically significant differences identified ($p > 0.05$). [Table 2]

Table 2 Comparison of glutamate receptor expression between seizure group and non-seizure group

Glutamate	Seizure	Non-seizure	Fisher-Exact p-value	Odd ratio
Inotropic glutamate receptors				
1. AMPA receptors : 4 subtypes : GRIA1 (GluA1), GRIA2 (GluA2), GRIA3 (GluA3), GRIA4 (GluA4)	2	2	1	1
2. Kainate receptors : 5 subtypes : GRIK1 (GluK1), GRIK2 (GluK2), GRIA3 (GluR3), GRIK4 (GluK4), GRIK5 (GluK5)	4	2	0.6285	2.536921
3. NMDA receptors : GRIN1 (GluN1), GRIN2A (GluN2), GRIN2B (GluN2), GRIN3A, GRIN3B	7	7	1	1
Metabotropic glutamate receptors				
1. Group 1 : GRM1 (mGluR1), GRM5 (mGluR5)	2	2	1	1
2. Group 2 : GRM2 (mGluR2), GRM3 (mGluR3)	2	2	1	1
3. Group 3 : GRM4 (mGluR4), GRM6 (mGluR6), GRM7 (mGluR7), GRM8 (mGluR8)	1	2	1	0.4624944

Discussion

Our study found no significant difference in genetic variations of glutamate receptor gene between the seizure group and the non-seizure group in patients with astrocytoma. This finding is contrary to the initial hypothesis that variations in the glutamate receptor gene might correlate with the neuropathophysiology of seizures in astrocytoma. The lack of discrepancy in the variations of the glutamate receptor gene between groups suggests that the development of seizures may not be driven by this gene alone.

Seizures in astrocytoma patients could be multifactored, influenced by a combination of other genetic factors, tumor location, size, inflammatory

processes, and alterations in neurotransmitter systems, rather than solely mediated by glutamate signaling pathways.

Our findings seem to diverge from the conclusions of prior studies, which have indicated that glutamate receptor genes contribute to epileptogenesis. This discrepancy can be influenced by multiple factors including the diversity of patient populations, the methodologies used for genetics and clinical assessments, and the distinct genetic backgrounds of the studied groups. The absence of differences in glutamate receptor gene between groups highlights the potential role of alternative mechanisms in seizure generation within the astrocytoma patient population. Astrocytoma pathology may itself alter neuronal circuitry or the tumor microenvironment in a manner that predisposes to seizures independent of glutamate receptor gene status. Given these findings, the clinical utility of screening for glutamate receptor gene mutations as a predictive biomarker for seizure risk in astrocytoma may be limited. Clinicians should continue to rely on a multifactorial approach to seizure risk assessment that incorporates tumor location, size, histological grade, and patient history.

One possible limitation affecting our results could be the sample size. A larger cohort of patients may provide sufficient power to discern subtle genetic differences impacting the occurrence of seizures. Additionally, we focused on specific gene previously implicated in seizure disorders, which may not encapsulate the full spectrum of genetic variants that influence seizure risk. Moreover, the study design was retrospective study, which might restrict the generalizability of the results and the ability to infer causality.

The strength of our study is 1) the investigation of glutamate in a specific subtype of glioma, astrocytoma, 2) the utilization of new method for genetic study, next generation sequencing.

In future, we are planning to investigate the gene variation of other neurotransmitter, as well as excitatory and inhibitory neurotransmitter in astrocytoma, along with the genetic landscape in astrocytoma with epilepsy.

Conclusion

Overall, the lack of difference in the presence of the glutamate receptor gene between groups with and without seizures in astrocytoma patients suggests that the etiology of seizures in this population is complex and not solely determined by this gene. Our study emphasizes the need for comprehensive research to elucidate the multitude of factors influencing seizure occurrence in order to enhance prognostic capabilities and therapeutic strategies for patients with astrocytoma.

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