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Original Article

Correlation between GCH1 mutation and glioma

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Abstract: Objective: This study aimed to investigate the correlation between guanosine triphosphate (GTP) cyclohydrolase 1 (GCH1) mutation and glioma. Methods: A total of 152 glioma patients diagnosed and treated in the Affiliated Hospital of Jining Medical University from February 2016 to February 2019 were randomly enrolled as the case group, while 218 healthy people who received physical examination in the same hospital during the same time period were included in the control group. The venous blood deoxyribonucleic acid (DNA) was extracted from all the subjects, and rs841 and rs3783637 of GCH1 gene were detected using TaqMan fluorescence quantitative polymerase chain reaction (qPCR). The distribution frequencies of diverse genotypes were recorded, and the associations of varying genotypes of single nucleotide polymorphisms (SNPs) with the risk of glioma were analyzed. Results: There were no statistical differences in the 3 genotype frequencies (CC, CT and TT) of rs841 and rs3783637 between the case group and the control group (P>0.05). In terms of genetic model analysis, no statistical differences in the dominance, excessiveness and overdominance of rs841 and rs3783637 were observed (P>0.05). However, the glioma patients with dual homozygous mutation of the two polymorphisms, namely TT vs. TT, had a higher risk value [odds ratio (OR) =4.053], displaying a statistical difference (P=0.016). The genotypes exhibited no statistically significant differences among grade I, II and III (P>0.05). In the case of grade IV, the incidence probability in patients with TT genotype of rs841 was significantly higher than those with CC and CT genotypes (P<0.05), and the incidence probability in patients with TT genotype of rs3783637 was statistically higher than those with CC genotype (P<0.05). Conclusion: There is an increasing risk of glioma in the patients with homozygous mutant TT genotype of GCH1 gene, which is certainly related to the WHO pathological grade.

Keywords: GTP cyclohydrolase, glioma, fluorescence quantitative PCR, gene polymorphism

Introduction

Glioma, also known as brain glioma, is a neuroepithelial tumor and belongs to craniocerebral malignant tumors, with a relatively high incidence rate. It has great harms to the body, and a malignant tumor has generally emerged and developed with different degrees when the patients are diagnosed with glioma. Besides, the symptoms and onset time of glioma are also different, from several weeks to several months or even more than a year [1, 2]. The etiology of glioma has not been clarified yet, and factors such as viral infections, changes in living environment as well as chemical and electromagnetic radiation are all able to trigger tumorigenesis. In the meantime, the heredity of population genes is an internal cause of tumorigenesis [3, 4], in which the gene polymorphism can reflect the diversified risks of the same disease in the same population. Currently, multiple genes correlated with glioma have been explored and studied from the aspect of pathways affected. It has been reported that guanosine triphosphate (GTP) cyclohydrolase 1 (GCH1) gene is closely related to neuropathic pain, dopa-responsive dystonia and cancer pain, serving as a key gene of neuroregulation [5, 6], but the relationship between its polymorphism and glioma is rarely reported. Therefore, based on the polymorphisms of two single nucleotide polymorphisms (SNPs) sites of GCH1 gene, the correlation of the polymorphisms with the occurrence of glioma was analyzed particularly in the present study.

Materials and methods

General data

A total of 152 glioma patients diagnosed and treated in the Affiliated Hospital of Jining Me-

Table 1. General clinical data

Parameter	Case group (n=152)	Control group (n=218)	χ^2/t	*P*
Gender				
Male	89 (58.6%)	122 (56%)	0.138	0.710
Female	63 (41.4%)	96 (44%)		
Age (years old)	49.2±12.3	46.5±11.7	2.126	0.866
Smoking addiction	34 (22.4%)	46 (21.1%)	0.05	0.823
Alcoholism	48 (31.6%)	55 (25.2%)	1.007	0.316

Table 2. Primer sequences

SNP	Primer sequence	Probe sequence
rs841	Forward: 5'-CAATTGTTACAGATGTGAAC-3'	HEX: 5'-AGTGTAAGTATGTGCACA-3'
	Reverse: 5'-TGACAGTTCGCACAGGACGTC-3'	FAM: 5'-AGTGTAAGTACGTGCACA-3'
rs3783637	Forward: 5'-AATCACATCCTGCAACTC-3'	HEX: 5'-TCTACCACTTGTTTGA-3'
	Reverse: 5'-AGTGAAAGCAGAGAGAGA-3'	FAM: 5'-TCTACCACCTGTTTGA-3'

dical University from February 2016 to February 2019 were randomly selected as the case group, including 29 cases of World Health Organization (WHO) pathological grade I, 46 cases of grade II, 48 cases of grade III and 29 cases of grade IV. In addition, 218 healthy people undergoing the physical examination in the same hospital during the same time period were enrolled as the control group. The individuals in this study were selected according to the following criteria. Inclusion criteria: (1) brain radiology (computed tomography or magnetic resonance imaging) to diagnose or exclude OPG; (2) GCH1 gene tested to determine pathogenic mutations; (3) age of patients without OPG of 10 years or more. Exclusion criteria: (1) inconclusive radiological diagnosis of OPG; (2) wrong assessment of gene mutations, due to cDNA changes not conforming to predicted amino acid alterations or the original cDNA base in the reported position not conforming to the corresponding base in the reference sequence. All the patients and their families understood the study information and had signed the informed consent. The study was approved by the ethics committee of our hospital. There were no statistically significant differences in gender, age difference, lifestyle habits, etc. ($P>0.05$) (**Table 1**), and the results were comparable and effective.

Methods

Extraction of genomic deoxyribonucleic acid (DNA): The whole blood (5 mL) was drawn from every patient by using an anticoagulant vacuum tube, and the genomic DNA was extracted from the blood using Omega Mag-Binds Forensic DNA Kit (Omega). After that, the concentration and purity of DNA were detected via NanoDrop, and the DNA was stored at -20°C.

SNP typing via reverse transcriptase-polymerase chain reaction (RT-PCR): The primer sequences and TaqMan probe sequences at the SNP sites were designed using Oligo 6.0 (**Table 2**), and the primers were synthesize by Sangon Biotech (Shanghai) Co., Ltd. 1 μL of DNA solution and 1.2 μL of prepared primer solution (including 0.4 μL of forward primer, 0.4 μL of reverse primer and 0.4 μL of probe primer) were added into 17.8 μL of TransStart Probe quantitative PCR (qPCR) SuperMix (TransGen Biotech Co., Ltd., Beijing) prepared in advance, which were mixed by shaking gently and placed in a CFX96 fluorescence qPCR instrument (Bio-Rad). The reaction conditions were as follows: 94°C for 3 min, 94°C for 15 s and 60°C for 30 s for 40 cycles. The experimental results were generated by the built-in software of the instruments. 3 duplicated wells were set for detection of each sample, DEPC-treated water was used for negative control, and positive plasmids containing the sequences [synthesize by Sangon Biotech (Shanghai) Co., Ltd.] were adopted for positive control. Genotyping: those close to the FAM abscissa belong to homozygous wild type, those close to the HEX ordinate belong to homozygous mutant type, and those close to the 45° line belong to heterozygous type.

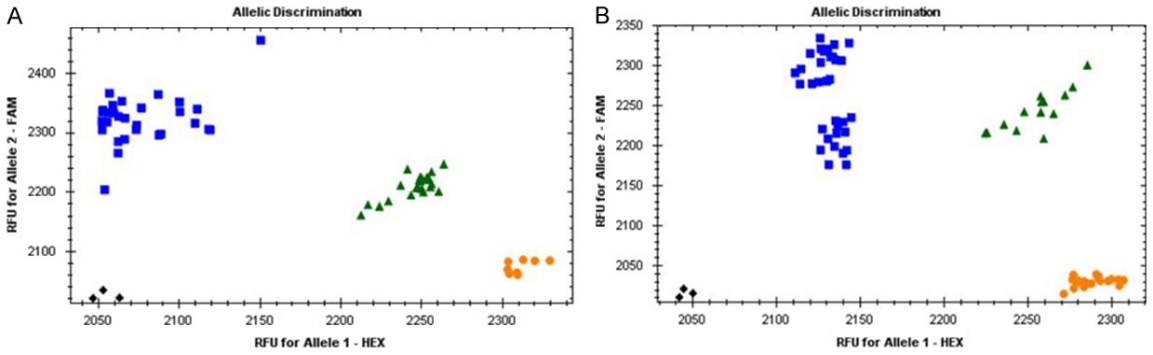

Figure 1. Genotyping results of rs841 and rs3783637. A. Genotyping results of rs841. B. Genotyping results of rs3783637. For rs841, the FAM stands for CC genotype, the HEX for TT genotype, and the green part for CT genotype. For rs3783637, the FAM stands for CC genotype, the HEX for TT genotype, and the green part for CT genotype.

Table 3. Distribution of genotype frequencies

Genotype	Case group		Control group		χ^2	*P*
	n	%	n	%		
rs841						
CC	62	40.8	114	52.3	2.658	0.103
CT	58	38.2	78	35.8	0.124	0.725
TT	32	21	26	11.9	3.013	0.083
C	182	59.9	306	70.2	2.333	0.127
T	122	40.1	130	29.8		
rs3783637						
CC	57	37.5	98	45	1.161	0.281
CT	59	38.8	90	41.3	0.13	0.718
TT	36	23.7	30	13.7	3.289	0.070
C	173	56.9	286	65.6	1.595	0.207
T	131	43.1	150	34.4		

Statistical analysis

SPSS 19.0 was employed for statistical analysis. The difference in the distribution of genotypes between the case group and the control group was recorded. Chi-square test was applied in statistical calculations for the analysis of variables. $P<0.05$ suggested that the difference was statistically significant.

Results

Genotyping results and distribution frequencies of GCH1 gene polymorphisms

The definite genotyping results were obtained from all the patients (**Figure 1**). Among the study population, there were no statistical differences in the frequencies of the 3 genotypes (CC, CT and TT) of both rs841 and rs3783637 between the case group and the control group ($P>0.05$) (**Table 3**). The distribution conformed to the requirement of Hardy-Weinberg equilibrium [*P* (rs841) =0.32, *P* (rs3783637) =0.46].

Correlation between rs841 and risk of glioma analyzed via models

The odds ratio (OR) of dominant model (TC+CC/TT), recessive model (CC/TT+TC) and over-dominant model (TT+CC/TC) was 1.969 ($P=0.018$), 1.591 ($P=0.03$) and 1.107 ($P=0.641$), respectively (**Table 4**).

Correlation between rs3783637 and risk of glioma analyzed via models

The OR of dominant model (TC+CC/TT), recessive model (CC/TT+TC) and over-dominant model (TT+CC/TC) was 1.945 ($P=0.014$), 1.361 ($P=0.153$) and 0.902 ($P=0.634$), respectively (**Table 5**).

Correlation of combinative mutation of rs841 and rs3783637 with the risk of glioma

Among the 4 types of double mutations, the glioma patients with dual homozygous mutation, namely TT vs. TT, were found with significantly high risk value (OR=4.053) ($P=0.016$), while no differences were detected in other mutation combinations ($P>0.05$) (**Table 6**).

Correlations of GCH1 gene polymorphisms with clinical WHO grades

The genotypes exhibited no statistically significant differences among grade I, II and III ($P>0.05$). In grade IV, the incidence probability in patients with TT genotype of rs841 was evi-

Table 4. Risk of glioma analyzed via different models of rs841

Model	Genotype	Case group (n=152)	Control group (n=218)	OR [95% confidence interval (CI)]	*P*
Dominant model	TT	32 (21)	26 (11.9)	1.969 (1.102-3.451)	0.018
	TC+CC	120 (79)	192 (88.1)		
Recessive model	TT+TC	90 (59.2)	104 (47.7)	1.591 (1.037-2.451)	0.030
	CC	62 (40.8)	114 (52.3)		
Overdominant model	TC	58 (38.2)	78 (35.5)	1.107 (0.716-1.700)	0.641
	TT+CC	94 (61.8)	140 (64.5)		

Table 5. Risk of glioma analyzed via different models of rs3783637

Model	Genotype	Case group (n=152)	Control group (n=218)	OR (95% CI)	*P*
Dominant model	TT	36 (23.7)	30 (13.7)	1.945 (1.152-3.342)	0.014
	TC+CC	116 (76.3)	188 (86.3)		
Recessive model	TT+TC	95 (62.5)	120 (55)	1.361 (0.899-2.087)	0.153
	CC	57 (37.5)	98 (45)		
Overdominant model	TC	59 (38.8)	90 (41.3)	0.902 (0.890-1.368)	0.634
	TT+CC	93 (61.2)	128 (58.7)		

Table 6. Distribution of genotype frequencies of rs841 combined with rs3783637 and their OR

Genotype		Case group		Control group		OR (95% CI)	*P*
		n	%	n	%		
rs841	rs3783637						
CC	CC	38	25	66	30.3	1	0.402
TT	TT	21	13.8	9	4.1	4.053 (3.124-6.038)	0.016
CT	CT	39	25.7	32	14.7	2.117 (1.565-2.738)	0.053
CT	TT	5	3.3	3	1.4	2.895 (1.589-1.935)	0.375
TT	CT	5	3.3	11	5	0.789 (0.611-0.912)	0.547

dently increased compared to those with CC and CT genotypes, and the incidence probability in patients with TT genotype of rs3783637 was also higher than those with CC genotype (**Tables 7** and **8**).

Discussion

As a type of tumor in nervous system, glioma is difficult to be cured and able to cause hazards to the health [7, 8]. Its pathogeny is complicated, which is not only related to external environmental factors but also greatly associated with the genetic predisposition of the patients themselves [9, 10]. The existing conventional therapies for the early treatment of glioma include radiotherapy and chemotherapy, and the prognosis and survival of the patients are quite optimistic. However, there are no substantially efficacious measures for advanced glioma; the postoperative effect is not satisfactory, and the 5-year survival rate is low [11, 12]. Hence, a growing number of studies tend to focus on the risk of the disease in organisms themselves, seeking to reduce the incidence probability of glioma through proactive prevention, early detection and in-time treatment [13, 14]. In recent years, a multitude of genes such as IDH, TERT and PPM1D have been discovered to have strong associations with glioma, the mutations and functional alterations of which are believed to drastically increase the incidence probability of glioma [15, 16].

GCH1 gene can encode GCH1 protein which acts as an initial and rate-limiting enzyme of GTP synthesis into tetrahydrobiopterin (BH4). It plays a vital role in the transmission of neurotransmitter and signal [17]. BH4 exerts crucial effects in the processing of several proteins (amino acids) in vivo and participates in the reactions producing neurotransmitter. Such a chemical substance transmits signals between nerve cells in the brain. Among the numerous functions, dopamine can transmit signals in the brain and stimulate stable body movements,

Table 7. Genotype frequencies of rs841 in glioma patients with WHO grades [n (%)]

Characteristic	n	rs841					
		CC (62)	CT (58)	TT (32)	X^2	df	P
Grade							
I	29	12 (19.4)	13 (22.4)	4 (12.5)	5.601	6	0.4693
II	46	22 (35.5)	15 (25.9)	9 (28.1)			
III	48	19 (30.6)	20 (34.5)	9 (28.1)			
II	29	9 (14.5)	10 (17.2)	10 (31.3)*,#			

*P<0.05 vs. CC genotype, #P<0.05 vs. CT genotype.

Table 8. Genotype frequencies of rs3783637 in glioma patients with WHO grades [n (%)]

Characteristic	n	rs3783637					
		CC (57)	CT (59)	TT (36)	X^2	df	P
Grade							
I	29	11 (19.3)	13 (22)	5 (13.9)	4.135	6	0.6585
II	46	20 (35.1)	15 (25.4)	11 (30.6)			
III	48	18 (31.6)	20 (33.9)	10 (27.8)			
IV	29	8 (14)	11 (18.6)	10 (27.8)*,#			

*P<0.05 vs. CC genotype, #P<0.05 vs. CT genotype.

while serotonin can regulate emotions, sleep and appetite [18]. A study revealed that GCH1 gene mutation caused abnormalities in the dopaminergic neurotransmission, resulting in dystonia [19]. Meanwhile, it was manifested in some studies that GCH1 can affect the activation of T cells in cancers, and it plays important roles in breast cancer and lung cancer [20]. Both rs841 and rs3783637 are located in the intron of GCH1 gene, the SNPs of which have critical relations with the gene expression regulation. In this study, the separate analyses on the susceptibility of rs841 and rs3783637 to glioma indicated that there were no statistical differences in the frequencies of the 3 genotypes (TT, TC and CC) compared with those in the control group, and no prominent correlations with the susceptibility to glioma were detected via the 3 kinds of genetic models (dominant, recessive and over-dominant). Nevertheless, if the homozygous mutant TT genotypes of the two polymorphisms were combined, that is, if the individuals carry 2 types of homozygous mutation simultaneously, the risk value can be significantly increased (OR=4.053, P=0.016). It is speculated that there are inter-individual variations in the occurrence and development of glioma because of genetic polymorphisms. In addition, it was discovered in the WHO pathological grades that the incidence probability in patients with TT genotype of rs841 was higher than those with CC and CT genotypes in the case of grade IV, while that in patients with TT genotype of rs3783637 was higher than that with CC genotype, suggesting that the TT genotypes at the 2 mutation sites are associated with high pathological grades, and people carrying the TT genotypes are more vulnerable to the deterioration of the disease, so early detection and treatment are of great necessity. Our data is similar to the previous findings that the TT homozygous mutation increased the risk of developing Crohn's disease and may contribute to perianal disease [21], while the enhanced UBAC2 expression associated with the homozygous risk allele (TT) of rs9517723 could induce overactivation of ubiquitination-related pathway, resulting in the development of ocular and CNS lesions in Behcet's disease [22]. However, the limitation still exists that differences and diversifications in SNP results may appear due to different races and sample sizes. Therefore, the research results will be more convincing if larger sample sizes and more specific nations and races are incorporated.

Conclusion

In conclusion, the patients with homozygous mutant TT genotype of GCH1 gene are found with increasing risk of glioma, which is certainly related to the WHO pathological grade.

Disclosure of conflict of interest

None.

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