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ORIGINAL RESEARCH

Association Between Genetic Polymorphisms In *TYMS* And Glioma Risk In Chinese Patients: A Case-Control Study

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Background: Thymidylate synthase (*TYMS*) polymorphisms are reported to be related to susceptibility to some cancers. However, no study exists on *TYMS* polymorphisms and glioma risk. This study aimed to evaluate the relationship between two common *TYMS* gene variants (rs1059394 C>T, rs2847153 G>A) and glioma susceptibility.

Methods: This case-control study included 605 patients and 1300 cancer-free individuals. Genotyping was performed using Sequenom Mass-ARRAY. We determined odds ratios (ORs) and their 95% confidence intervals (CIs) to estimate the correlations.

Results: The analysis revealed that rs1059394 TT and CT+TT genotype had significantly low glioma risk (TT to CC: OR = 0.71, 95% CI = 0.52–0.97, $P = 0.03$; CT+TT to CC: OR = 0.74, 95% CI = 0.55–0.99, $P = 0.04$). However, no significant difference was found between rs2847153 and glioma risk in any genetic model ($P > 0.05$). In high-grade gliomas, the GA and GA+AA genotypes of rs2847153 made the majority of genotypes, compared with GG genotype (GA to GG: OR = 2.01, 95% CI = 1.39–2.91, $P < 0.001$; GA+AA to GG: OR = 1.78, 95% CI =1.25–2.54, $P < 0.001$). Moreover, online expression quantitative trait locus (eQTL) analysis indicated that these two polymorphisms may alter TYMS gene expression in transformed fibroblast cells.

Conclusion: Our study provides evidence of the effect of *TYMS* rs1059394 on the susceptibility of glioma. In high-grade gliomas, compared with GG genotype, the GA and GA+AA genotypes of rs2847153 comprise a larger proportion.

Keywords: *TYMS*, glioma, gene variant, susceptibility, case-control study

Introduction

Glioma was the most common type of brain cancer, accounting for almost 80% of brain malignancies.[1] Gliomas were divided into grades I to IV, based on the World Health Organization (WHO) classification scheme.[2] The 5-year survival rate for glioblastoma patients, accounting for 45% of all gliomas, was just 5–6%.[3,4] Various risk factors were considered to be associated with gliomas, such as exposing to high doses of ionizing radiation, allergies or atopic disease, and hereditary genetic disorders (family history).[5,6] Similar to other tumors, hereditary factors seem to be an important factor in the occurrence of glioma. It was reported that single-nucleotide polymorphisms (SNPs) were the most frequent single-nucleotide variations that occur in a specific position. Numerous SNPs, such as those in XRCC1/4, ERCC1/4, MGMT, PARP1, and MTHFR have been demonstrated to contribute to glioma susceptibility.[1,7]

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The thymidylate synthase (*TYMS*) gene is located at human chromosome band 18p11.32. *TYMS* is essential for *de novo* biosynthesis of thymidylate (TMP), cell proliferation and survival.[8] Inhibition of *TYMS* expression leads to thymidylate depletion and thymineless death, accompanied by DNA damage, apoptosis, and chromosome aberrations.[9] Currently, several *TYMS* SNPs have been reported to be correlated with susceptibility to cancers including breast, lung, gastric, colorectal, and ovarian cancers.[10–14]

A previous study presented that *TYMS* expressed positively in 27.39% of lymph node of low-grade glioma patients.[15] However, no studies illuminated the association between *TYMS* gene polymorphism and the glioma risk. Therefore, this case-control study aimed to clarify the correlation between two common *TYMS* gene variants (rs1059394 C>T, rs2847153 G>A) and glioma susceptibility.

Materials And Methods

Study Population

The protocol of this study was approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University Shaanxi Province (Xi'an, China). All patients gave written informed consent prior to participation in the study. This study was conducted in accordance with the Declaration of Helsinki.

This study consisted of 605 patients with gliomas (mean age: 40.71±18.28 years) who underwent surgical resection; they were consecutively recruited between September 2010 and May 2014 at Tangdu Hospital, which is affiliated with the Fourth Military Medical University in China. Eligible patients were diagnosed with glioma based on imaging and pathology, and were untreated with chemotherapy or radiotherapy before surgery. Healthy controls included 1,300 age- and sex-matched healthy individuals (mean age: 41.68 ±13.54 years) who underwent a checkup at the same hospital during the same period of time. Basic characteristics of patients and controls were collected, including ethnicity, age, sex, WHO grade, extent of resection, radiotherapy, and chemotherapy strategy.

Genotyping Assay

Peripheral blood was collected in ethylenediaminetetraacetic acid tubes and stored at −80°C after centrifugation. We then extracted genomic DNA from whole blood using the Universal Genomic DNA Extraction Kit (TaKaRa, Kyoto, Japan). DNA concentrations were assessed using spectrophotometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). In total, two tag-SNPs (rs1059394 and rs2847153) were selected in our study. The Multiplexed SNP Mass EXTEND assay was designed by Sequenom Mass ARRAY Assay Design (version3.0, Agena Bioscience, San Diego, CA, USA),[16] which was referred to in previous studies.[17–19] SNP genotyping was carried out using Sequenom Mass-ARRAY RS1000. Sequenom Typer 4.0 software was used to analyze data.[16,20] Primers of each SNP are presented in Table 1.

Genotype-Phenotype Association

eQTL are regions of the genome containing DNA sequence variants that influence the expression level of one or more genes. We conducted the expression quantitative trait loci (eQTL) analysis using GTEx portal web site (http://www.gtexportal.org/home/) to predict potential associations between the two SNPs and TYMS gene expression levels.[21] The GTEx Portal provides open access to data including gene expression, QTLs, and histology images.

Statistical Analysis

Statistical analyses were performed using the software R (version 3.5.1). The Chi-square test was used to examine Hardy- Weinberg equilibrium (HWE) based on gene frequencies in individuals. We used univariate logistic regression analysis to evaluate differences in the genotype distributions of the two SNPs between the cases and controls. The glioma risk associated with the *TYMS* rs1059394 and rs2847153 genotypes were estimated using odds ratios (ORs) and their 95% confidence intervals (CIs). For all

Table 1 Primers Used For This Study

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
rs1059394	ACGTTGGATGGTATCGACAGGATCATACTC	ACGTTGGATGCGACCTGTTGTAATTGCTCC	cATTGCTCCTCATGTCC
rs2847153	ACGTTGGATGTCTTTAAGTAGGCTGGT CCC	ACGTTGGATGAGAAAAGATCTGGGAGG GTG	gCAAAGAAGGGATCAG ACT

Notes: 1st-PCRP, reverse primer; 2nd-PCRP, forward primer.

tests, a two-tailed P-value < 0.05 was considered statistically significant.

Results

Characteristics Of The Study Population

All the participants were of Han Chinese Ethnicity. There were no significant differences between the two groups regarding age or sex (P = 0.195 and, P = 0.534, respectively). The patients included 335 (55.4%) men and 270 (44.6%) women, with 267 patients younger than 40 years of age, and 338 patients older than 40 years of age. A total of 382 (63.1%) patients were classified with low-grade glioma (WHO grades I–II) and 223 (36.9%) with high-grade glioma (WHO grades III–IV). There were 416 (68.8%) patients with glioma who underwent gross-total tumor surgical resection and 189 (31.2%) who underwent near-total or sub-total resection. In total, 545 (90.1%) patients received radiotherapy treatment, and 250 (41.3%) patients received chemotherapy. The basic characteristics of the participants are listed in Table 2.

Table 2 The Characteristics Of Gliomas Cases And Cancer-Free Controls

Characteristics	Cases	Control	*P* value*
Number	605	1300	
Age (mean ± SD)	40.71±18.28	41.68±13.54	0.195
<40 years	267	561	
≥40 years	338	739	0.688
Sex			
Male	335	700	
Female	270	600	0.534
WHO Grade			
I-II	382		
III-IV	223		
Surgery			
STR & NTR	189		
GTR	416		
Radiotherapy			
No	60		
Yes	545		
Chemotherapy			
No	355		
Yes	250		

Note: *T-test or two-sided χ^2-test.
Abbreviations: STR, subtotal resection; NTR, near total resection; GTR, gross total resection; SD, Standard Deviation.

TYMS Polymorphisms In The Patients With Glioma And Controls

The genotypic frequency for the *TYMS* rs1059394 and rs2847153 polymorphisms conformed to HWE (P = 0.53 and P = 0.47, respectively). The genotypic and allelic frequencies of *TYMS* rs1059394 and rs2847153 are presented in Table 3. Compared with the wildtype genotype of rs1059394, we found that TT and CT+TT genotype carriers had significantly decreased glioma risk (TT to CC: OR = 0.71, 95% CI = 0.52–0.97, P = 0.03; CT+TT to CC: OR = 0.74, 95% CI =0.55–0.99, P = 0.04). However, no statistically significant difference was found between rs2847153 and glioma risk in genetic models (P > 0.05).

Relationship Between TYMS SNPs And Clinical Characteristics Of Glioma

We evaluated the correlations between the rs1059394 and rs2847153 polymorphisms and clinical characteristics of patients with glioma, including age, sex, and WHO grade. As shown in Table 4, in high-grade gliomas, the GA and GA+AA genotypes of rs2847153 were significantly increased, with the GG genotype as the reference (GA to GG: OR = 2.01, 95% CI = 1.39–2.91, P < 0.001; GA+AA to GG: OR = 1.78, 95% CI =1.25–2.54, P < 0.001). There was a balanced genotype distribution in rs1059394 polymorphisms (Table 5).

Expression Quantitative Trait Loci

To investigate the potential biological effects of the two significant SNPs on the TYMS gene expression, we explored eQTL analysis by GTEx portal. The results indicated that genotypes of both SNPs were significantly associated with TYMS gene expression in transformed fibroblasts cells (Figure 1).

Discussion

Gliomas are highly malignant with a poor prognosis, although early diagnosis and improved treatment are widely implemented. In addition, there were 296,851 new cases of brain and nervous system cancer, and glioma accounted for the majority of brain cancers.[22,23] In China, 1,016,000 new cases of brain and central nervous system cancer were reported in 2015.[24] It was suggested that genetic factors were primarily responsible for glioma genesis,[25] and there was still a lack of prospective molecular biomarkers for glioma.

TYMS is reported to be associated with folate metabolism, and it catalyzes conversion of deoxyuridine-5́- monophosphate

Table 3 Genotype Frequencies Of *TYMS* Polymorphisms In Cases And Controls

Model	Genotype	Control (n, %)	Case (n, %)	OR (95% CI)	*P*-value*
rs1059394 HWE: *P=0.53*					
Co-dominant	CC	131(10.1%)	80 (13.2%)	1.00 (reference)	
Heterozygote	CT	548(42.1%)	255 (42.2%)	0.76(0.56–1.04)	0.09
Homozygote	TT	621(47.8%)	270 (44.6%)	0.71(0.52–0.97)	0.03
Dominant	CC	131(10.1%)	80 (13.2%)	1.00 (reference)	
	CT+TT	1169(89.9%)	525(86.8%)	0.74(0.55–0.99)	0.04
Recessive	CC+CT	679(52.2%)	335(55.4%)	1.00 (reference)	
	TT	621(47.8%)	270(44.6%)	0.88(0.73–1.07)	0.20
Overdominant	CC+TT	752(51.9%)	350(57.8%)	1.00 (reference)	
	CT	548(42.1%)	255(42.2%)	1.00(0.82–1.22)	1.00
Allele	C	810(31.2%)	415(34.5%)	1.00 (reference)	
	T	1790(68.8%)	795(65.5%)	0.87(0.75–1.00)	0.05
rs2847153[a] HWE: *P=0.47*					
Co-dominant	GG	534(41.1%)	223(36.9%)	1.00 (reference)	
Heterozygote	GA	589(45.3%)	295(48.9%)	1.20(0.97–1.48)	0.09
Homozygote	AA	177(13.6%)	86(14.2%)	1.16(0.86–1.57)	0.32
Dominant	GG	534(41.1%)	223(36.9%)	1.00 (reference)	
	GA+AA	766(58.9%)	381(63.1%)	1.19(0.98–1.45)	0.09
Recessive	GG+GA	1123(86.4%)	518(85.8%)	1.00 (reference)	
	AA	177(13.6%)	86(14.2%)	1.05(0.80–1.39)	0.71
Overdominant	GG+AA	711(44.7%)	309(51.2%)	1.00 (reference)	
	GA	589(45.3%)	295(48.8%)	1.15(0.95–1.39)	0.15
Allele	G	1657(63.7%)	741(61.3%)	1.00 (reference)	
	A	943(36.3%)	467(38.7%)	1.11(0.96–1.28)	0.16

Notes: *Univariate logistic regression analysis for the distributions of genotype and allele frequencies. Adjusted for age and sex. [a]Genotype deletion: cases n=1. The Co-dominant, Dominant, Recessive, Overdominant, Allele represented five models.

Abbreviations: HWE, Hardy–Weinberg Equilibrium; OR, Odd Ratio; CI, Confidence Interval.

into deoxythymidine-5́-monophosphate. It is suggested that *TYMS* down regulation can influence DNA repair mechanisms, which is related to cell transformation and cancer development.[26] *TYMS* is also an important target of 5-fluorouracil (5-FU), inhibition of *TYMS* by fluorodeoxyuridine monophosphate (an active metabolite of 5-FU) results in DNA damage and cell death.[15,27] Therefore, functional genetic variants of *TYMS* may lead to cancer, and *TYMS* maybe a molecular biomarker. It is indicated that *TYMS* genetic polymorphisms are correlated with the susceptibility of different cancers.

The *TYMS* polymorphisms rs1059394 (C>T) and rs2847153 (G>A) have been investigated in a few cancers. Rs1059394 TT genotypes were found to be correlated with a significantly increased risk of gastric cancer.[12] Further stratified analysis indicated that the rs1059394 T variant allele was associated with a significantly decreased risk of breast cancers in patients with a smoking history.[10] In addition, as for patients with non-small cell lung cancer, rs2847153 in *TYMS* may be helpful for prognosis and personalized treatment.[28] There have been no studies about *TYMS* polymorphisms and glioma risk previously.

Our study evaluated the relationship between *TYMS* polymorphisms (rs1059394 and rs2847153) and glioma risk. Compared with the wildtype genotype of rs1059394, we found that TT and CT+TT genotype carriers had a significantly decreased glioma risk, indicating that rs1059394 C>T was associated with the low susceptibility of glioma. In high-grade gliomas, the GA and GA+AA genotypes of rs2847153 were significantly increased,

Table 4 The Associations Between The *TYMS* rs2847153 Polymorphisms And Clinical Characteristics Of Gliomas Patients

Characteristics	Genotype Distributions			
	GG	GA	AA	GA+AA
Age				
<40/≥40	106/117	123/172	38/48	161/220
OR (95% CI)	1.00 (Reference)	1.27 (0.82–1.80)	1.14 (0.69–1.89)	1.23 (0.89–1.73)
P-value*		0.185	0.597	0.208
Sex				
Male/Female	116/107	169/126	50/36	219/162
OR (95% CI)	1.00 (Reference)	0.81 (0.57–1.15)	0.78 (0.47–1.29)	0.80 (0.58–1.12)
P-value*		0.233	0.334	0.193
WHO Grade				
I+II/III+IV	159/64	163/132	59/27	222/159
OR (95% CI)	1.00 (Reference)	2.01 (1.39–2.91)	1.14 (0.66–1.95)	1.78 (1.25–2.54)
P-value*		<0.001	0.641	0.001

Notes: *Univariate logistic regression analysis for the distributions of genotype frequencies. Genotype distributions including all the genotype of *TYMS* rs2847153 polymorphisms.
Abbreviations: OR, Odd Ratio; CI, Confidence Interval.

Table 5 The Associations Between The *TYMS* Rs1059394 Polymorphisms And Clinical Characteristics Of Gliomas Patients

Characteristics	Genotype Distributions			
	CC	CT	TT	CT+TT
Age				
<40/≥40	38/42	118/137	111/159	229/296
OR (95% CI)	1.00 (reference)	1.05 (0.63–1.74)	1.30 (0.78–2.14)	1.17 (0.73–1.87)
P-value*		0.848	0.311	0.515
Sex				
Male/Female	13/37	145/110	147/123	292/233
OR (95% CI)	1.00 (reference)	0.88 (0.53–1.46)	0.97 (0.59–1.61)	0.93 (0.58–1.49)
P-value*		0.625	0.913	0.754
WHO Grade				
I+II/III+IV	46/34	162/93	174/96	336/189
OR (95% CI)	1.00 (reference)	0.78 (0.47–1.30)	0.75 (0.45–1.25)	0.76 (0.47–1.23)
P-value*		0.333	0.26	0.263

Note: *Univariate logistic regression analysis for the distributions of genotype frequencies.
Abbreviations: OR, Odd Ratio; CI, Confidence Interval.

which means that GA or GA+AA genotypes may predict a worse prognosis. Therefore, the polymorphism of *TYMS* may be biomarkers of clinical outcomes and personalized treatment. A study evaluated the expression of *TYMS* gene in the metastatic lymph node and primary foci of low-grade glioma, with a significant positive TYMS expression.[15] The specific mechanism of this is unclear, which is a potential subject on high-grade glioma for further evaluation.

Our study also had some limitations. Firstly, all samples originated from a hospital in the northwest region of China, which inevitably led to selection bias. Second, we did not stratify our analysis for tumor subtypes because the sample size was circumscribed. Third, due to the limits of data, we did not analyze the impact of other factors, such as dose radiation exposure, lifestyle, family history, tumor size and outcome. Hence, this deserves further investigation with a multi-center, case-control study in the future.

To summarize, our study indicated that *TYMS* polymorphisms were associated with glioma susceptibility. The rs1059394 C>T variant could decrease the risk of glioma. In addition, the rs2847153 G>A variant might predict worse survival in glioma patients. Further functional and multi-center

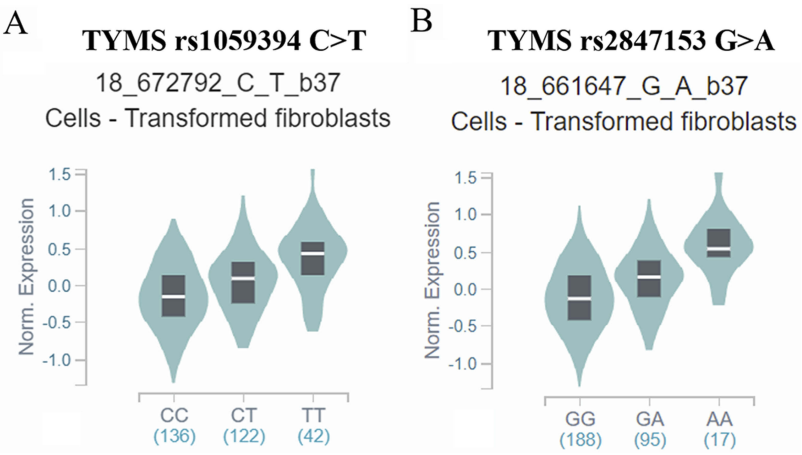

Figure 1 Analysis of the rs1059394 and rs2847153 polymorphisms in the TYMS gene in transformed fibroblast cells. Shown is the eQTL analysis for the (**A**) rs1059394 and (**B**) rs2847153 polymorphisms in the TYMS gene in transformed fibroblast cells (GTEx portal).

case-control studies are needed to clarify the association between *TYMS* polymorphisms and the susceptibility of glioma.

Ethical Approval And Informed Consent

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration. Informed consent was obtained from all individual participants included in the study.

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Author Contributions

All authors contributed towards data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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