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

Li Yao1,*, Linghui Zhou2,3,*, Yujiao Deng2,3,*, Yi Zheng2,3, Pengtao Yang2, Meng Wang2, Shanshan Dong4, Qian Hao2,3, Peng Xu2,3, Na Li2,3, Ying Wu2,3, Zhen Zhai2,3, Lijuan Lyu2,3, Zhijun Dai1,2,3

1Department of Neurology, The Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710004, People’s Republic of China; 2Department of Oncology, The Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710004, People’s Republic of China; 3Department of Breast Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, People’s Republic of China; 4School of Life Science and Technology, Xi’an Jiaotong University, Xi’an 710049, People’s Republic of China

*These authors contributed equally to this work

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
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. Inhibition of TYMS expression leads to thymidylate depletion and thymineless death, accompanied by DNA damage, apoptosis, and chromosome aberrations. Currently, several TYMS SNPs have been reported to be correlated with susceptibility to cancers including breast, lung, gastric, colorectal, and ovarian cancers.

A previous study presented that TYMS expressed positively in 27.39% of lymph node of low-grade glioma patients. 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), which was referred to in previous studies. SNP genotyping was carried out using Sequenom Mass-ARRAY RS1000. Sequenom Typer 4.0 software was used to analyze data. 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. 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 | ACGTTGGATGGTATCGACAGGATCATACCTC | ACGTTGGATGGTATCGACAGGATCATACCTC |         |
| rs2847153 | ACGTTGGATGGTATCGACAGGATCATACCTC | ACGTTGGATGGTATCGACAGGATCATACCTC |         |

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.

**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. In China, 1,016,000 new cases of brain and central nervous system cancer were reported in 2015. It was suggested that genetic factors were primarily responsible for glioma genesis, 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-5'-monophosphate

---

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

| Characteristics | Cases | Control | $P$ value* |
|-----------------|-------|---------|-----------|
| Number          | 605   | 1300    |           |
| Age (mean ± SD) |       |         |           |
| <40 years       | 40.71±18.28 | 41.68±13.54 | 0.195    |
| ≥40 years       | 338   | 739     | 0.688     |
| Sex             |       |         |           |
| Male            | 335   | 700     |           |
| Female          | 270   | 600     | 0.534     |
| WHO Grade       |       |         |           |
| I-II            | 382   | 561     |           |
| III-IV          | 223   | 739     |           |
| Surgery         |       |         |           |
| STR & NTR       | 189   | 561     |           |
| GTR             | 416   | 739     |           |
| Radiotherapy    |       |         |           |
| No              | 60    | 130     |           |
| Yes             | 545   | 1170    |           |
| Chemotherapy    |       |         |           |
| No              | 355   | 561     |           |
| Yes             | 250   | 739     |           |

*Note: *$T$*-test or two-sided $\chi^2$-test.

**Abbreviations:** STR, subtotal resection; NTR, near total resection; GTR, gross total resection; SD, Standard Deviation.
into deoxythymidine-5’-monophosphate. It is suggested that TYMS down regulation can influence DNA repair mechanisms, which is related to cell transformation and cancer development. 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. 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. 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. In addition, as for patients with non-small cell lung cancer, rs2847153 in TYMS may be helpful for prognosis and personalized treatment. 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 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      |
| Heterozygote     | CC       | 131(10.1%)     | 80 (13.2%)  | 1.00 (reference) |
|                  | CT       | 548(42.1%)     | 255 (42.2%) | 0.76(0.56–1.04) | 0.09     |
|                  | TT       | 621(47.8%)     | 270 (44.6%) | 0.71(0.52–0.97) | 0.03     |
| Dominant         |
| CC               | 131(10.1%)  |
| CT+TT            | 1169(89.9%) |
|                  | 80 (13.2%)  | 525(86.8%)     | 1.00 (reference) |
| Recessive        |
| CC+CT            | 679(52.2%)  |
| CT               | 621(47.8%)  |
|                  | 335(55.4%)  | 270(44.6%)     | 1.00 (reference) |
|                  | 0.88(0.73–1.07) | 0.20     |
| Overdominant     |
| CC+TT            | 752(51.9%)  |
| CT               | 548(42.1%)  |
|                  | 350(57.8%)  | 255(42.2%)     | 1.00 (reference) |
|                  | 1.00(0.82–1.22) | 1.00     |
| Allele           |
| C                | 810(31.2%)  |
| T                | 1790(68.8%) |
|                  | 415(34.5%)  | 795(65.5%)     | 1.00 (reference) |
|                  | 0.87(0.75–1.00) | 0.05     |

| rs2847153 HWE: P=0.47 |
|-----------------------|
| Co-dominant           |
| Heterozygote          |
| GG                    | 534(41.1%)  |
| GA                   | 589(45.3%)  |
| AA                   | 177(13.6%)  |
| Dominant              |
| GG                    | 534(41.1%)  |
| GA+AA                | 766(58.9%)  |
| Recessive             |
| GG+GA                | 1123(86.4%) |
| AA                   | 177(13.6%)  |
| Overdominant          |
| GG+AA                | 711(44.7%)  |
| GA                   | 589(45.3%)  |
| Allele                |
| G                    | 1657(63.7%) |
| A                    | 943(36.3%)  |

Notes: *Univariate logistic regression analysis for the distributions of genotype and allele frequencies. Adjusted for age and sex. 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.
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.\textsuperscript{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
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.

Acknowledgments
We thank all members of our study team for their whole-hearted cooperation and the included participants for their wonderful cooperation.

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.

References
1. Liu K, Jiang Y. Polymorphisms in DNA repair gene and susceptibility to glioma: a systematic review and meta-analysis based on 33 studies with 15 SNPs in 9 genes. Cell Mol Neurobiol. 2017;37(2):263–274. doi:10.1007/s10571-016-0367-y
2. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114(2):97–109. doi:10.1007/s00401-007-0243-4
3. Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a “state of the science” review. Neuro-oncology. 2014;16(7):896–913. doi:10.1093/neuonc/nou087
4. Visser O, Ardanaz E, Botta L, et al. Survival of adults with primary malignant brain tumours in Europe; Results of the EUROCARE-5 study. Eur J Cancer. 2015;51(15):2231–2241. doi:10.1016/j.ejca.2015.07.032
5. Bauchet L, Ostrom QT. Epidemiology and molecular epidemiology. Neurooncol N Am. 2019;30(1):1–16. doi:10.1016/j.neu.2018.08.010
6. Savage N. Searching for the roots of brain cancer. Nature. 2018;561(7724):S50–S51. doi:10.1038/d41586-018-06709-2
7. Kumawat R, Gowda SH, Deb Nath E, et al. Association of Single Nucleotide Polymorphisms (SNPs) in genes encoding for folate metabolising enzymes with glioma and meningioma in Indian population. Asian Pac J Cancer Prev. 2018;19(12):3415–3425. doi:10.31557/APJCP.2018.19.12.3415
8. Hori T, Takahashi E, Ayusawa D, Takeishi K, Kaneda S, Seno T. Regional assignment of the human thymidylate synthase (TS) gene to chromosome band 18p11.32 by nonisotopic in situ hybridization. Hum Genet. 1990;85(6):576–580. doi:10.1007/bf00193577
9. Chen D, Jansson A, Sim D, Larsson A, Nordlund P. Structural analyses of human thymidylate synthase reveal a site that may control conformational switching between active and inactive states. J Biol Chem. 2017;292(32):13449–13458. doi:10.1074/jbc.M117.787267
10. Guan X, Liu H, Ju J, et al. Genetic variant rs16430 6bp > 0bp at the microRNA-binding site in TYMS and risk of sporadic breast cancer risk in non-Hispanic white women aged <= 55 years. Mol Carcinog. 2015;54(4):281–290. doi:10.1002/mc.22097
11. Feng W, Guo X, Huang H, et al. Polymorphism rs3819102 in thymidylate synthase and environmental factors: effects on lung cancer in Chinese population. Mol Carcinog. 2015;54(9):880–888. doi:10.1002/mc.22160
12. Shen R, Liu H, Wen J, et al. Genetic polymorphisms in the microRNA binding-sites of the thymidylate synthase gene predict risk and survival in gastric cancer. Mol Carcinog. 2015;54(9):880–888. doi:10.1002/mc.22160
13. Amirfallah A, Kocal GC, Unal OU, Ellidokuzu H, Oztok I, Basbınar Y. DPYD, TYMS and MTHFR genes polymorphism frequencies in a series of Turkish colorectal cancer patients. J Pers Med. 2018;8:4. doi:10.3390/jpm8040045
14. Kelemen LE, Earp M, Fridley BL, et al. rs495139 in the TYMS-ENOSF1 region and risk of ovarian carcinoma of mucinous histology. Int J Mol Sci. 2018;19:9. doi:10.3390/ijms19092473
15. Ding B, Gao M, Li Z, Xu C, Fan S, He W. Expression of TYMS in lymph node metastasis from low-grade glioma. Oncol Lett. 2015;10(3):1569–1574. doi:10.3892/ol.2015.3419
16. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protocols Human Genet. 2009. Chapter 2:Unit2.12.

17. Lin S, Wang M, Liu X, et al. FEN1 gene variants confer reduced risk of breast cancer in Chinese women: a case-control study. Oncotarget. 2016;7(47):78110–78118. doi:10.18632/oncotarget.12948

18. Tian T, Wang M, Zheng Y, et al. Association of two FOXP3 polymorphisms with breast cancer susceptibility in Chinese Han women. Cancer Manag Res. 2018;10:867–872. doi:10.2147/CMAR.S158433

19. Dai Z, Tian T, Wang M, et al. Genetic polymorphisms of estrogen receptor genes are associated with breast cancer susceptibility in Chinese women. Cancer Cell Int. 2019;19:11. doi:10.1186/s12935-019-0727-z

20. Thomas RK, Baker AC, Debiasi RM, et al. High-throughput oncoprotein mutation profiling in human cancer. Nat Genet. 2007;39(3):347–351. doi:10.1038/ng1975

21. GTex Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45(6):580–585. doi:10.1038/ng.2653

22. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.21492

23. Benson VS, Pirie K, Schuz J, Reeves GK, Beral V, Green J. Mobile phone use and risk of brain neoplasms and other cancers: prospective study. Int J Epidemiol. 2013;42(3):792–802. doi:10.1093/ije/dyt072

24. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–132. doi:10.3322/caac.21338

25. Haque A, Banik NL, Ray SK. Molecular alterations in glioblastoma: potential targets for immunotherapy. Prog Mol Biol Transl Sci. 2011;98:187–234. doi:10.1016/B978-0-12-385506-0.00005-3

26. Mandola MV, Stoechmacher J, Zhang W, et al. A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics. 2004;14(5):319–327.

27. Mitchell LA, Lopez Espinoza F, Mendoza D, et al. Toca 511 gene transfer and treatment with the prodrug, 5-fluorocytosine, promotes durable antitumor immunity in a mouse glioma model. Neuro-oncol. 2017;19(7):930–939. doi:10.1093/neuonc/nox037

28. Dong H, Bao D, Guo X, et al. Effect of thymidylate synthase gene polymorphism on the response to chemotherapy and clinical outcome of non-small cell lung cancer patients. Tumour Biol. 2015;36(9):7151–7157. doi:10.1007/s13277-015-3447-6